出國報告（出國類別：其他）

赴美國參加 31st Symposium on Biotechnology for Fuels and Chemicals

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摘 要

本次公差赴美目的爲參加 31st Symposium on Biotechnology for Fuels and Chemicals (簡稱 SBFC)，並受邀於會中發表本所纖維酒精計畫研究成果。論文名稱爲：Transcriptomic analysis of carbohydrate metabolism during ethanol fermentation by *Pichia stipitis*。SBFC 爲全美規模最大之生質能源研討會，今年與會的各界專家學者人數近千人，共有五百多篇的論文發表。會議議程涵蓋範圍相當廣泛，自學術研究、產業發展乃至於政策走向等均有所涉略。通過本研討會發現，目前纖維酒精的發展已進入製程整合的階段，如何根據不同生質原料的特性，搭配適當的轉化技術，並選擇高轉化率的生質能源輸出爲當今各界的研發重點。在單元技術發展方面，生質原料的收集及物流管理系統逐漸受到重視、纖維酵素的研發爲影響酒精成本的關鍵因素，高丁醇耐受性菌種的發現使生質丁醇的未來發展引人矚目。參與此次會議除可收集最新技術研發成果外，能與各界專家學者直接的交流與經驗分享，更有助於日後進行國際合作的研究。

目　次

一、目的

本所於 94 年起開始致力於纖維酒精技術之研發，迄今已陸續完成 Lab scale 技術發展、Bench scale 程序驗證、10 公斤級小型纖維酒精程序單元測試系統建立與噸級纖維酒精測試廠設置。現階段工作除進行噸級酒精測試廠程序研究之外，對於製程中各單元技術的效能精進仍持續進行。

Symposium on Biotechnology for Fuels and Chemicals 爲全美規模最大之生質能源相關之研討會，本次大會假舊金山舉辦爲第 31 屆會議,會中討論範圍自基礎研究到產業現況均有所涉略。本次受邀於會議中進行論文發表，說明本所纖維酒精技術之發展現況。一方面可拓展本所國際能見度另一方面也希望能收集國際上最新的技術發展現況，提供本所纖維酒精研發人員參考。

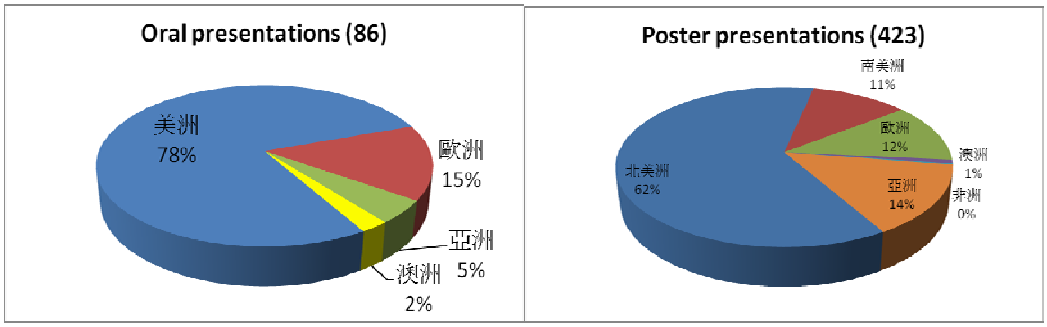
二、過程

(一)行前準備

本次公務人員赴美公差主要行程爲參加 31st Symposium on Biotechnology for Fuels and Chemicals，並受邀於會中進行論文發表。作品名稱爲”Transcriptomic analysis of carbohydrate metabolism during ethanol fermentation by *Pichia stipitis*”(如附錄一)。在纖維酒精生產過程中，*Pichia stipitis* 可以發酵葡萄糖及木糖生成酒精，爲一相當有潛力的發酵菌種。利用客製化、高專一性的基因晶片大規模分析在不同碳源存在的條件下，*Pichia stipitis* 於發酵過程中的基因表現差異。釅清酵母菌對應不同碳源時細胞內的基因表現，有助於建立後續利用基因工程行菌種改殖的基礎。

研討會詳細議程於會議舉辦前一週公布於 SBFC 的官方網站: http://www.simhq.org/meetings/sbfc2009/index.html。研討會議程分爲 oral presentation 與 poster presentation 兩大部分。oral presentation 部分共計有 14 個 session (包括兩個 Special Topic)，86 場演講。依演講者所屬國家來區分，美國占

78%、歐洲占 15%、亞洲及澳洲各占 5%及 2%。Poster presentation 部分共計有 12 個 session，423 篇 poster。依第一作者的國籍進行統計，北美洲占 62%、亞洲占 14%、歐洲占 12%、南美洲占 11%、澳洲與非洲則各有 3 篇及 1 篇發表。(圖一)



圖一、研討會論文發表國籍統計

(二)赴美行程

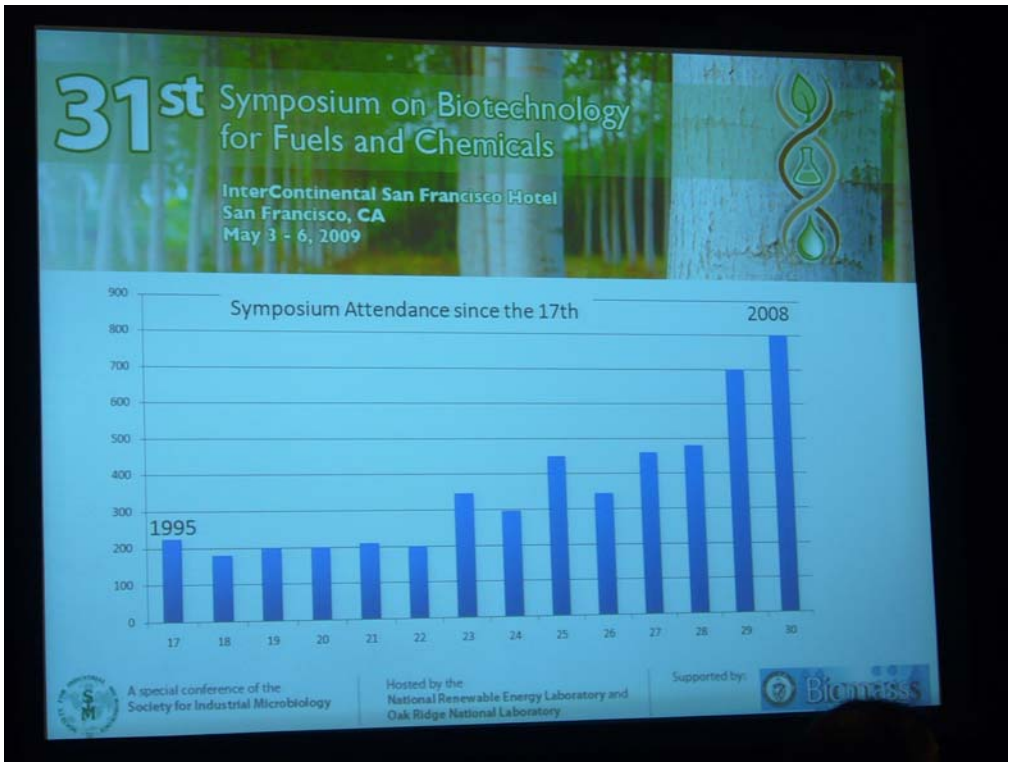
5/1：搭乘長榮航空(BR18)由桃園國際機場出發至舊金山國際機場。

5/3~5/6：參加 31st Symposium on Biotechnology for Fuels and Chemicals

5/7：搭乘長榮航空(BR27)由舊金山國際機場返回桃園國際機場

(三)會議內容

本次公務出國主要目的係參加 31st Symposium on Biotechnology for Fuels and Chemicals。本次大會於美國舊金山市區 InterContinental San Francisco Hotel 舉行，為期四天(5/3~5/6)，超過 9 百多人與會參加。(圖二) 研討議程相當豐富，包括：(1)Plant Science and Technology、(2)Microbial Science and Technology、(3)Biomass Pretreatment and Fractionation、(4)Translational Genomics for Bioenergy Feedstocks and Microbes、(5)Enzyme Science and Technology、(6)Biorefinery Deployment、(7)Biofuels logistics and Sustainability、(8)International Commercialization of 2nd Generation Biofuels、(9)Development and Commercialization of Algal-based 、Biofuels、(10)Bioprocessing and Separations Technology、(11)Emerging Biofuels and Chemicals、(12)Biomass Recalcitrance 等多項研究主題。



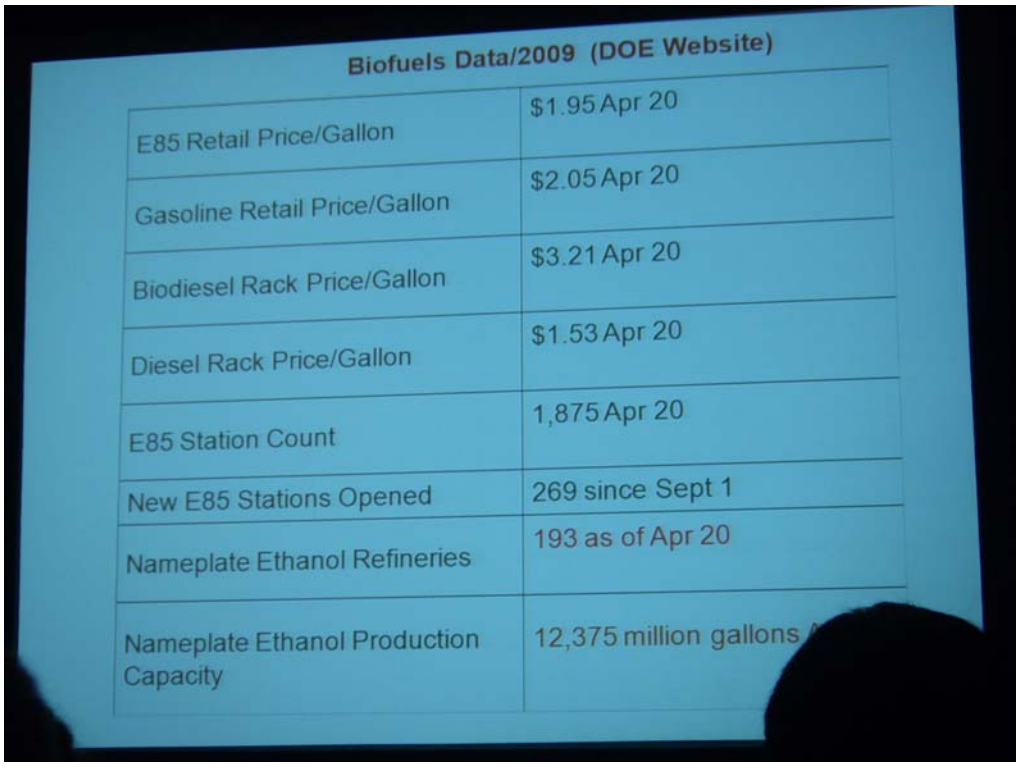
圖二、SBFC的參與人數呈逐年增加趨勢

議程內容包括實驗室基礎研究、生質燃料的商業化發展乃至於政策方向均有所涉略，資料相當豐富。由於討論主題眾多，同一時段係有兩個session同時進行，加上此次會議本所只有一人參加，因此僅能挑選計畫較爲著重的主題參與，難免有遺珠之憾。茲將本次與會所收集之生質燃料技術研發現況彙整如下：

1.研討會第一天

(1) Texas Tech University的Dr. Karim根據DOE最新的資料(圖三)指出，目前每加侖E85酒精的零售價$1.95已經低於

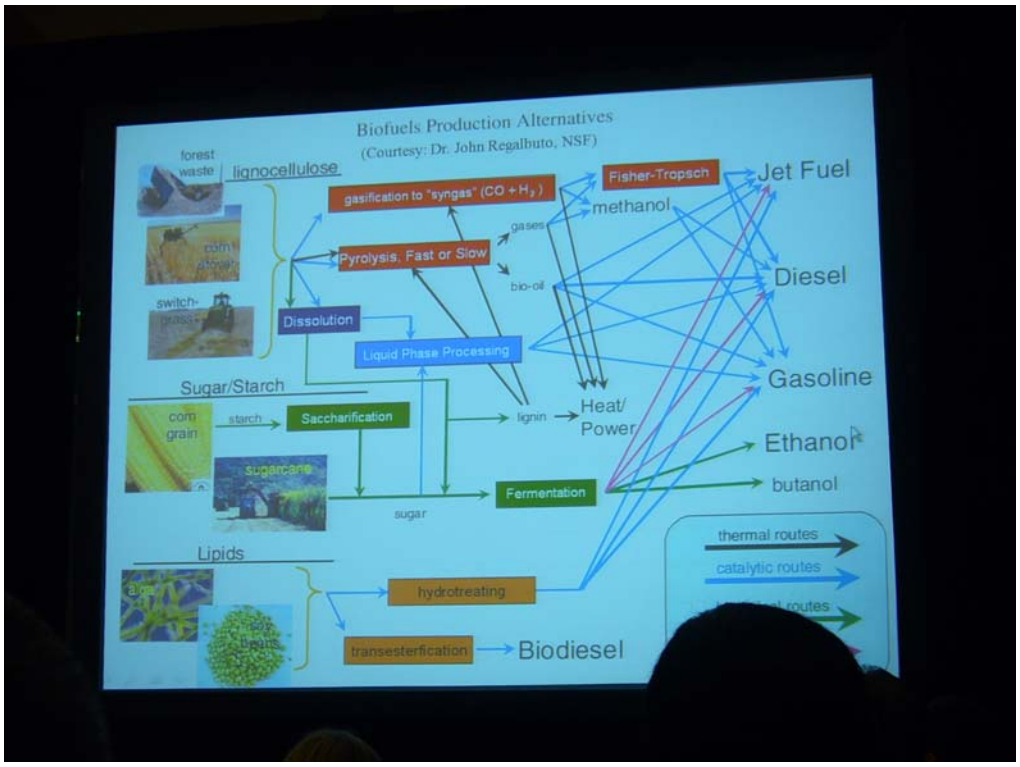
汽油的零售價$2.05，全美現今共有193個酒精精煉廠，可生產12,375百萬加侖(million gallons)的酒精，並且有超過1800座的E85加油站，這些數據顯示酒精汽油已的確成爲穩定的燃料供給鏈的一員。發展纖維酒精生產技術不單是學術界的實驗研究而是現實上的迫切需求。以多樣化的生質原料轉化策略 (包括熱化學、化學催化、生物反應)進行各種生質燃料的生產 (包括燃油、柴油、汽油、酒精、丁醇)將是今後發展的趨勢。



圖三、DOE統計全美生質燃料供應現況

生質酒精的發展已從第一代的澱粉酒精生產、第二代的木質纖維素酒精生產邁向第三代的整合式生質燃料

生產計畫 (An integrated approach renewable biofuels program)(圖四)。整合式生質燃料生產計畫係指充分利用不同生質能源間的特性，整合利用以達到最佳的能源效益，此概念相當受到與會各界專家學者的認同，值得我們參考。

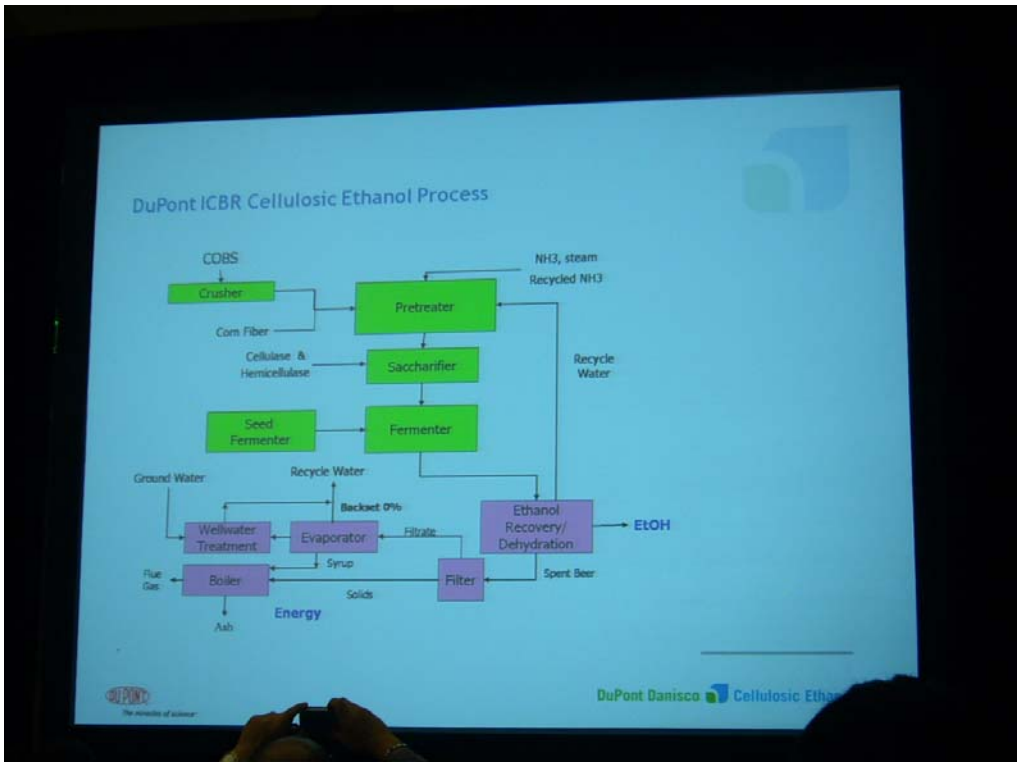


圖四、整合式生質燃料生產示意圖

而根據NREL所建立的評估資料，在2012年酒精價格可望降至$1.0，其中酵素的成本降低仍是一個關鍵的因素。德州大學在先前的研究指出高固液比的酵素反應中，質傳效率 (mass transfer rate)爲影響反應產率的關鍵因素。研究團隊利用基因工程技術在高酒精耐受度*E.coli*

LY01的細胞膜上表現纖維酵素，使其能同步進行酵素水解及發酵兩種反應。在實驗室階段已完成了利用玉米桿水解液 (corn stover hydrolyzate)以SSF程序進行酒精生產的研究。

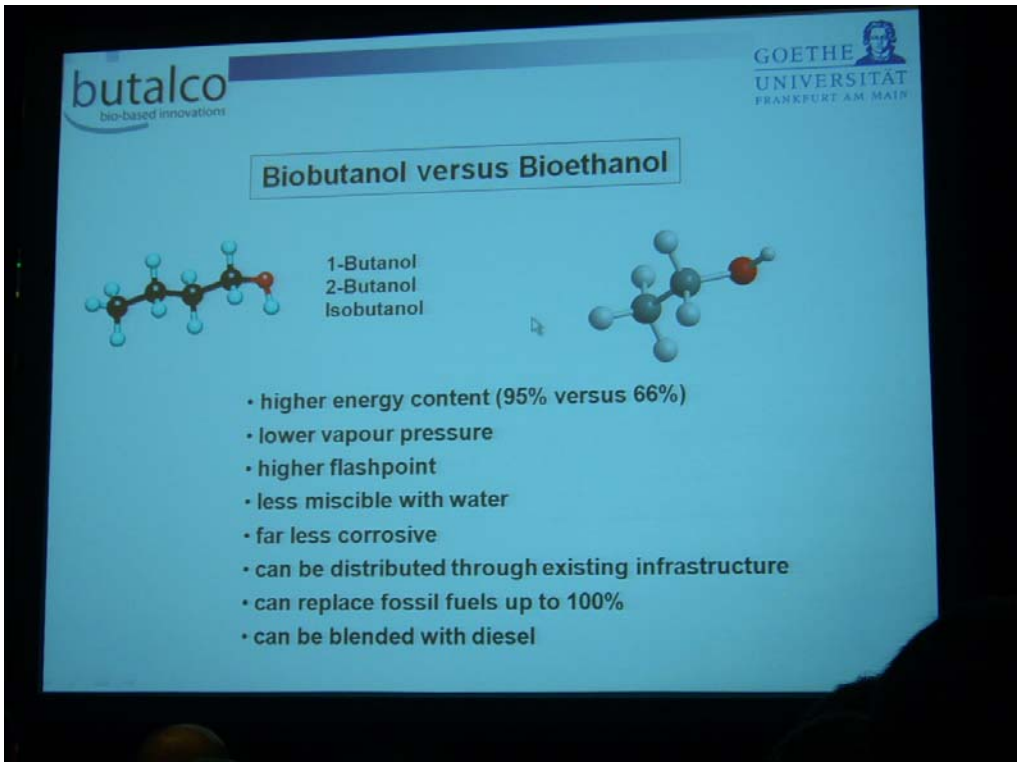
(2) DuPont與NREL的合作研究報告中指出，利用基因工程改良的*Z.mobilis*進行高濃度混合糖質的同步發酵(圖五)。DuPont ICBR Cellulosic Ethanol Process以玉米穗軸(corn cob)做爲原料，利用氨氣(NH3)進行前處理，配合纖維酵素與半纖維酵素進行水解反應。以此製程在200L的發酵槽中進行測試，以ZW800作爲發酵菌種在50小時左右可以消耗掉70g/L葡萄糖與50g/L木糖並產生82g/L酒精，酒精產率爲1.45g/L/h，且並沒有發現明顯副產物的生成。不過分析其酒精產量竟然超過理論產率，使得其研究成果有待更進一步確認。



圖五、DuPont ICBR的纖維酒精製程

(3) Goethe University與Butalco公司合作，利用基因工程技術轉殖細菌的木糖異構酶(xylose isomerase, XI)基因到工業用酵母菌*Saccharomyces cerevisiae*中，發展共發酵酵母菌的研究結果相當出色。理想的發酵菌株須具備以下特性：爲強健的(robust)工業用菌種、穩定性高、環境與抑制物的耐受性高、高產率與高發酵速率且具備共發酵能力。本篇研究的策略與所採取的實驗方法相當完整，經由分析不同來源的XI基因優缺點，篩選出最佳的基因來源後再針對其基因缺陷處進行人工修飾，配合專一性五碳糖傳送因子的選殖以及轉殖菌種的穩定性馴化策略，初步完成了高效率

的木糖、阿拉伯糖共發酵菌的建構。

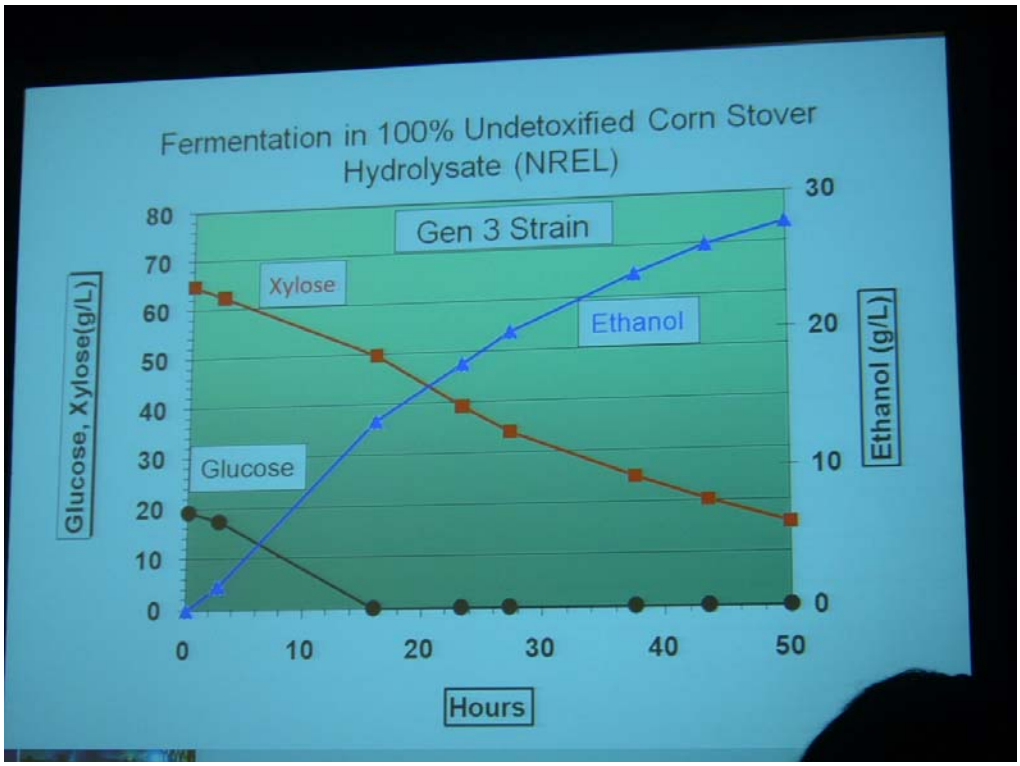


圖六、生質丁醇與酒精的比較

此外Butalco亦著手發展生質丁醇(biobutanol)的研究，與酒精相比，以丁醇作爲燃料的優點很多，有熱值高、較低的蒸氣壓、高閃點、與水相容性低、腐蝕性低、可以現有管線運輸、可完全替代石化燃料與可與柴油互相參配等(圖六)。Butalco業已開始進行生產生質丁醇的菌株研發，並獲得初步成果。一般而言，丁醇對於微生物的毒性較高，限制了以微生物進行丁醇生產的發展。不過Dr. Boles實驗室經過持續的菌種篩選，發現一株最高丁醇耐受度可達50g/L以上的菌種，提供了發展生質丁醇一個新

的契機。

(4) 在發酵過程中，若能控制在較低的pH值下進行，可以大幅降低雜菌汙染的風險。減少鹼液的使用量，對於降低成本亦有很大的幫助。Cargill’s yeast是一株具有高環境耐受性的微生物，對於醋酸、水解抑制物、酒精及溫度都有很高的容忍度。在pH4.5同時含有1%醋酸的發酵條件下，Cargill’s yeast可以在24小時內將超過150 g/kg的葡萄糖與甘露糖轉化成74 g/L的酒精，酒精產率爲94%，整體酒精生產速率達3.3g/L/h，是一株相當有潛力的酒精發酵菌。Dr. Suiminen利用XI的轉殖策略，將木糖代謝基因殖入Cargill’s yeast的基因中，進行共發酵菌種的研發。目前最新一代的基因工程轉殖株能在40度、pH<5的環境下進行發酵。以NREL所提供的稀酸水解液(xylose 66.8 g/L、glucose 22.2 g/L、Acetate 13.07 g/L、furfural 2.4 g/L)進行測試，在50小時之內可以發酵產生大約30 g/L的酒精。(圖七)



圖七、Cargill’ s yeast的發酵圖示

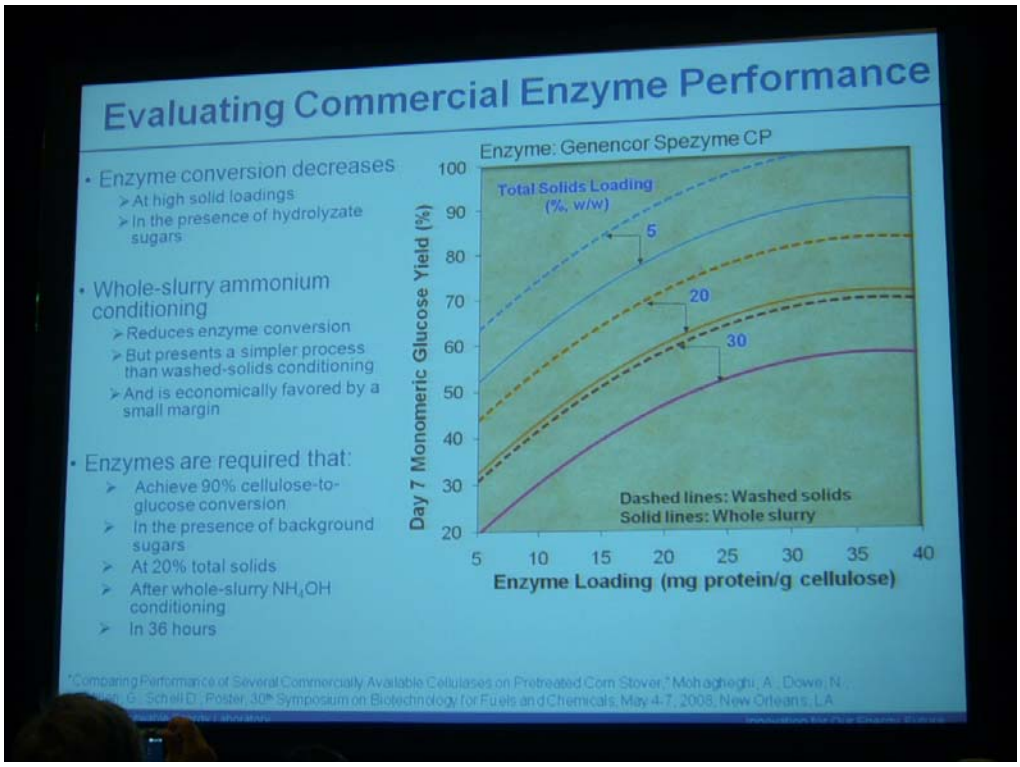
(5) UC Berkeley & Joint BioEnergy Institute共同發表基因工程丁醇發酵酵母菌的研究。*Clostridium acetobutylicum* (Cac)是傳統用來生產丁醇(n-butanol)的微生物。利用基因轉殖策略，將Cac合成n-butanol的代謝途徑殖入酵母菌*Saccharomyces cerevisiae*中，並調控代謝途徑中各種不同酵素的表現，期能達成最好的代謝效率。已經完成了轉殖菌株的初步建構，後續將由代謝產物的分析，持續進行菌種的改良。

2. 研討會第二天

(1) DSM是一家國際級的生技公司，酵素生產與研發爲其重要技術平台。DSM與DOE簽有合作計畫進行纖維生質原料的酵素研發工作，同時與Abengoa、LA National Lab等都有合作關係。在研討會中DSM發表其最新的纖維素水解酵素研發成果。DSM所採用的酵素生產系統爲真菌(fungal host system)，利用真菌系統做爲生產同源或異源性酵素的平台，用以水解纖維素生質原料。High cell density, high volumetric productivity, low interfering enzymatic activities 以及low enzyme costs是DSM生產酵素的四大重要原則。其所研發的熱穩定的糖化酵素組合，已配合不同的纖維原料與各種前處理技術進行測試。

(2) NREL利用經濟學的模式評估酵素反應的固液比對於生產酒精的成本所造成的影響。MESP (Minimum Ethanol Selling Price)是NREL用來評估生產成本的指標，係指每生產一加侖酒精所需的損益兩平售價(圖八)。影響MESP的因素很多，包括化學藥劑的添加量、酒精的轉化率、製程所需時間…等，整體而言，酵素成本是影響MESP最關鍵的因素。在實際的酵素反應模式中，提高反應的固液比必然

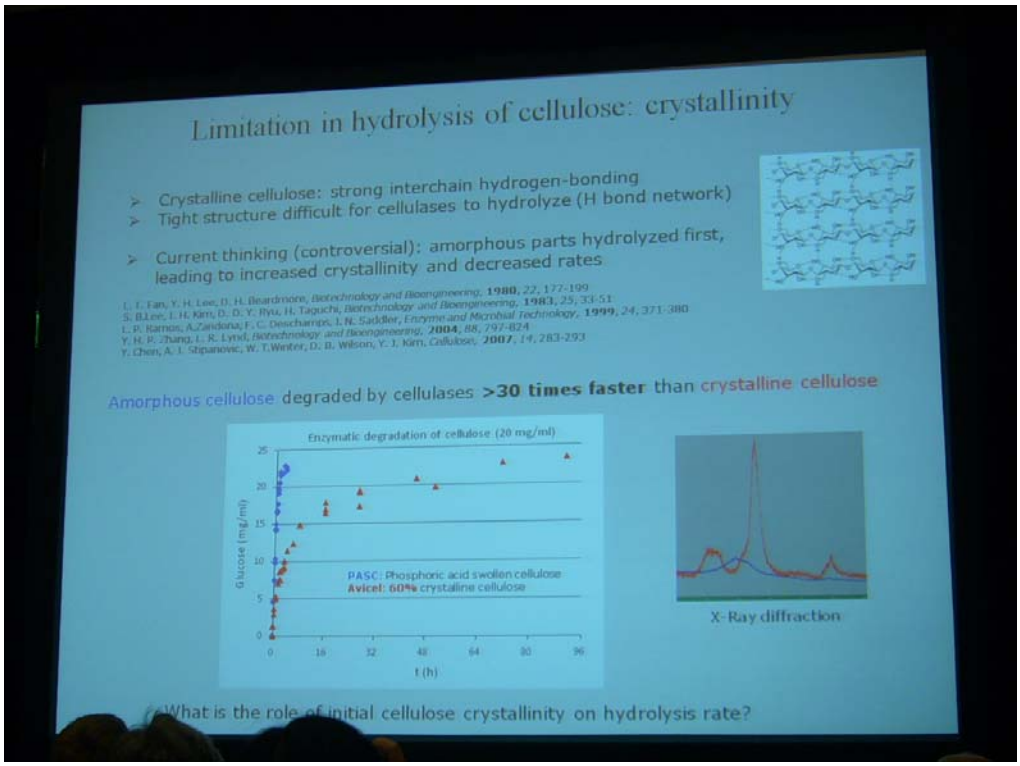
會導致酒精轉化率下降，增加酵素的使用量儘管可以彌補因爲高固液比所導致的轉化率下降，不過同時也需要考量增加酵素用量所提高的成本。在程序中加入纖維渣料水洗的步驟可以提升整體酒精轉化率約10%。以高固液比進行酵素水解的好處有減少設備投資、提高發酵酒精濃度等。以NREL所建立的模式作爲評估標準，在20mg/g的酵素使用量下，以19%的固液比進行酵素水解會得到最低的MESP。當未來酵素成本持續降低時，有利於發展更高固液比的水解程序。



圖八、NREL評估商業酵素表現

(3) Georgia Institute of Technology的Dr. Hall所進行的研

究，目的在尋找影響纖維酵素水解的關鍵因素。在酵素水解反應的過程中，50%的纖維素會在6小時內完全水解成葡萄糖，而在接下來的48~72小時內僅有約20%的纖維素會再被水解成葡萄糖。根據Dr. Hall的研究發現，這種不同速率的水解現象是由於纖維素的結構不同所導致的影響。纖維素可分爲結晶型纖維素(crystalline cellulose)與非結晶型(amorphous cellulose)纖維素兩種，結晶型纖維素以氫鍵做爲鍵結，而氫鍵較難以被打斷。在反應初期所產生的葡萄糖多半是由非結晶型的纖維素水解而得，因此反應速率較快。反應後期因爲原料中只剩下結晶型的纖維素，所以反應速率明顯下降。利用磷酸前處理技術可以有效降低生質原料的結晶性(crystallinity)，配合XRD技術分析生質原料結晶性的高低可以有效預測酵素水解的效率(圖九)。不過研究也指出，不同生質原料的基礎特性是影響酵素水解的主要因素。



圖九、生質原料的結晶性是影響纖維素水解因子

3. 研討會第三天

(1) POET爲一國際級的酒精生產公司，有超過20年以上的運轉經驗，年產量可達15億加侖(1.5 billion gallons) 。POET在全球擁有26個運轉中的酒精廠，有由上游到下游垂直整合的商業模式。其所發展的纖維酒精技術以玉米穗軸(corn cob)作爲原料，根據其統計玉米穗軸有超過50億加侖酒精的產值。使用玉米穗軸的優點有形狀固定容易收集、酒精產量高，收集玉米穗軸可爲農場每年增加30億的收入，也是由玉米酒精轉變至纖維酒精的最理想途徑。經過不同收割方式的測試與收割設備的改良，加上各種的儲存試驗，

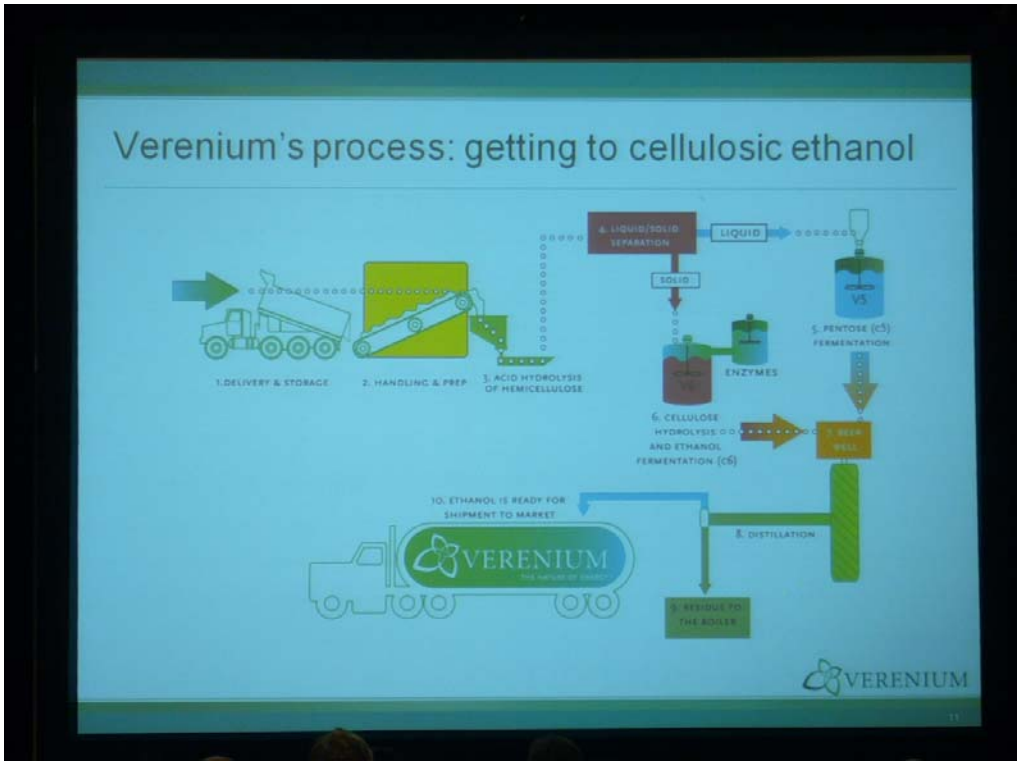
POET已經成功的建立玉米穗軸收集機制。POET’s pilot scale facility已經於2008年正式運轉，目前年產量爲2萬加侖。下一步POET將著手建立商轉工廠，預計總投資金額超過$200million。其中DOE資助$80million，加上Iowa州政府的資助，預計將於2011完成興建。POET’s commercial facility爲一整合型的生物煉製廠(biorefinery)。預計年產量可達125百萬加侖，其中的25百萬加侖由纖維素原料提供，運轉之後將是全世界第一個成功整合澱粉酒精和纖維酒精生產的系統。

(2) Novozymes於亞洲地區已累積多年的研發能量，在本研討會中說明近年來在中國發展生質酒精的經驗與潛力。根據統計資料顯示在2020年全中國每年可製造600百萬公噸(million tons)的農業廢棄物，其中約有200百萬公噸可用於生質酒精生產，年酒精產量爲55百萬公噸。預估在2015年每加侖纖維酒精的生產價格可降低至1.5美金，相當具有競爭力。爲了達成纖維酒精商轉的目標，自2006年開始，Novozymes便與中石化、中糧進行技術合作，成立Sinopec/COFCO/Novozymes biomass project。合作的兩大關

鍵技術爲：發展適用於玉米桿(corn stover)的最佳酵素組合、研發新的酒精生產製程。其前處理技術係採用自造紙廠購得的連續式蒸氣爆裂(steam explosion)設備。酵素水解採高固液比30~35%操作，目的爲提高酒精濃度及降低蒸餾能耗。由於渣料水洗程序需耗用大量的水及增加成本，最新製程採取非水洗及無去毒的PCS程序，對於玉米桿有很好效果。目前所研發出的酵素已具有經濟競爭力，並持續自各種不同黴菌中，研發新酵素，期望能再進一步降低酵素成本。在發酵方面採高糖質濃度的發酵模式，六碳糖水解液經過發酵可產出7%的酒精溶液。適用於玉米桿生質原料的五碳糖發酵菌種仍在評估中，並持續與美國Novozymes研究中心合作進行共發菌的研發。Novozymes在中國已經完成纖維酒精的初步研究工作。未來主要的工作方向，將朝向五碳糖的利用、生質原料的後勤管理、商轉工廠設計與整合系統的開發。

(3) 美國Verenium是全球第一家擁有全流程技術的纖維酒精生產公司。Verenium’s process 是採取六碳糖SSF與五碳糖發酵並行的模式，已有示範工廠(Demonstration plant)

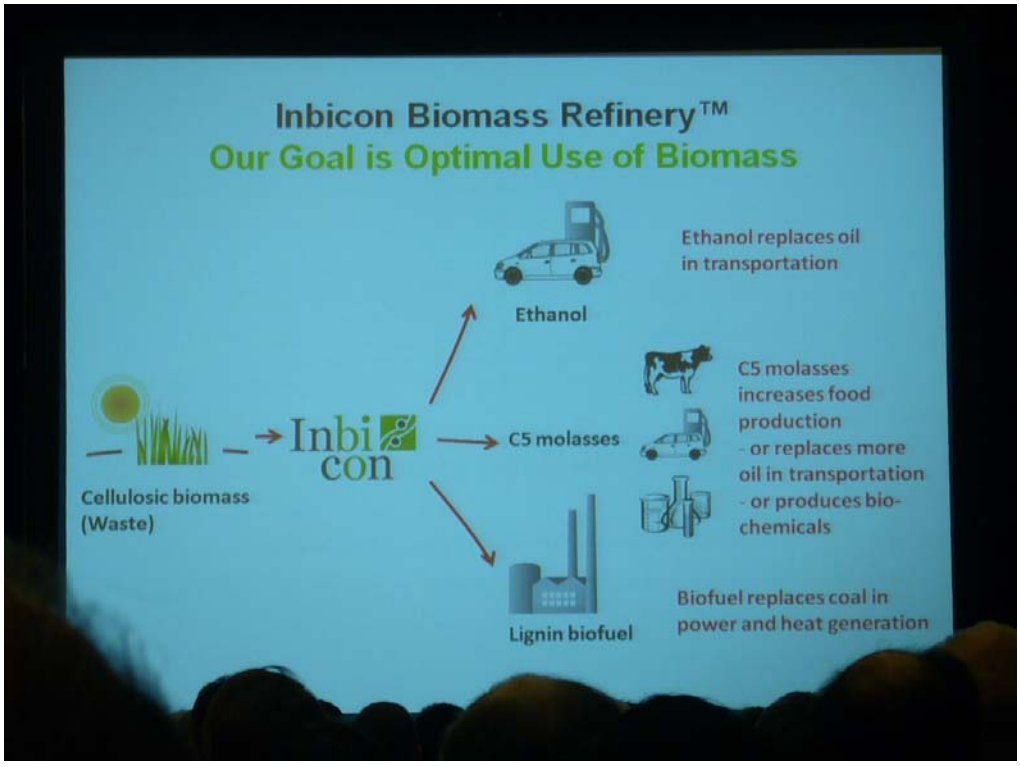
的建置(圖十)。Verenium以*E.coli*作爲五碳糖發酵的菌種，而六碳糖部分則以纖維酵素加上*K.oxytoca*的SSF程序進行處理。Verenium的研發程序與本所相當類似，先由實驗室建立基礎技術，再逐步進行放大研究，在放大過程中所遭遇問題，便再回饋至實驗室尋求解決方法。目前Verenium已經擁有10000加侖的發酵槽系統，相關的研發經驗值得我們參考。



圖十、Verenium的纖維酒精製程

(4) Inbicon展示在丹麥成功實現麥稈 (wheat straw)酒精的生產經驗。Inbicon Biomass Refinery的目標爲建立最佳化的生質原料利用模式。生質原料經過處理後，依不同組成

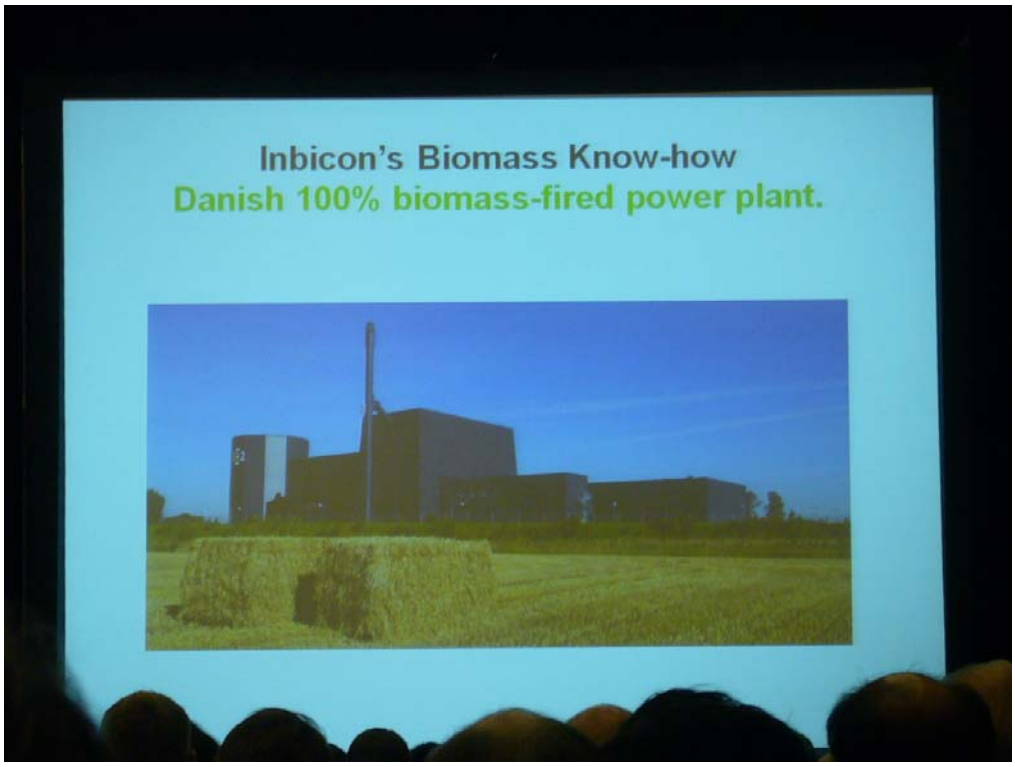
的特性，產出各種不同的產物如：酒精、食品加工物、化學原料及以木質素替代煤做爲發電用(圖十一)。Inbicon將纖維酒精生產製程中的各個單元，依其技術困難性與成熟度分爲：Know-How、核心技術與傳統技術。Inbicon把生質原料的預處理(Mech. Treatment)作爲其公司發展know-how。原料前處理、酵素水解與酒精發酵則列入核心技術。



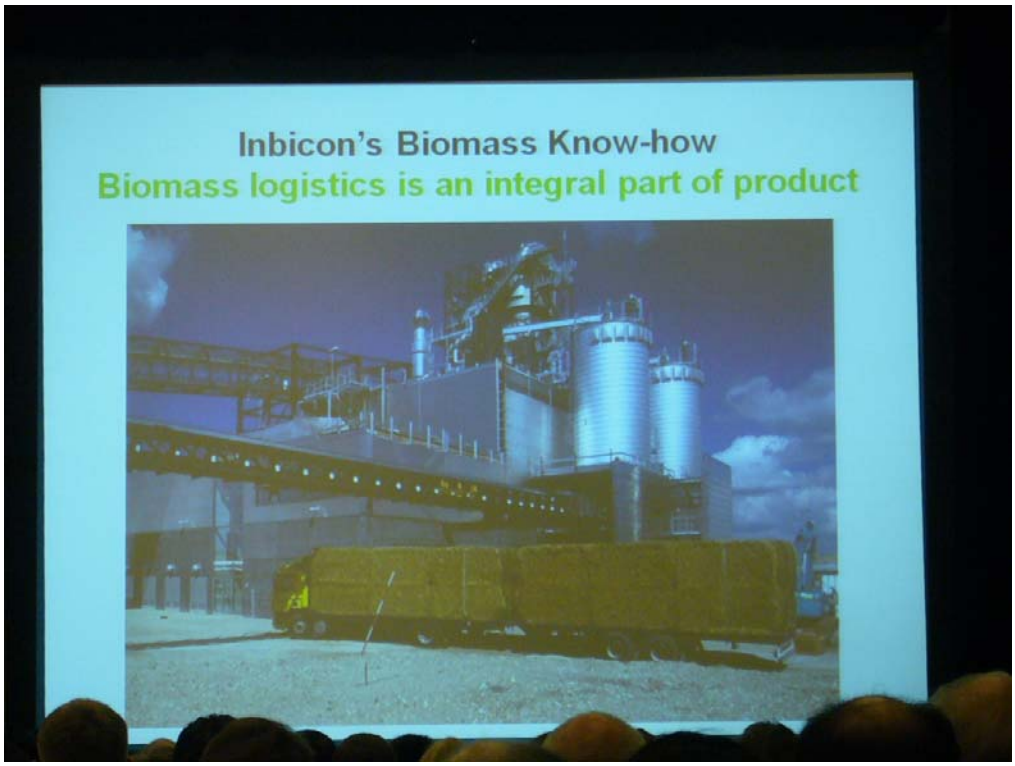
圖十一、Inbicon的生質原料利用策略

Inbicon採用水熱法技術(Hydrothemical treatment)來進行生質原料的前處理。生質原料經前處理後以橫躺式的反應槽來進行酵素水解反應。酵素水解操作的固液比設定在

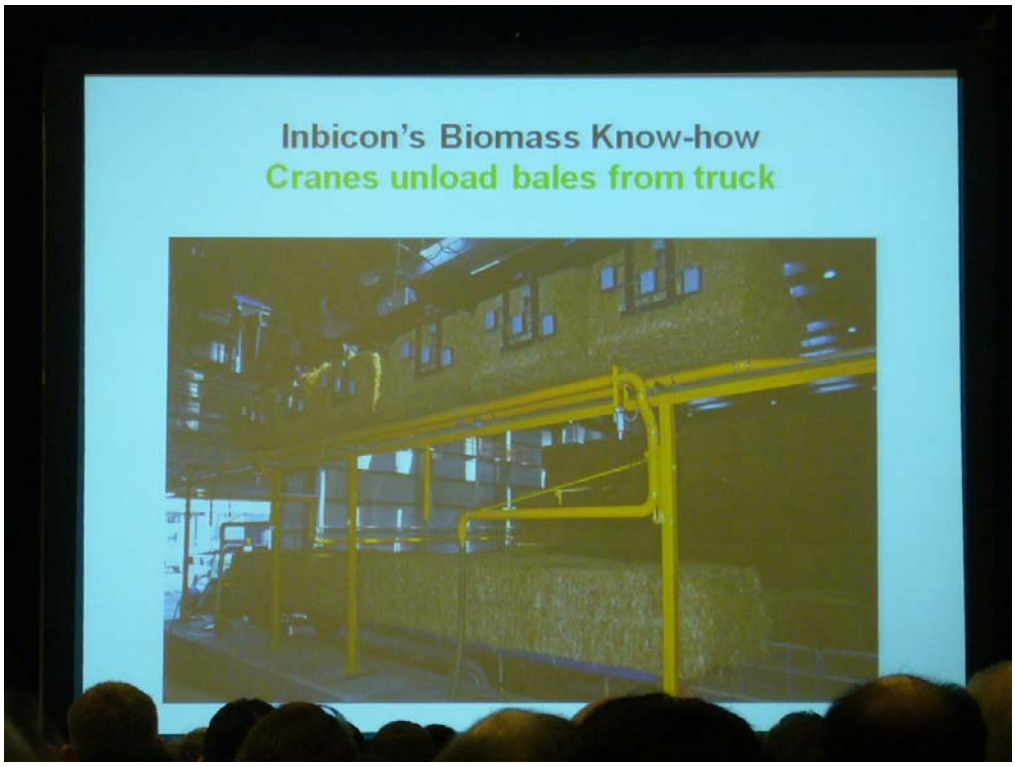
20~40%，由於高固液比的酵素反應，經過發酵反應後可獲得濃度高達8%的酒精發酵液。Inbicon在2003年完成了日進料2.4MT的纖維酒精轉化系統，經過製程放大的努力，2005年增加規模爲日進料24MT，2009年更進一步擴大爲日進料100MT的生產系統。此100MT/day的生產系統年進料量爲30000MT麥稈，可產出5.4ML酒精、8250MT生質燃料(biofuel)和11250MT糖蜜(C5-molasses)。廠內纖維素水解酵素係由Genencor與Novozymes提供。預計在2011年將有日進料1200MT的工廠完成建置(圖十二~十五)。



圖十二、丹麥100%生質燃料發電廠



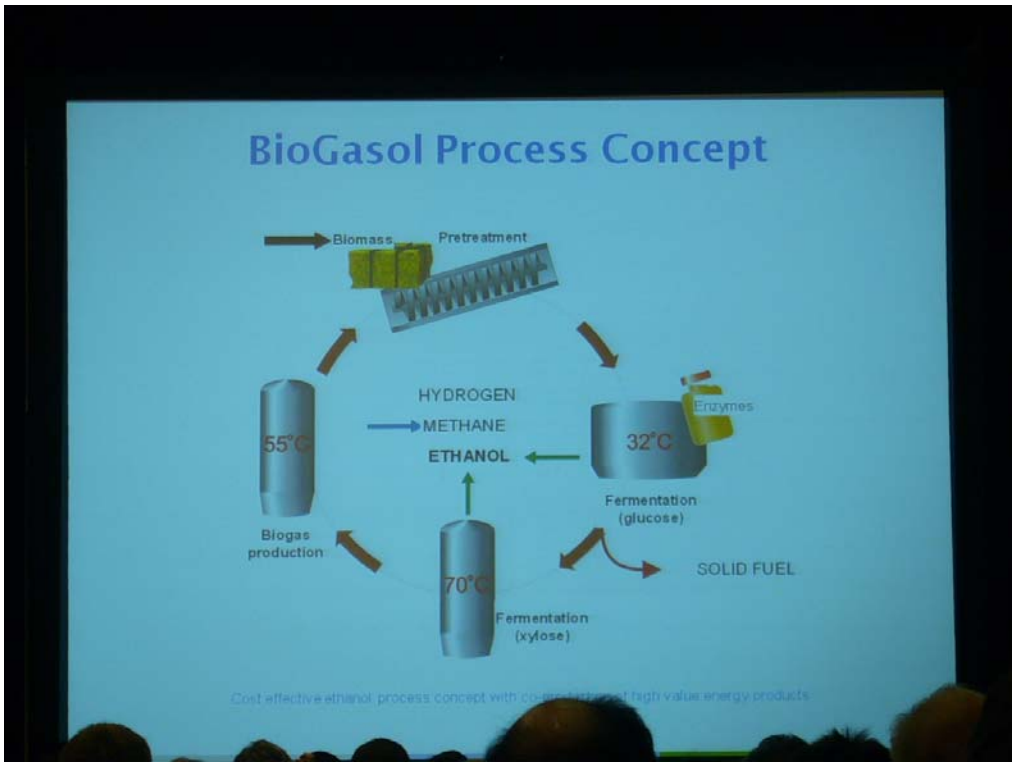
圖十三、生質原料物流系統



圖十四、生質原料裝卸

圖十五、生質原料儲存與傳送

(5) 華盛頓州大與BIOGASOL發表其對於第三代生質燃料的研究構想。根據其提出的BioGasol Process Concept，每公噸的生質原料經過轉化可產生酒精203公斤、甲烷47公斤、氫氣2.9公斤、木質纖維燃料240公斤與其他化學副產物。BioGasol Process所使用的菌種爲厭氣菌 *Thermoanaerobacter* BG1，最高可在70度的環境下進行發酵。同時亦利用基因工程技術，進行微生物代謝途徑修飾，降低發酵過程中副產物乳酸以及醋酸的生成(圖十六)。



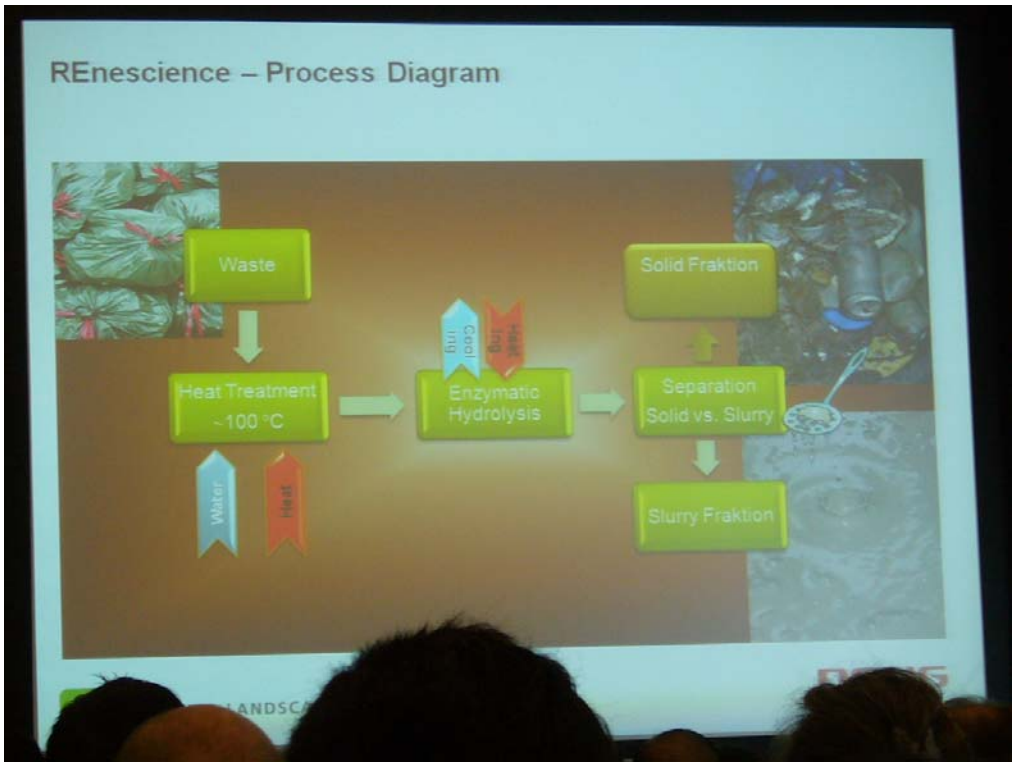
圖十六、BioGasol製程

4. 研討會第四天

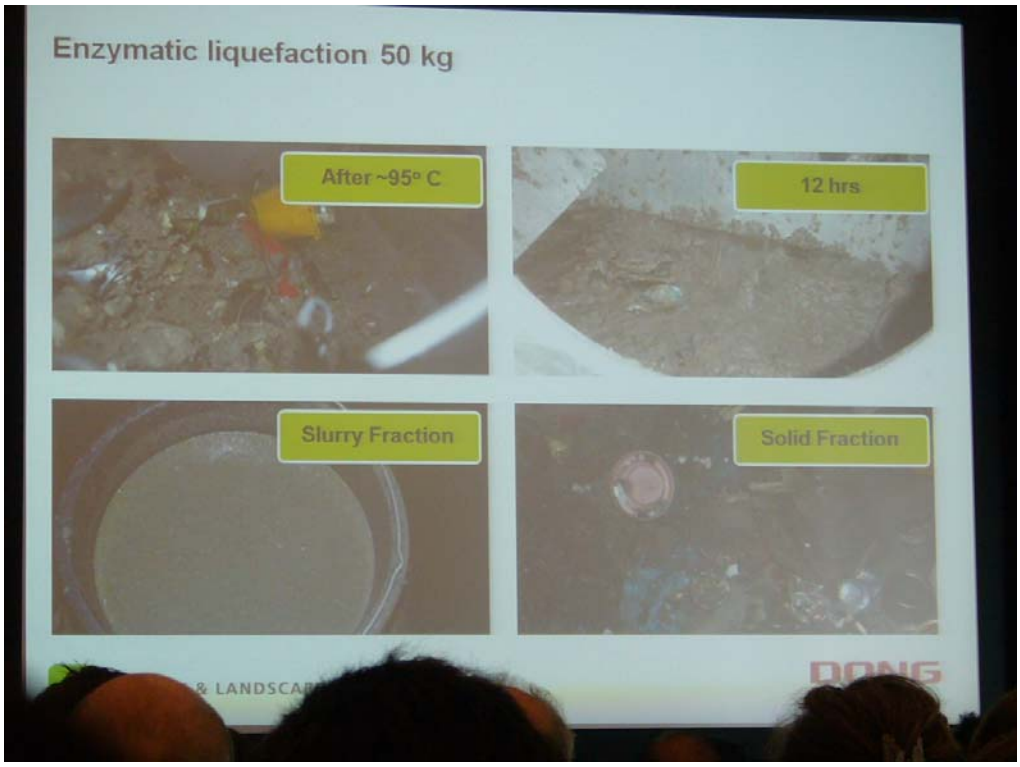
(1)NREL發表高效率的生質原料組成分析設備 (High throughput biomass compositional analysis)，繼生質原料的組成分析方法標準化後再將組成分析帶入機械化的時代。利用機械化進行組成分析的優點有：1.大幅增加分析效率，可同時進行不同生質原料的組成分析2.操作程序一致化，可減少人為操作誤差，有助於發展相關分析技術3.可大幅降低分析成本。

(2) Copenhagen University和 Dong Energy company所發表

的資料：將都市廢棄物轉化成生質能源的研究。都市廢棄物(Municipal Solid Waste, MSW)的數量隨著都市化的發展有日漸增多的趨勢，根據統計在2006年全美及歐洲城市一共生產了481百萬公噸的MSW。這些MSW大多數未被妥善利用，僅以掩埋或燃燒處理。但由於MSW的來源複雜，其中的有機成分由於高含水率導致燃燒效率降低。單純以掩埋法處理又無法有效回收MSW中的高價金屬。有鑑於此，Dong Energy設計一套MSW的處理程序。首先將MSW集中存放，加水並同時加熱至100℃先進行液化反應，降低溫度後加入酵素進行有機成分的水解。經過液化與水解反應後，以固液分離設備將MSW分離，液體部分經由適當微生物的代謝轉化爲生質能源，固體再依金屬類、塑膠類等不同性質回收利用。目前此套系統在丹麥已建立每小時100公斤的先導工廠(pilot plant)，正在評估後續興建示範工廠(demo plant)的可行性(圖十七、十八)。



圖十七、都市廢棄物(MSW)處理流程圖



圖十八、MSW經過酵素液化結果

三、心得

本次赴美參與第31屆SBFC研討會有兩大目的：一是收集世界各國最新研究成果，二是發表本所纖維酒精研發成果。在資料收集方面，有以下幾點令人印象深刻：

1.整合性與多元性爲現今發展生質能源的關鍵。在此次研討會議程中發現，世界各大主要的研究機構均不約而同的把”整合性生質能源生產程序”列爲其重要發展項目。整合性指不同世代生質原料之間的整合，例如以玉米做爲原料的澱粉酒精廠可導入以玉米穗軸作爲原料第二代纖維酒精製程。如此一來，生質原料收集處理設備均可共用，由於澱粉類原料的加入，亦可解決纖維酒精濃度過低的能耗問題，並可以達成全株生質原料的充分利用，有效降低生產成本。多元性則指生質原料轉化的最終產物可以有多種選擇性，如甲烷、乙醇、丁醇、柴油、氫氣、化學原料及木質素燃料等。依照各種生質原料的組成特性搭配不同的生產製程，可以達成生質原料最大的轉換效益。

2.除了酒精之外，生質丁醇的發展值得關注。以丁醇作爲燃料的主要優點有熱值高、不易吸水、腐蝕性低、可以適用現有石化管線運輸等。在本屆研討會中，有許多篇關於

生質丁醇的研究成果。過去利用生物法生產丁醇最大的瓶頸在於丁醇對於微生物而言爲一高毒性物質，導致無法產生足夠濃度的生質丁醇。如今已有研究單位發表可耐高濃度丁醇的菌種，加上丁醇代謝途徑的持續改良，以丁醇作爲生質能源的選項值得我們參考。

另外在成果發表方面，SBFC所安排poster session是在第一天、第二天的晚上以雞尾酒會的形式進行。來自各國的與會專家學者在輕鬆的心情下流覽各家的研究成果，並做適當的討論交流。本所發表的研究成果”Transcriptomic analysis of carbohydrate metabolism during ethanol fermentation by *Pichia stipitis*”與製程放大的建廠流程，吸引了不少研究學者的目光。值得一提的是University of Wisconsin Madison分校的Dr. Jeffery也於會中發表與本所相似的研究成果”The effect of carbon source and oxygen level on global gene expression analysis in *Pichia stipitis*”，顯示本計畫的研究能量的確具有國際一流的水準。

四、建議事項

本次本人有幸代表計畫參與第 31 屆 SBFC 研討會，在爲期四天的議程中獲益良多。以下提供幾點建議供大家參考：

1.參與國際級研討會，除了可以收集第一手的研究成果外、與世界各地的專家學者能有面對面的交流機會更是彌足珍貴。SBFC 爲生質能源最具規模的研討會，全世界與纖維酒精發展相關的研究團隊多會參與此一盛會。本計畫應固定每年派員參與此一研討會，隨時掌握最新研發趨勢。

2.目前國際上發展生質能源已逐漸趨向整合型的生產概念。在本所噸級纖維酒精測試廠即將完工之際，如何有效整合國內各種生質原料供應鏈與建立完整生質能源產出分析系統更益發重要。國際上生質丁醇的研究成果值得我們注意，建議應審慎評估其可行性。

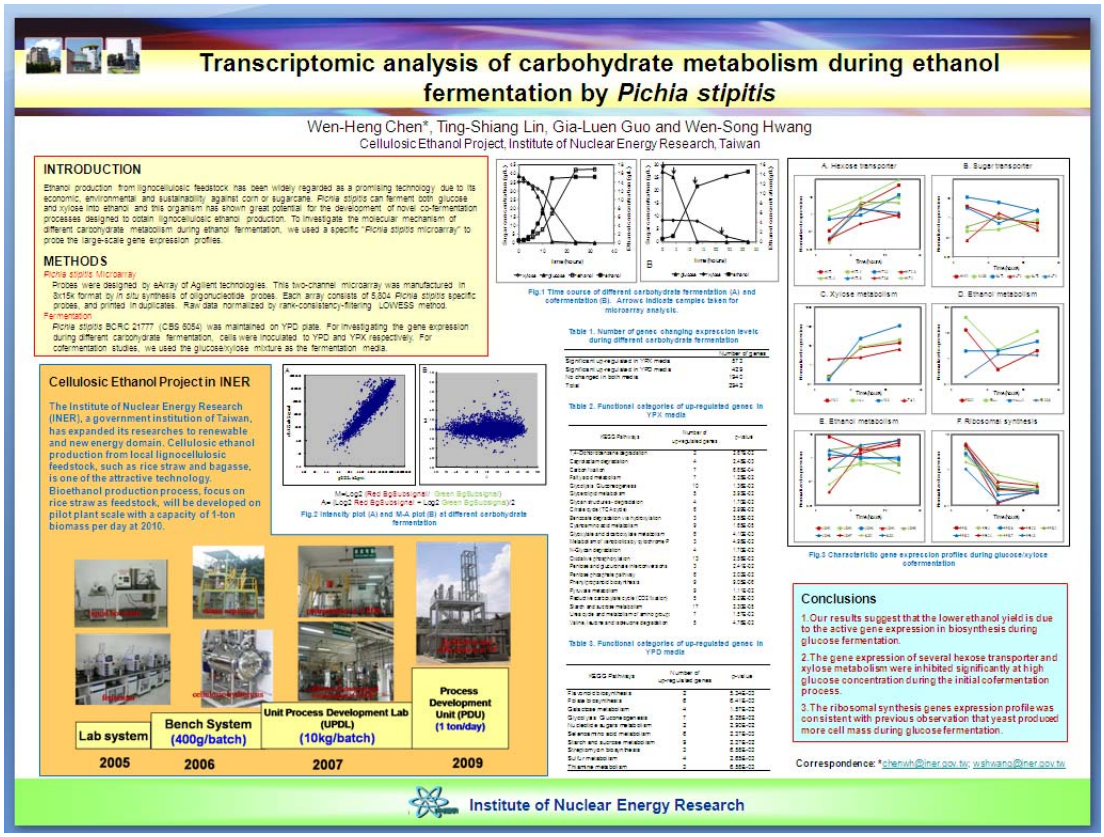
附錄

附錄一、本次於研討會所發表論文全文

附錄二、酒精工業發展現況整理表

附錄三、31st SBFC 議程及論文摘要集

附錄一


Transcriptomic analysis of carbohydrate metabolism during ethanol fermentation by Pichia stipitis
Wen-Heng Chen*, Ting-Shiang Lin, Gia-Luen Guo and Wen-Song Hwang
Cellulosic Ethanol Project, Institute of Nuclear Energy Research, Taiwan
INTRODUCTION
Ethanol production from lignocellulosic feedstock has been widely regarded as a promising technology due to its economic, environmental and sustainability against corn or sugarcane. Pichia stipitis can ferment both glucose and xylose into ethanol and this organism has shown great potential for the development of novel co-fermentation processes designed to obtain lignocellulosic ethanol production. To investigate the molecular mechanism of different carbohydrate metabolism during ethanol fermentation, we used a specific "Pichia stipitis microarray" to probe the large-scale gene expression profiles.
METHODS
Pichia stipitis Microarray
Probes were designed by eArray of Agilent technologies. This two-channel microarray was manufactured in 8x15k format by in situ synthesis of oligonucleotide probes. Each array consists of 5,804 Pichia stipitis specific probes, and printed in duplicates. Raw data normalized by rank-consistency-filtering LOWESS method.
Fermentation
Pichia stipitis BCRC 21777 (CBS 5054) was maintained on YPD plate. For investigating the gene expression during different carbohydrate fermentation, cells were inoculated to YPD and YPX respectively. For cofermentation studies, we used the glucose/xylose mixture as the fermentation media.
Fig.1 Time course of different carbohydrate fermentation (A) and cofermentation (B). Arrows indicate samples taken for microarray analysis.
Table 1. Number of genes changing expression levels during different carbohydrate fermentation
Table 2. Functional categories of up-regulated genes in YPX media
Table 3. Functional categories of up-regulated genes in YPD media
Fig.2 Intensity plot (A) and M-A plot (B) of different carbohydrate fermentation
Fig.3 Characteristic gene expression profiles during glucose/xylose cofermentation
A. Hexose transporter
B. Sugar transporter
C. Xylose metabolism
D. Ethanol metabolism
E. Ethanol metabolism
F. Ribosomal synthesis
Cellulosic Ethanol Project in INER
The Institute of Nuclear Energy Research (INER), a government institution of Taiwan, has expanded its researches to renewable and new energy domain. Cellulosic ethanol production from local lignocellulosic feedstock, such as rice straw and bagasse, is one of the attractive technology. Bioethanol production process, focus on rice straw as feedstock, will be developed on pilot plant scale with a capacity of 1-ton biomass per day at 2010.
Lab system
Bench System (400g/batch)
Unit Process Development Lab (UPDL) (10kg/batch)
Process Development Unit (PDU) (1 ton/day)
2005
2006
2007
2009
Conclusions
1.Our results suggest that the lower ethanol yield is due to the active gene expression in biosynthesis during glucose fermentation.
2.The gene expression of several hexose transporter and xylose metabolism were inhibited significantly at high glucose concentration during the initial cofermentation process.
3.The ribosomal synthesis genes expression profile was consistent with previous observation that yeast produced more cell mass during glucose fermentation.
Correspondence: *chenwh@iner.gov.tw; wshwang@iner.gov.tw
Institute of Nuclear Energy Research

附錄二

國家/公司	內容
美國IOWA州/ POET (美國最大酒精製造商)	1. 在全球共有26個運轉中的酒精工廠，年產量15億加侖(以玉米為原料) 2. Pilot scale facility已於2008年運轉，年產量2萬加侖(以玉米穗軸為原料)，廠址位於Scotland, S.D. (USA)，並利用木質素及甲烷作為廠用燃料。 3. 投資超過2億美金的Commercial facility "POET LIBERTY"，預計2011年完工。年酒精產量為125百萬加侖，其中25百萬加侖來自玉米穗軸。
丹麥/ Inbicon	1. 以麥稈做為原料，前處理採取水熱法技術，以酵素水解與IBUS程序生產酒精 2. 生產規模：年進料量30000MT(100MT/day)，可產出5.4ML酒精、8250MTbiofuel與11250MT C5-molasses 3. 預計2011年完成日進料1200MT生產廠
美國 Massachusetts/ Verenium (Celunol)	1. 前處理採取稀酸法，配合五碳糖發酵(E.coli)與六碳糖SSF(商業酵素加K.oxytoca)模式生產 2. Pilot Plant位於Jennings LA,，日進料約2噸biomass 3. Demonstration Plant位置同上，發酵槽規模>1萬加侖，最大年產量1.4百萬加侖，仍持續進行程序驗證與製程最佳化測試 4. Commercial Projects仍在評估階段，預估年產量30百萬加侖。 5. 技術授權給日本Tokyo-based Marubeni Corp，在Osaka興建世界第一座以wood waste做為原料的酒精工廠BioEthanol Japan，年產量1.4百萬公升
美國/ BioGasol	1. BioGasol process Concept：前處理採wet explosion (wet oxidation+steam explosion)，不加酸，反應溫度為170~200℃。發酵採五碳糖發酵(Thermoanaerobacter BG1，反應溫度70℃)與六碳糖SSF模式，最後再加上anacrobic digestion程序生成氫氣與甲烷做為廠用燃料。 2. 預估每公噸原料可產出酒精203公斤、甲烷47公斤、氫氣2.9公斤、木質素240公斤與其他化學產物。 3. Demo Plant正運轉測試中，預計在2011年可進行商業生產

Hosted by the ***National Renewable Energy Laboratory***
and ***Oak Ridge National Laboratory***

Final Schedule

31st Symposium on Biotechnology for Fuels and Chemicals

A special conference of the ***Society for Industrial Microbiology***

CALL FOR ABSTRACTS
Extended Deadline: 1/5/2009

The 31st Symposium program chairs invite you to submit abstracts for the posters or oral presentations in the technical program.

All submissions must be submitted electronically. You will receive automatic confirmation of receipt of your abstract.

Courtesy of DOE/NREL

To submit an abstract, please go to:

www.simhq.org/meetings/sbfc2009/index.html

InterContinental San Francisco Hotel
San Francisco, CA
May 3-6, 2009

Welcome to the 31st Symposium on Biotechnology for Fuels and Chemicals

There has been a tremendous upsurge of interest in sustainable fuels and chemicals production over the past several years. Government and private sector support for research, development and deployment of renewable fuels and chemicals technologies is at an all time high. This year's Symposium provides a superb forum for experts from around the world to gather to discuss the latest research breakthroughs and results in this exciting and growing field. This year's scientific program is designed to facilitate effective exchange of new information and technical progress among attendees from industrial, academic, and government sectors. The program includes a variety of oral presentation sessions, poster sessions and special topic sessions as well as ample opportunities to participate in informal discussions. Participants' experiences will also be enhanced by social activities and optional tours to explore the many offerings available in the greater northern California bay area region.

San Francisco

The city

San Francisco is one of the great cities of the world and by far the most interesting and intriguing. Located on a peninsula between the Pacific Ocean and San Francisco Bay, the city is very compact, hilly, and scenic.

The greatest thing about the city is the number of different neighborhoods that blend together to form the cohesive whole. Five minutes from the Transamerica Pyramid is Chinatown, and just beyond is North Beach, the ethnic Italian neighborhood. The Marina, Pacific Heights and the Embarcadero are just a few of the areas that are worth visiting. San Francisco's attractions are almost too numerous to mention but include Golden Gate Park, Chinatown, Japantown, Cliff House, and Twin Peaks.

San Francisco's food is also fantastic! A culinary center of the United States, the city excels at every type of cuisine, including the best vegetarian and local flavors.

Outside the city limits is Napa and the wine country to the north and Monterey to the south. Overall, San Francisco is a great place to spend a week - or longer!

The weather

Even in mid-July, sweaters or light coats are suggested. The wind coming off the Bay can be harsh, especially at the top of the taller hills or near the water. The famous fog rolls in over the Golden Gate but dissipates leaving a blue sky on most days.

Getting around

Rental cars are not necessary within the city as its compact nature encourages walking from Market Street to the Wharf, allowing for stops along the way. Of course, the famous San Francisco Cable Car is a other option, as well as BART (Bay Area Rapid Transit). If you have a few days, buy one of the MUNI passes. The F-line steetcar is another good option, going from Market along the Embarcadero and finishing at the Wharf.

Meeting Venue

InterContinental San Francisco Hotel
888 Howard St.
San Francisco, CA 94103
tel: 1-800-381-9552

The InterContinental San Francisco, San Francisco's newest luxury hotel in the vibrant South of Market (SoMa) neighborhood, is within easy walking distance of premium shopping areas, a diverse collection of trendy and upscale restaurants and bars, and a number of major tourist attractions such as Union Square, Chinatown and the Powell Street Cable Car Turnaround.

The InterContinental's rooms are luxurious and elegantly appointed with the latest amenities. The InterContinental San Francisco features a 10-room spa offering exquisite treatments and signature experiences, an indoor heated lap-pool, and a fitness center.

Languages spoken by hotel staff are: Chinese, English, French, German, Spanish, and Tagalog.

A block of rooms has been reserved for the conference dates of May 3 - 6, 2009. The conference rate is $206 + tax per night and may be offered three days before and three days after the conference dates if rooms are available. A limited block of rooms is available at the government rate.

Contact Information

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Deadlines	Date
Abstract Deadline (extended)	**1/5/2009**
Author Notification	**2/01/2009**
Manuscripts Due (optional)	**5/03/2009**

Hotel Reimbursement for Academics

For 2009, limited hotel reimbursement is available for students and academics to defray a portion of the cost of hotel rooms at the InterContinental San Francisco.

Reimbursement will take the form of a $40 reduction of each night's stay, up to a maximum of 5 nights, at the InterContinental San Francisco Hotel. Other hotels do not qualify. Funds are limited and will be made available on a first-come, first-served basis. Please see the requirements for eligibility below.

Eligibility

- **Students presenting in the poster or oral sessions**
- **Academic invited speakers**
- **Academic poster presenters**

Criteria for selection are:

- **Submit your abstract by the extended deadline of January 5, 2009.**
- **Register for the meeting by the pre-registration deadline of April 3, 2009.**
- **State the number of room nights requested (up to 5).**
- **Attend the meeting and present the poster or talk.**

How to apply and file for reimbursement:

- **Please check the box on the abstract submission form to apply for reimbursement.**
- **After notification that your abstract has been accepted for presentation, register for the meeting and make your hotel reservations by the advanced registration deadline.**
- **Request travel expense vouchers at the SIM registration desk at the meeting site.**
- **Submit travel vouchers to SIM, with all required receipts, within 30 days of the close of the meeting.**

Sponsorship Opportunities

Sponsorship opportunities are available. In addition to inclusion in conference publicity, sponsors may receive complimentary registrations. For more information about sponsorship opportunities, go to www.simhq.org/meetings/sbfc2009/index.html and download the sponsorship pdf.

Tabletop Exhibits

Tabletop exhibits will be on display during the meeting. To download the exhibit prospectus, go to www.simhq.org/meetings/sbfc2009/exhibitors.html or contact: nancy.gorell@simhq.org.

Advertising

Program advertising opportunities available. The deadline for submitting advertising insertion forms is March 3. The deadline for submitting artwork is March 21. For rates and details, please contact: suzi.eller@simhq.org

Symposium Registration

Registration begins January 5, 2009

To register please visit: www.simhq.org/meetings/sbfc2009/index.html.

Full conference registration includes the continental breakfasts Monday-Wednesday, daily refreshments, poster session receptions, conference banquet, conference program and abstracts, and conference proceedings. Only full registrations will receive the conference proceedings.

Cancellations: All requests for refunds must be submitted in writing to meetings@simhq.org and sent no later than April 3, 2009. Refunds will be issued, less a $75 administrative fee. No refunds will be issued after April 3, 2009.

NOTE: *Personnel substitutions may be made in lieu of cancellation.*

Before 4/03/2009	SIM Member	Nonmember
Full Registration (includes proceedings)	$475	$575
One day Registration (does not include proceedings)	$275	$325
Student Registration (does not include proceedings)	$100	$100

After April 3, 2009 add $50 to all categories except students

SUNDAY AFTERNOON, MAY 3

Opening Plenary Session

Session 1: Plant Science and Technology

This session will highlight advances in plant science and agronomic technologies to enhance the quantity and quality of plant materials as a renewable feedstocks base. Papers are sought describing progress across all relevant research areas, from breeding to improve plant yield or processing characteristics to in planta expression of enzymes or coproducts, to advances in harvesting technology and supply chain logistics.

Session 2 : Microbial Science and Technology I

Microorganisms are powerful catalysts capable of synthesizing an ever wider range of fuels and chemicals from renewable feedstocks (sugars, synthesis gas, etc). Papers in this session will describe recent progress in developing and implementing new or improved microbes for biorefining applications.

SUNDAY EVENING, MAY 3

Poster Session 1/Reception

Courtesy of DOE/NREL

MONDAY MORNING, MAY 4

Session 3: Biomass Pretreatment and Fractionation

Lignocellulosic plant material (biomass) is difficult to economically disassemble at high yield due to the presence of structural carbohydrates (holocellulose) and aromatic (lignin) polymers. Papers in this session will discuss recent developments in chemical and biochemical pretreatment or fractionation processes - both existing and new approaches - that make biomass more amenable to subsequent bioconversion.

Session 4: Translational Genomics for Bioenergy Feedstocks

Recent advances in systems biology promise to revolutionize the speed and precision with which lignocellulosic plants (biomass feedstocks) can be engineered to improve their value for bioenergy/biofuels applications. Papers in this session will describe recent progress in using genomics tools to characterize and understand plant cell wall synthesis and deconstruction.

MONDAY AFTERNOON, MAY 4

Session 5: Enzyme Science and Technology I

This session will highlight advances in enzyme discovery, characterization and modification to improve enzyme performance as well as progress on cost effectively producing and applying enzymes to biorefinery processes.

Session 6: Microbial Science and Technology 2

Microorganisms are powerful catalysts capable of synthesizing an ever wider range of fuels and chemicals from renewable feedstocks (sugars, synthesis gas, etc). Papers in this session will describe recent progress in developing and implementing new or improved microbes for biorefining applications.

MONDAY EVENING, MAY 4

Poster Session 2/Reception

TUESDAY MORNING, MAY 5

Session 7: Biorefinery Deployment

Significant efforts are underway around the world to commercialize technologies for producing fuels, chemicals and other value-added bioproducts from renewable feedstocks. Presentations in this session will highlight recent progress in developing and operating demonstration-scale and commercial-scale integrated biorefineries.

Session 8 – Biofuels Logistics and Sustainability

The potential of lignocellulosic biomass to supply humanity with renewable fuels and chemicals is significant but constrained by feedstock logistics and environmental sustainability considerations such as land availability, soil quality and water use. Papers in this session will examine these and other sustainability issues that must be understood for the bioenergy industry to rapidly grow.

TUESDAY EVENING, MAY 5

Special Topic A: International Commercialization of 2nd Generation Biofuels

(co-sponsored by IEA Bioenergy Task 39 and 40)

Speakers in this special session will highlight recent international progress to accelerate deployment of advanced biofuels technologies. Presenters will include members of IEA Bioenergy Tasks working to bring forward advanced biofuels technologies.

Special Topic B: Development and Commercialization of Algal-based Biofuels

Speakers in this special session will describe some of the many efforts underway around the world to develop and commericalize algal-based biofuels technologies.

Courtesy of DOE/NREL

WEDNESDAY MORNING, MAY 6

Session 9 – Bioprocessing and Separations Technology

An economically viable bioprocess requires effective material handling and bioconversion technologies in combination with efficient downstream product separation and recovery. Papers in this session will describe advances in the development, testing and demonstration of bioconversion and separations processes at various stages of process integration.

Session 10 – Enzyme Science and Technology II

This session will highlight advances in enzyme discovery, characterization and modification to improve enzyme performance as well as progress on cost effectively producing and applying enzymes to biorefinery processes.

WEDNESDAY AFTERNOON, May 6

Session 11 – Emerging Biofuels and Chemicals

This session will focus on the research and development of new fuels and chemicals from renewable feedstocks. Presentations will discuss recent progress in developing technologies to produce new fuels such as biogasoline and higher alcohols, advances in merging thermochemical processes with biological conversion, as well as new targets for biomass-derived chemicals.

Session 12: Biomass Recalcitrance

It remains challenging to cost-effectively deconstruct biomass, particularly the secondary cell wall of lignocellulosic materials. This session will highlight advances in the study and understanding of plant cell wall structure and composition, emphasizing efforts to improve biomass deconstruction efficiency.

WEDNESDAY EVENING, May 6

Reception and Banquet

3929 Old Lee Highway
Suite 92 A
Fairfax, VA 22030
www.simhq.org

» Program at a Glance

Sunday, May 3, 2009

7:30 AM — 9:00 PM	**Internet Lounge** Mission, 3rd Fl
8:30 AM — 6:00 PM	**Registration/Editor's Desk** Grand Foyer, 3rd Fl
8:30 AM — 5:00 PM	**Poster Setup** InterCont. Ballroom, 5th Fl
8:30 AM — 2:00 PM	**Exhibit Setup** Grand Foyer, 3rd Fl
12:15 PM — 12:30 PM	**Opening Remarks/Keynote** Grand Ballroom, 3rd Fl
1:00 PM — 5:00 PM	**Session 1 Plant Sci.** Grand Ballroom C, 3rd Fl
1:00 PM — 5:00 PM	**Session 2 Microbial Sci. I** Grand Ballroom A–B, 3rd Fl
2:30 PM — 3:30 PM	**Exhibits Open** Grand Foyer, 3rd Fl
5:00 PM — 7:00 PM	**Exhibits Open/Reception** Grand Foyer, 2nd Fl
6:00 PM — 9:00 PM	**Poster Session I (Sect. 1–4, 6–8)** Intercon. Ballroom, 5th Fl Pacific Terrace Foyer, 4th Fl

Monday, May 4, 2009

7:15 AM — 5:00 PM	**Registration/Editor's Desk** Grand Foyer, 3rd Fl
7:15 AM — 8:00 AM	**Continental Breakfast** Grand Foyer, 3rd Fl
7:15 AM — 8:00 AM	**Speaker's Breakfast** SoMa, 3rd Fl
7:30 AM — 10:30 AM	**Exhibits Open** Grand Foyer, 3rd Fl
7:30 AM — 9:00 PM	**Internet Lounge** Mission, 3rd Fl
8:00 AM — 11:30 AM .	**Session 3 Biomass Pretreat** Grand Ballroom A–B, 3rd Fl
8:00 AM — 11:30 AM	**Session 4 Translat. Genomics** Grand Ballroom C, 5th Fl
11:30 AM — 1:00 PM	**Lunch on your own**
1:00 PM — 5:00 PM	**Session 5 Enzyme Sci. I** Grand Ballroom, A–B, 3rd Fl
1:00 PM — 5:00 PM	**Session 6 Microbial Sci. II** Grand Ballroom, C, 3rd Fl
2:30 PM — 3:30 PM	**Exhibits Open** Grand Foyer, 3rd Fl
5:00 PM — 7:00 PM	**Exhibits open/Reception** Grand Foyer, 3rd Fl
6:00 PM — 9:00 PM	**Poster Session II:** **(Sections 5, 9–12)** Intercon. Ballroom, 5th Fl Pacific Terrace Foyer, 4th Fl

Tuesday, May 5, 2009

7:15 AM — 12:00 noon	**Registration/Editor's Desk** Grand Foyer, 3rd Fl
7:15 AM — 8:00 AM	**Continental Breakfast** Grand Foyer, 3rd Fl
7:15 AM — 8:00 AM	**Speaker's Breakfast** SoMa, 3rd Fl
7:30 AM — 10:30 AM	**Exhibits Open** Grand Foyer, 3rd Fl
7:30 AM — 9:00 PM	**Internet Lounge** Mission, 3rd Fl
8:00 AM — 11:30 AM	**Session 7 Biorefinery Deploy** Grand Ballroom A–B, 3rd Fl
8:00 AM — 11:30 AM	**Session 8 Biofuels Log.&Sust.** Grand Ballroom C, 3rd Fl
12:15 PM — 1:45 PM	**Organizing Committee lunch** Sutter, 5th Fl
Free afternoon	
7:00 PM — 9:00 PM	**Special Topic I:** **Intl. 2nd Gen. Biofuels** Grand Ballroom C, 3rd FL
7:00 PM — 9:00 PM	**Special Topic II:** **Algal–based Biofuels** Grand Ballroom A–B, 3rd Fl

Wednesday, May 6, 2009

7:15 AM — 5:00 PM	**Registration/Editor's Desk** Grand Foyer, 3rd Fl
7:15 AM — 8:00 AM	**Continental Breakfast** Grand Foyer, 3rd Fl
7:15 AM — 8:00 AM	**Speakers Breakfast** SoMa, 3rd Fl
7:30 AM — 9:00 PM	**Internet Lounge** Mission, 3rd Fl
8:00 AM—11:30 AM	**Session 9 Bioprocessing** Grand Ballroom, C, 3rd Fl
8:00 AM —11:30 AM	**Session 10 Enzyme Sci. II** Grand Ballroom A–B, 3rd Fl
11:30 AM — 1:00 PM	**Lunch on your own**
1:00 PM — 5:00 PM	**Session 11:** **Emerg. Biofuels/Chem.** Grand Ballroom C, 3rd Fl
1:00 PM — 5:00 PM	**Session 12 Biomass Recal.** Grand Ballroom A–B, 3rd Fl
6:00 PM	**Reception** Grand Foyer, 3rd Fl
7:00 PM	**Banquet** Grand Ballroom, 3rd Fl

Registration

On-site registration and distribution of meeting packets to pre-registrants.

Grand Ballroom Foyer, 3rd Floor

Sunday, May 3	8:30 AM – 6:00 PM
Monday, May 4	7:15 AM – 5:00 PM
Tuesday, May 5	7:15 AM – 12:00 PM
Wednesday, May 6	7:15 AM – 5:00 PM

Programs will be distributed at the meeting to all attendees. (Extra copies of the meeting program at the meeting site are $50.)

Name badges must be worn for admittance to the scientific sessions, exhibits and special functions.

Smoking is not permitted in the hotel.

Exhibit Setup

Sunday, May 3
8:30 AM – 2:00 PM

Grand Ballroom Foyer, 3rd Floor

Poster Setup

Sunday, May 3
8:30 AM – 5:00 PM
(posters must be set up by 5:00 PM)

InterContinental Ballroom and Foyer, 5th Floor and Pacific Terrace Foyer, 4th Floor

Special Needs

The Organizing Committee and SIM want to ensure your comfort and convenience at the Symposium. If you have any Special Needs, please let us know at the registration desk.

Grand Ballroom Foyer, 3rd Floor

Editor's Desk

The editor's desk will be open Sunday, May 3 - Wednesday, May 6 during registration hours.

Grand Ballroom Foyer,3rd Floor

30th Symposium Proceedings

If you attended the 30th Symposium and are to receive a copy of the proceedings book or DVD, please pick up your copy at the Editor's Desk during registration hours.

Internet Lounge

An Internet lounge is available to all attendees.

Sunday - Wednesday 7:30 AM - 9:00 PM

Mission, 3rd Floor

Speaker ready room

Speakers may review their presentations during the following hours:

Sunday–Wednesday 7:30 AM – 9:00 PM

Marina, 3rd Floor

Placement Service

SIM will feature a Placement Service during Symposium hours. Post your resume or view available positions.

In addition, the National Renewable Energy Laboratory (NREL) in Golden, CO will have a representative present to discuss employment at NREL and current opportunities available. Resumes will be accepted for open positions.

Grand Ballroom Foyer, 3rd Floor

Membership Table

The SIM Membership Committee would like to welcome new members, first-time attendees and students to the Symposium. Please visit the membership table to learn more about SIM and its memberships benefits.

Grand Ballroom Foyer, 3rd Floor

Information and Message Center

Messages and announcements will be posted on the bulletin board in the Registration Area. In case of emergency, registrants may be contacted through the InterContinental San Francisco Hotel, 1-888-811-4273.

Meals

Poster Reception/Light Buffet

Sunday-Monday 6:00 PM – 9:00 PM

Grand Ballroom Foyer, 3rd Floor/
InterContinental Ballroom, 5th Floor

Continental Breakfast (All attendees)

Monday – Wednesday 7:15 AM – 8:00 AM

Grand Ballroom Foyer, 3rd Floor

Invited Speaker Breakfast

Breakfast for invited speakers/session chairs on day of presentation.

Sunday 9:00 AM – 10:00 AM

Monday–Wednesday 7:15 AM – 8:00 AM

SoMa, 3rd Floor

Lunch – on your own

Wednesday Night Banquet Information

Reception

6:00 PM

Grand Ballroom Foyer, 3rd Floor

Banquet and Award Presentations

Banquet Speaker: Jennie Hunter-Cevera, President, University of Maryland Biotechnology Institute

"Fueling around with Biotechnology"

There are a lot of intellectual and financial resources being spent on alternative fuels. How can we as a nation maximize our investments? What works as collaborative efforts and what does not? Are we re-inventing the wheel or maybe there within is the real focus - transportation modes have not changed in over a hundred years. If we are to learn from the past and from nature to better our ability to provide reliable cost efficient forms of energy in the near future, what lessons have we learned and what have we forgotten?

7:00 PM

Grand Ballroom, 3rd Floor

Hospitality Suite

Monday - Wednesday Opens at the close of the Evening Sessions

Sutter, 5th Floor

Sponsored by KATZEN International

InterContinental San Francisco Hotel

888 Howard St.
San Francisco, 94103

Tel: 1-888-424-6835
Tel: 1-415-616-6500
Fax: 1-415-616-6581

LUXURY HIGH ABOVE THE HEART OF THE CITY
InterContinental San Francisco is the Bay Area's newest luxury destination. The 32-story glass tower sparkles high above the city, standing sentry over the South of Market (SoMa) area. The smoke-free hotel is convenient to everything, including the Moscone Convention Center, Union Square, Chinatown and the Powell Street Cable Car Turnaround.

Check In/Out

Check-In Time 3:00 PM

Check-Out Time 12:00 PM

Late Check-Out Available

Restaurants & Bars

Luce puts a local spin on American fare for breakfast and lunch. At night it transforms into a celebrity wine restaurant serving Italian cuisine with a California twist. Bar 888 is the place for tantalising cocktails and conversation.

Amenities & Services

Our 24-hour fitness centre offers 2,200 sq ft of space equipped with free-weights, cardio and resistance equipment and an indoor lap pool (6 AM - 10 PM). I-Spa offers body and facial treatments in its 10 private spa rooms.

Have you picked up your 30th Symposium Proceedings Book or DVD?

Now available at the editor's desk.

Transportation

San Francisco International Airport (SFO)

Distance 13 MI / 20.92 KM NORTH to Hotel

Shuttle Charge (one way): $25.00 (USD)

Taxi Charge (one way): $45.00 (USD)

Time by taxi: 20 minutes

101 N to 80 East towards Bay Bridge. Take Fourth Street exit towards Embarcadero. Turn left onto Bryant St then left onto Third St. Left turn on Howard St. Hotel is on the right.

Oakland International Airport (OAK)

Distance 19 MI / 30.58 KM WEST to Hotel

Shuttle Charge (one way): $35.00 (USD)

Taxi Charge (one way): $50.00 (USD)

Time by taxi: 35 minutes

80 West Bay Bridge and cross over Bridge. Exit Fremont Street and turn left onto Howard Street (first street off ramp). Continue on Howard Street for 0.7 miles and Hotel is on the right.

San Jose International Airport (SJC)

Distance 46 MI / 74.03 KM NORTH to Hotel

Shuttle Charge (one way): $55.00 (USD)

Taxi Charge (one way): $85.00 (USD)

Time by taxi: 55 minutes

Head NE on Airport Pkwy to Technology Dr. Continue on E Brokaw Rd. Merge onto US-101 N to San Francisco and drive for 44 miles. Take exit 2 for Fourth St. Slight left at Bryant St. Turn left at 3rd St. Turn left at Howard St.

Train

Station Name: Amtrak

Distance 5.0 MI / 8.05 KM WEST to Hotel

Take 80 East to Bay Bridge towards San Francisco. Take Fremont Exit and make left onto Howard Street (first street off ramp). Continue on Howard Street for 0.7 miles and Hotel is on the right.

Subway

Subway Name: Powell Station

Distance 0.1 MI / 0.16 KM EAST to Hotel

Exit hotel and turn right. Turn right onto 5th street and right on Market.

SAN FRANCISCO (0 MI / 0 KM)

Westfield Shopping Center (0.1 MI / 0.16 KM)

Union Square Shopping Area (0.3 MI / 0.48 KM)

SF Museum of Modern Art (0.2 MI / 0.32 KM)

Fisherman's Wharf/Pier 39 (1 MI / 1.61 KM)

Chinatown (0.4 MI / 0.64 KM)

Ferry Building at Embarcadero (0.5 MI / 0.8 KM)

Shopping Center at Embarcadero (0.5 MI / 0.8 KM)

AT&T Park (0.7 MI / 1.13 KM)

Museum of the African Diaspora (0.1 MI / 0.16 KM)

Yerba Buena Gardens/Zeum (0.1 MI / 0.16 KM)

Coit Tower (2.5 MI / 4.02 KM)

Metreon (0.1 MI / 0.16 KM)

Golden Gate Park (5.5 MI / 8.85 KM)

Asian Art Museum (3.5 MI / 5.63 KM)

De Young Museum (3.5 MI / 5.63 KM)

San Francisco Local Information

Visitor Information Center

San Francisco's Official Travel Resource

What speaks 12 languages and always has the scoop on what's happening in San Francisco? The answer is San Francisco's Visitor Information Center.

Location

900 Market Street, on the lower level of Hallidie Plaza, next to the cable car turntable at Powell and Market streets.

Hours

May through October

9:00 AM to 5:00 PM - Monday through Friday
9:00 AM to 3:00 PM - Weekends and Holidays

November through April

9:00 AM to 5:00 PM - Monday through Friday
9:00 AM to 3:00 PM - Saturday
Closed - Sunday

Recorded Events

To hear our listing of monthly events in English call (415) 391-2001.

The condensed version is available in Spanish, French, Italian, German and Japanese languages:

French Francais (415) 391-2003

Spanish Espanol (415) 391-2122

Japanese (415) 391-2101

German Deutsch (415) 391-2004

Italian Italiano (415) 391-2002

Getting Around

Public transportation: Bay Area Rapid Transit (BART)
www.bart.gov

Visit six San Francisco attractions and ride the famous cable car - for one low price! And take as many as nine days to use your pass. Just added: The new California Academy of Sciences is now part of the **San Francisco city pass**. Book online: *http://san-francisco.tourcorp.com*

Glide With the Original San Francisco & Sausalito **Segway** Tour Company - Since 2004. Reservations required. 415-474-3130 or toll-free 1-877-474-3130; *http://san-francisco.tourcorp.com*

More fun than walking, more eco-friendly than driving -- the Segway is the best way to see San Francisco or Sausalito!

- Six daily departures
- Experienced guides
- Easy to learn
- Locally owned and operated
- Great for families, couples, or singles!
- Fully narrated sightseeing

Fisherman's Wharf

www.visitfishermanswharf.com

San Francisco's Fisherman's Wharf is a world famous tourist attraction and a thriving and vibrant local neighborhood and commercial area. Home to world-class dining, shopping, hotels and endless entertainment opportunities, the Wharf is truly the place to start your San Francisco experience.

As the home of San Francisco's fishing fleet, docked along Jefferson Street, Fisherman's Wharf is the important center of our city's historic fishing industry. Along our neighborhood's "Fish Alley" you can still see fishermen at work, which is always a fun and unique San Francisco experience. The Wharf area is also the launching point for Bay cruises and charters.

Family entertainment is a neighborhood specialty. With our famous sea lions, Wax Museum, Ripley's Believe It or Not! Museum, The Aquarium of the Bay at PIER 39 and the World War II submarine, USS Pampanito, Fisherman's Wharf is the perfect place to bring the kids. Specialty shops and restaurants line the Wharf--including PIER 39, Anchorage Square and THE CANNERY shopping complexes. The world famous Ghirardelli Square has been converted to an open-air center filled with fun shops and restaurants. Here you can even see the company's original chocolate-making machines.

Alcatraz Island

http://san-francisco.tourcorp.com

Cold, foreboding, and isolated, Alcatraz Island made escape impossible for its inmates. See for yourself the subject of legend, lore, and more than a few Hollywood movies.

California Academy of Sciences

www.calacademy.org

55 Music Concourse Drive Golden Gate Park
San Francisco, CA 94118

Phone (415) 379-8000

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Hours of Operation

Monday – Saturday	9:30 AM – 5:00 PM
Sunday	11:00 AM – 5:00 PM

Home to Steinhart Aquarium, Kimball Natural History Museum, Morrison Planetarium, and world-class research and education programs, the new California Academy of Sciences is the world's largest "green" museum and one of San Francisco's must-see destinations. From the splashing penguins in African Hall to the wildflowers on the roof, the building is bursting with life. A four-story living rainforest and awe-inspiring coral reef ecosystem will delight visitors of all ages, while interactive space shows will transport audiences beyond the boundaries of our planet. Opportunities abound to meet Academy scientists, share in their discoveries, and join the journey to make our world a greener, more sustainable place to live.

SFJAZZ Spring Season 2009

www.sfjazz.org

March 6-June 21, 2009

Various Venues Citywide

Tickets: (866) 920-5299

Asian Art Museum

www.asianart.org

The Asian Art Museum of San Francisco is one of the largest museums in the Western world devoted exclusively to Asian art. Your ticket to Asia. Here, you can travel through 6,000 years of history, trek across seven major regions, and sample the cultures of numerous countries.

Tabletop Exhibits

Company representatives will be available during the Symposium. Please visit the exhibitors during the show hours listed.

Grand Ballroom, 3rd Floor

Sunday, May 3	Monday, May 4	Tuesday, May 5
2:30 PM – 3:30 PM 5:00 PM – 7:00 PM	7:30 AM – 10:30 AM 2:30 PM – 3:30 PM 5:00 PM – 7:00 PM	7:30 AM – 10:30 AM

Accelrys, San Diego, CA

Accelrys develops and commercializes scientific business intelligence software and solutions that help accelerate science for clients in the Life Sciences, Energy, Chemicals, Aerospace, and Consumer Packaged Goods industries. Our solutions are used by biologists, chemists, materials scientists, IT and business professionals to aggregate, analyze, simulate, and visualize scientific data.

Accugenix, Inc., Newark, DE

Accugenix, Inc. is the world's leading service laboratory for microbial identification and characterization. We test and analyze environmental isolates common in pharmaceutical and other manufacturing industries using our validated DNA sequence library. Identification of 300,000+ microorganisms. FDA-registered, cGMP compliant, competitive global standards. Accugenix—the leader in genetic microbial identification.

Applikon Biotechnology, Foster City, CA

Appropriate Technical Resources, Inc. (ATR), Laurel, MD

ATR is the US partner of Infors. With 40 years of experience, INFORS provides innovative fermenter and control systems for research and process engineering. The Multitron environmental stackable shakers; provide cooling, lighting, humidity and CO2 control. Terrafors solid state fermenter is a unique instrument for the study of bioremediation and biodegradation.

Beckman Coulter, Inc., Fullerton, CA

Beckman Coulter provides systems and solutions for both the analysis of fermentable sugars as well as related changes in gene expression. The PA 800 capillary electrophoresis system rapidly performs superior automated analysis of fermentable sugars. The GenomeLab™ GeXP Genetic Analysis System is a multiplexed quantitative solution that measures subtle, biologically relevant changes in expression profiling.

Ceres, Inc. — The Energy Crop Company™, Thousand Oaks, CA

Ceres, Inc. is a leading developer and marketing of high-yielding, dedicated energy crops that can be planted as raw materials for biofuels, biopower and other biomass conversion processes. The plant breeding and biotechnology company markets its products under its Blade Energy Crops brand. The privately held company also licenses its technology and traits to other organizations.

DASGIP Biotools LLC, Shrewsbury, MA

DASGIP develops and manufactures technologically advanced Parallel Bioreactor Systems for cultivation of microbial, animal and human cells at bench top scale.

DNA2.0, Menlo Park, CA

DNA2.0is a synthetic biology company providing gene synthesis and protein engineering products and services. The company enables scientists to accomplish new endeavors in molecular biology through a range of novel synthetic applications and backed by expert scientific support. Please see www.DNA20.com for more information on the free Gene Designer software, increased expression of recombinant proteins, RNAi resistant genes, protein engineering, and more.

Energy Biosciences Institute, Berkeley, CA

The Energy Biosciences Institute is the world's largest public/private consortium dedicated to the development of bioenergy. Its partners – BP, the global energy company which has committed $500 million to the 10-year program; the University of California, Berkeley; Lawrence Berkeley National Laboratory; and the University of Illinois at Urbana-Champaign – are exploring the application of biological processes, materials and mechanisms to the energy sector.

Lucigen/C5-6 Technologies, Middleton, WI

Lucigen is the only source for pure, recombinant CAZyme™ Carbohydrases: thermostable, multi-functional enzymes with numerous applications including biofuel production. We also construct custom genomic and metagenomic libraries for genomics and enzyme discovery; provide PCR kits, cloning systems, and competent cells for cloning and protein expression—for even very difficult genes.

Microbiology International, Frederick, MD

Microbiology International is the exclusive distributor for a complete suite of automated laboratory solutions for research microbiologists. Specialists in anaerobic and hypoxic workstations for the Biofuel researcher. Supplier of Systec autoclaves and media preparation units. Offering complete support, service and calibration on all equipment.

MIDI, Newark, DE

MIDI Inc. is the developer of the Sherlock Microbial Identification System which is used to identify bacteria and yeast by gas chromatographic analysis. The system is being widely used by the government and private sector to research and identify alternate fuel sources for use in the deployment of renewable energy.

National Renewable Energy Laboratory (NREL), Golden, CO

The National Renewable Energy Laboratory (NREL) is the nation's primary laboratory for renewable energy and energy efficiency research and development. NREL's mission and strategy are focused on advancing the U.S. Department of Energy's and our nation's energy goals. The laboratory's scientists and researchers support critical market objectives to accelerate research from scientific innovations to market-viable alternative energy solutions.

New Brunswick Scientific Co., Inc., Edison, NJ

New Brunswick Scientific offers a wide range of fermentation systems in 1 - 3,000 Liter capacities, ideal for research & pilot production of Biofuels. Our advanced benchtop systems feature an easy-to-use touchscreen controller to regulate up to 28 parameters including external devices like scales, analyzers and ancillary pumps. Large-scale BioFlo Pro systems now feature Allen Bradley PLC controls.

Oak Ridge National Laboratory (ORNL), Oak, Ridge, TN

Oak Ridge National Laboratory is the Department of Energy's largest science and energy laboratory. ORNL has six major mission roles: neutron science, energy, high-performance computing, systems biology, materials science at the nanoscale, and national security, plus one of the three Office of Science-sponsored Bioenergy Science Centers in the United States.

Phoenix BioConsulting LLC, Fanwood, NJ

Phoenix BioConsulting, LLC provides scientific consulting services for the industrial microbiology and biotechnology sectors. Leveraging on-line meeting and collaboration tools, the consultant becomes a "virtual team member" for scientific, due diligence, or business development projects. This is a flexible and cost-effective means of infusing expertise into your organization.

QMI, Oakdale, MN

You can be safe with Safe-Septum from QMI. It keeps bacteria, yeast, mold and other unwanted organisms from contaminating your fermentation process during sampling. Safe-Septum is aseptic, pressure and temperature safe, and pre-sterilized. Our multi-port design is easy to retrofit to your bioreactor. In fact, most applications require no engineering, cutting or welding modifications to use Safe-Septum.

The U.S. Department of Energy Office of Energy Efficiency and Renewable Energy's Biomass Program, Washington D.C.

The U.S. Department of Energy Office of Energy Efficiency and Renewable Energy's Biomass Program works with industry, academia and national laboratory partners on a balanced portfolio of research, development, and demonstration efforts geared to develop feedstocks, conversion technologies, and integrated biorefineries with necessary supporting infrastructure. The Biomass Program and its partners seek to transform the nation's renewable and abundant biomass resources into cost competitive, high performance biofuels, value-added bioproducts, and biopower. The Program's vision is to use these accomplishments for enhanced U.S. energy security, reduced dependence on oil, environmental benefits including reduced greenhouse gas emissions, and creation of economic opportunities across the nation.

The U.S. Department of Energy GENOMICS:GTL, Oak Ridge, TN

The U.S. Department of Energy's Genomics: GTL (Genomes to Life) research program uses genomic data and high-throughput technologies for studying the proteins encoded by microbial and plant genomes to develop a predictive understanding of the biological systems relevant to solving energy and environmental challenges including bioenergy production, environmental remediation, and climate stabilization.

U.S. Department of Agriculture, ARS NCAUR, Peoria, IL

The USDA, Agricultural Research Service conducts research to develop and transfer solutions to agricultural problems of high national priority and provide information access and dissemination to ensure high-quality, safe food, and other agricultural products; assess the nutritional needs of Americans; sustain a competitive agricultural economy; enhance the natural resource base and the environment, and provide economic opportunities for rural citizens, communities, and society as a whole.

YSI, Inc., Yellowsprings, OH

YSI, Inc. offers Biochemistry Analyzers for the rapid measurement of xylose, glucose, sucrose, ethanol and methanol in cellulosic ethanol research and process development. The YSI 2700 and 7100 Analyzers are employed daily to gain an understanding of process dynamics in cellulosic biomass saccharification and fermentation processes and to evaluate enzyme performance.

2010

32nd Symposium on Biotechnology for Fuels and Chemicals

April 19-22

Hilton Clearwater Beach

Clearwater, Florida

NOTE: New Meeting Pattern – Monday-Thursday

DEADLINE FOR ABSTRACT SUBMISSION DECEMBER 7, 2009

Sunday, May 3, 2009

7:30 AM — 9:00 PM	**Internet Lounge** Mission, 3rd Fl
8:30 AM — 6:00 PM	**Registration/Editor's Desk** Grand Foyer, 3rd Fl
8:30 AM — 5:00 PM	**Poster Setup** InterCont. Ballroom, 5th Fl
8:30 AM — 2:00 PM	**Exhibit Setup** Grand Foyer, 3rd Fl
12:15 PM — 12:30 PM	**Opening Remarks/Keynote** Grand Ballroom, 3rd Fl
1:00 PM — 5:00 PM	**Session 1 Plant Sci.** Grand Ballroom C, 3rd Fl
1:00 PM — 5:00 PM	**Session 2 Microbial Sci. I** Grand Ballroom A–B, 3rd Fl
2:30 PM — 3:30 PM	**Exhibits Open** Grand Foyer, 3rd Fl
5:00 PM — 7:00 PM	**Exhibits Open/Reception** Grand Foyer, 2nd Fl
6:00 PM — 9:00 PM	**Poster Session I (Sect. 1–4, 6–8)** InterCont. Ballroom, 5th Fl Pacific Terrace Foyer, 4th Fl

Monday, May 4, 2009

7:15 AM — 5:00 PM	**Registration/Editor's Desk** Grand Foyer, 3rd Fl
7:15 AM — 8:00 AM	**Continental Breakfast** Grand Foyer, 3rd Fl
7:15 AM — 8:00 AM	**Speaker's Breakfast** SoMa, 3rd Fl
7:30 AM — 10:30 AM	**Exhibits Open** Grand Foyer, 3rd Fl
7:30 AM — 9:00 PM	**Internet Lounge** Mission, 3rd Fl
8:00 AM — 11:30 AM .	**Session 3 Biomass Pretreat** Grand Ballroom A–B, 3rd Fl
8:00 AM — 11:30 AM	**Session 4 Translat. Genomics** Grand Ballroom C, 5th Fl
11:30 AM — 1:00 PM	**Lunch on your own**
1:00 PM — 5:00 PM	**Session 5 Enzyme Sci. I** Grand Ballroom, A–B, 3rd Fl
1:00 PM — 5:00 PM	**Session 6 Microbial Sci. II** Grand Ballroom, C, 3rd Fl
2:30 PM — 3:30 PM	**Exhibits Open** Grand Foyer, 3rd Fl
5:00 PM — 7:00 PM	**Exhibits open/Reception** Grand Foyer, 3rd Fl
6:00 PM — 9:00 PM	**Poster Session II:** **(Sections 5, 9–12)** InterCont. Ballroom, 5th Fl Pacific Terrace Foyer, 4th Fl

Tuesday, May 5, 2009

7:15 AM — 12:00 noon	**Registration/Editor's Desk** Grand Foyer, 3rd Fl
7:15 AM — 8:00 AM	**Continental Breakfast** Grand Foyer, 3rd Fl
7:15 AM — 8:00 AM	**Speaker's Breakfast** SoMa, 3rd Fl
7:30 AM — 10:30 AM	**Exhibits Open** Grand Foyer, 3rd Fl
7:30 AM — 9:00 PM	**Internet Lounge** Mission, 3rd Fl
8:00 AM — 11:30 AM	**Session 7 Biorefinery Deploy** Grand Ballroom A–B, 3rd Fl
8:00 AM — 11:30 AM	**Session 8 Biofuels Log.&Sust.** Grand Ballroom C, 3rd Fl
12:15 PM — 1:45 PM	**Organizing Committee lunch** Sutter, 5th Fl
Free afternoon	
7:00 PM — 9:00 PM	**Special Topic I:** **Intl. 2nd Gen. Biofuels** Grand Ballroom C, 3rd FL
7:00 PM — 9:00 PM	**Special Topic II:** **Algal–based Biofuels** Grand Ballroom A–B, 3rd Fl

Wednesday, May 6, 2009

7:15 AM — 5:00 PM	**Registration/Editor's Desk** Grand Foyer, 3rd Fl
7:15 AM — 8:00 AM	**Continental Breakfast** Grand Foyer, 3rd Fl
7:15 AM — 8:00 AM	**Speakers Breakfast** SoMa, 3rd Fl
7:30 AM — 9:00 PM	**Internet Lounge** Mission, 3rd Fl
8:00 AM—11:30 AM	**Session 9 Bioprocessing** Grand Ballroom, C, 3rd Fl
8:00 AM — 12 noon	**Session 10 Enzyme Sci. II** Grand Ballroom A–B, 3rd Fl
11:30 AM — 1:00 PM	**Lunch on your own**
1:00 PM — 5:00 PM	**Session 11:** **Emerg. Biofuels/Chem.** Grand Ballroom C, 3rd Fl
1:00 PM — 5:00 PM	**Session 12 Biomass Recal.** Grand Ballroom A–B, 3rd Fl
6:00 PM	**Reception** Grand Foyer, 3rd Fl
7:00 PM	**Banquet** Grand Ballroom, 3rd Fl

Technical Program

Sunday Morning, May 3

Registration/Editor's Desk

Grand Ballroom Foyer, 3rd Fl oor

8:30 AM-6:00 PM

Exhibit Setup

Grand Ballroom F, 3rd Floor

8:30 AM-2:00 PM

Invited Speaker Breakfast-Sunday speakers

SoMa, 3rd Floor

9:00 AM-10:00 AM

Poster Setup

InterContinental Ballroom, 5th Floor

8:30 AM-5:00 PM

Sunday Afternoon, May 3

Welcome Remarks

Grand Ballroom, 3rd Floor

12:15 PM

Session 1: Plant Science and Technology

Chairs: Z. Wang, The Noble Foundation, Ardmore, OK and S. Chaudhuri, Syngenta, Durham, NC

Grand Ballroom C, 3rd Floor

1:00 PM **1-01** **The French initiative on renewable carbon for green chemistry and bioenergies**

P. Colonna*, F. Houllier and A. Kammoun, INRA, Paris, France; X. Montagne, IFP, Rueil-Malmaison, France; C. Sales, CIRAD, Montpellier, France

1:30 PM **1-02** **Plant growth promoting microorganisms allow for sustainable growth and increased biomass production of poplar on marginal soils**

D. van der Lelie*, S. Monchy, L. Newman and S. Taghavi, Brookhaven National Laboratory, Upton, NY

2:00 PM **1-03** **Plants begetting plants: Lignocellulose saccharification by plant-expressed cellulases**

J.D. Nichols*, B. Link, S. Miles, M. Kim, B. Ember, S. Arellano and P. Oeller, Syngenta Biotechnology, Inc., Research Triangle Park, NC

2:30 PM **1-04** **Transgenic expression of endoglucanase and xylanase genes increases tobacco digestibility and biomass conversion**

K.L. Pappan*, D. Corredor and B. Gerdes, Edenspace Systems Corporation, Manhattan, KS; D.A. Lee and S.A. Yelundur, Edenspace Systems Corporation, Chantilly, VA; X. Wu and D. Wang, Kansas State University, Manhattan, KS

3:00 PM **Break**

Sponsored by Sartorius-Stedim

3:30 PM **1-05** **Enhanced bioprocessing of maize cell wall mutant**

W. Vermerris* and H.M. Caicedo, University of Florida, Gainesville, FL; N.S. Mosier and M.R. Ladisch, Purdue University, West Lafayette, IN

4:00 PM **1-06** **Impact of divergent selection on the abundance and activities of lignin biosynthetic enzymes in switchgrass, and characterization of recombinant switchgrass CAD and COMT proteins**

A.J. Saathoff*, N.A. Palmer, S.E. Sattler, R.B. Mitchell, K.P. Vogel and G. Sarath, USDA-ARS, Lincoln, NE; C. Tobias, USDA, Agricultural Research Service, Albany, CA; P. Twigg, University of Nebraska, Kearney, Kearney, NE; E.J. Haas, Creighton University, Omaha, NE

4:30 PM **1-06a** **Functional genomic analysis of plant biomass deconstruction by extremely thermophilic, cellulolytic bacteria in pure and co-culture**

Derrick L. Lewis*1, Sara Blumer-Schuette1, Inci Ozdemir1, Amy L. VanFossen1, Ira Kataeva2, Sung-Jae Yang2, Michael W.W. Adams2 and Robert M. Kelly1, (1)Chemical and Biomolecular Engineering, North Carolina State University, Raleigh, NC, (2) Biochemistry & Molecular Biology, University of Georgia, Athens, GA

Session 2: Microbial Science and Technology 1

Chairs: S. Picataggio, Verdezyne, Carlsbad, CA and R. Gonzalez, Rice University, Houston, TX

Grand Ballroom A-B, 3rd Fl oor

1:00 PM **2-01** **One-step cellulosic ethanol: Can we really do this?**

S. Ryu and M.N. Karim*, Texas Tech University, Lubbock, TX

1:30 PM **2-02** **Genetically engineering yeast for CO2 capture during ethanol fermentation**

Z. Dai*, K. Panther, S. Baker, J. Magnuson and L. Lasure, Pacific Northwest National Laboratory, Richland, WA

2:00 PM **2-03** **Production of a xylose utilizing *Zymomonas mobilis* strain for ethanol production from high concentrations of mixed sugars**

P. Viitanen*, C. McCutchen, M. Emptage and P. Caimi, DuPont Co., Wilmington, DE; M. Zhang, Y.C. Chou and M.A. Franden, NREL, Golden, CO

2:30 PM **2-04** **Construction of pentose fermenting industrial *Saccharomyces cerevisiae* strains expressing a bacterial xylose isomerase**

E. Boles*, D. Brat and B. Wiedemann, Goethe-University Frankfurt, Frankfurt, Germany

3:00 PM **Break**

Sponsored by Sartorius-Stedim

3:30 PM **2-05** **Development of a Robust Yeast Biocatalyst for Low pH Lactic Acid and Cellulosic Ethanol Fermentation.**

Pirkko Suominen1, Dan Beacom1, Tom McMullin1, Arlene Fosmer1, Chris Miller1, Brian Rush1, Jon Veldhouse1, Gary Folkert1, Liz Dierickx1, Ken Finley1, Beth Mastel1, Holly Jessen2, Josh Lundorff2, Ana Negrete-Raymond2 and Jian Yi2, (1)Biotechnology Development Center, Cargill, Minnetonka, MN, (2)Biotechnology Development Center, Cargill, Navarre, MN

4:00 PM **2-06** **Metabolic engineering of *Saccharomyces cerevisiae* for the production of n-butanol**

E.J. Steen*, S. Myers and A. Redding, UC Berkeley, UC San Francisco, Berkeley, CA; R.C. Chan, N.P. Prasad and M. Ouellet, Lawrence Berkeley National Laboratory, Berkeley, CA; C. Petzold, Joint BioEnergy Institute, Emeryville, CA; J.D. Keasling, UC Berkeley; Lawrence Berkeley National Laboratory, Emeryville, CA

4:30 PM **2-06a** **Integration of genomics and bioinformatics to identify genetic differences in an ethanol tolerant *Clostridium thermocellum* ATCC27405 strain**

S.D. Brown*, T. Karpinets, J.R. Mielenz, S. Yang, D.M. Klingeman, M.L. Land, L.J. Hauser, B. Raman, M. Rodriguez, T. Yan, T.A. Vishnivetskaya and M. Keller, Oak Ridge National Laboratory, Oak Ridge, TN; H. Strobel, University of Kentucky, Lexington, KY; Y. Xu and P. Dam, University of Georgia, Athens, GA; L.R. Lynd, Dartmouth College, Hanover, NH

Tabletop Exhibits

Grand Ballroom Foyer, 3rd Floor

2:30 PM-3:30 PM

Sunday Evening, May 3

Tabletop Exhibits

Grand Ballroom Foyer, 3rd Floor

5:00 PM-7:00 PM

Poster Session 1 / Reception

Chairs: M. Resch, C. Gerk, NREL, Golden, CO and J. Langhton, Univ. of California, Davis, CA

Sessions 1-4, 6-8

InterContinental Ballroom, 5th Floor

Pacific Terrace Foyer, 4th Floor

6:00 PM - 9:00 PM

Plant Science and Technology

1-07 **New method for fast detection of improved biodegradability in genetically modified plants**

E.A. Ximenes*, Y. Kim, X. Li, R. Meilan, M. Ladisch and C. Chapple, Purdue University, West Lafayette, IN

1-08 **Progress toward a renewable, plant-based production system for methacrylate**

J.H. Park*, E. Doukhanina, A. Park, A. Ragab, L. Zhang, R. Miller, S. Thomas and S. Bobzin, Ceres, Inc, Thousand Oaks, CA; D. Hawkins, Rohm and Haas Chemicals LLC

1-09 **Towards a realistic model of plant cell walls via correlative Raman imaging and EM tomography**

P. Sarkar*, E. Bosneaga, P. Jess and K. McDonald, University of California, Berkeley, Berkeley, CA; J. Han, K.H. Downing, B. Parvin and M. Auer, Lawrence Berkeley National Laboratory, Berkeley, CA; A. Carroll, Energy Biosciences Institute, Berkeley, CA; J. Liphardt, University of California, Berkeley, Lawrence Berkeley National Laboratory, Berkeley, CA

1-10 **Expression of lignocellulosic-degrading enzymes in transgenic plants**

S. Austin-Phillips*, S. Chaiwongsar, J.A. Raasch and T. Ziegelhoffer, University of Wisconsin, Madison, WI

1-11 **Withdrawn**

1-12 **A rapid analytical method for investigating genetic modification of lignin pathways in alfalfa (*Medicago sativa*)**

D. Astling*, R. Sykes, A. Ziebell, K. Reichel, C. Doeppke and M. Davis, National Renewable Energy Laboratory, Golden, CO

1-13 **Structural glycan composition of Pacific Northwest grass-derived biomass: A survey**

D. Masrungson, D. Smith and M.H. Penner*, Oregon State University, Corvallis, OR; H. El-Nashaar, S.M. Griffith and G.M. Banowetz, USDA-ARS, Corvallis, OR

1-14 **Biofuel feed stock source – *Jatropha curcas***

K. Sengar*, Sardar Vallabh Bhai Patel University of Agriculture & Technology, Meerut, India and A. Innani, University of Newcastle, Newcastle upon Tyne, United Kingdom

1-15 **Characterization of sorghum *bmr* mutants for biofuel applications**

A. Saballos* and W. Vermerris, University of Florida, Gainesville, FL; G. Ejeta, Purdue University, West Lafayette, IN; C. Kang and E. Sanchez, Washington State University, Pullman, WA

1-16 **Superior dedicated herbaceous energy crop varieties**

S.R. Thomas, Ceres, Inc., Thousand Oaks, CA

Microbial Science and Technology 1

2-07 **Tracking microbial community changes during decomposition of switchgrass**

A.P. Reddy* and M. Allgaier, Joint BioEnergy Institute, Emeryville, CA; P. Hugenholtz, Joint Genome Institute, Walnut Creek, CA; B.A. Simmons, Sandia National Laboratories, Livermore, CA; T.C. Hazen, Lawrence Berkeley National Laboratory, Berkeley, CA; J. VanderGheynst, University of California, Davis, Davis, CA

2-08 **Current status of the Department of Energy's Aquatic Species Program lipid-focused algae collection**

E.P. Knoshaug*, E.E. Jarvis, Y.C. Chou, P.T. Pienkos and A. Darzins, National Renewable Energy Laboratory, Golden, CO

2-09 **Withdrawn**

2-10 **Biological production of ethanol, xylitol and arabitol by novel, naturally occurring yeast**

A. Vajzovic*, R. Bura and S. Doty, University of Washington, Seattle, WA

2-11 **Comparative genomics of *Oligotropha carboxidovorans* OM5, a chemolithoautotrophic bacterium**

D. Paul*, B. Nanduri, S. Bridges, W. Holmes and M.L. Lawrence, Mississippi State University, Starkville, MS; R. Kumar, S. Burgess and Y. Dandass, Mississippi State University, Mississippi State, MS; T. French, Mississippi State University, MS State, MS; A. Brown, Mississippi State Chemical Lab, Mississippi State, MS

2-12 **Withdrawn**

2-13 **Pyruvate induced metabolic shift in cellobiose bed *Clostridium thermocellum* ATCC27405 batch cultures**

T. Rydzak*, D. Levin, N. Cicek and R. Sparling, University of Manitoba, Winnipeg, MB, Canada

2-14 **Effect of gas sparging on pyruvate catabolism during batch fermentation of *Clostridium thermocellum* ATCC 27405**

C.R. Carere*, T. Rydzak, R. Sparling, N. Cicek and D.B. Levin, University of Manitoba, Winnipeg, MB, Canada

2-15 **Development of a robust yeast biocatalyst for low pH lactic acid and cellulosic ethanol fermentation**

P. Suominen*, D. Beacom, T. McMullin, A. Fosmer, C. Miller, B. Rush, J. Veldhouse, G. Folkert, L. Dierickx, K. Finley and B. Mastel, Cargill, Minnetonka, MN; H. Jessen, J. Lundorff, A. Negrete-Raymond and J. Yi, Cargill, Navarre, MN

2-16 **Fed-batch schemes and yeast adaptation for improving ethanol production at high substrate loading**

E. Tomás-Pejó, J.M. Oliva and M. Ballesteros, CIEMAT, Research Centre for Energy, Environment and Technology, Madrid, Spain; L. Olsson*, Chalmers University of Technology, Göteborg, Sweden

2-17 **Improved xylose consumption in recombinant *Saccharomyces cerevisiae* strains**

N.S. Parachin*, B. Hahn-Hägerdal and M.F. Gorwa-Grauslund, Lund University, Lund, Sweden

2-18 **Yeast strains for ethanol production from lignocellulosic hydrolysates in situ detoxification**

Y. Xlushan* and T. Shen, Capital Normal University, Beijing, China

2-19 **The effect of carbon source and oxygen level on global gene expression analysis in *Pichia stipitis***

J.R.H. Van Vleet*, University of Wisconsin, Madison, WI and T.W. Jeffries, USDA Forest Service, Madison, WI

2-20 **Withdrawn**

2-21 **Metabolic engineering of *Escherichia coli* for efficient conversion of glycerol into ethanol**

C.T. Trinh* and F. Srienc, University of Minnesota, St. Paul, MN

2-22 **Withdrawn**

2-23 **Identification of *Saccharomyces cerevisiae* genes involved in the resistance to phenolic fermentation inhibitors**

L. Björklund, AstraZeneca R&D Lund, Lund, Sweden, S. Larsson, Riga Technical University, Riga, Latvia and L.J. Jönsson*, Umeå University, Umeå, Sweden

2-24 ***Aspergillus fumigatus* JF1: An ionic liquid tolerant fungus isolated from compost**

S. Singer* and A.P. Reddy, Joint BioEnergy Institute, Emeryville, CA; J. VanderGheynst, University of California, Davis, Davis, CA; B.A. Simmons, Sandia National Laboratories, Livermore, CA

2-25 **Converting C5 and C6 sugars from hydrolyzed pretreated lignocellulosic materials using *Thermoanaerobacter* BG1L1**

M.J. Mikkelsen* and B.K. Ahring, BioGasol ApS, Ballerup, Denmark

2-26 **The model filamentous fungus *Neurospora crassa*, a great system for studying lignincellulose degradation**

C. Tian*, J. Sun and N.L. Glass, University of California, Berkeley, Berkeley, CA; W. Beeson and J. Cate, University of California, Berkeley

2-27 ***Zymomonas mobilis* systems biology studies to elucidate process relevant inhibitor stress responses and tolerance mechanisms**

S. Yang*, D. Pelletier, T. Tschaplinski, G.B. Hurst, C. Pan, M.L. Land, L.J. Hauser, T.Y.S. Lu, G.L. Chen, Y.J. Chang, D.M. Klingeman, N. Engle, M. Rodriguez, B.H. Davison, T. Palumbo and S.D. Brown, Oak Ridge National Laboratory, Oak Ridge, TN; S.L. Martin, North Carolina State University, Raleigh, NC

2-28 **Characterization of four *Clostridium* species for ethanol production**

K.C. Williams*, R. Zhang, H. Chan, Y. Zheng and J. Fan, University of California, Davis, Davis, CA; J.A. McGarvey, USDA/ARS/FCR, Albany, CA

2-29 **The endogenous molecular basis for improved xylose utilization of *Saccharomyces cerevisiae***

N. Liu*, Z. Li and S. Chen, Washington State University, Pullman, WA

2-30 **Improved galactose fermentation by *Saccharomyces cerevisiae* through inverse metabolic engineering**

K.S. Lee, M.E. Hong, Y.J. Sung and D.H. Kweon, School of Biotechnology and Bioengineering, Sungkyunkwan University, Suwon, South Korea; J.C. Park, S.M. Park and B.J. Yu, Samsung Advanced Insitute of Technology; Y.S. Jin*, University of Illinois at Urbana-Champaign, Urbana, IL

2-31 **Molecular and physiological characterization of heterologous xylose transporters in recombinant xylose-utilizing *Saccharomyces cerevisiae***

D. Runquist*, P. Rådström and B. Hahn-Hägerdal, Lund University, Lund, Sweden

2-32 **Regulation of *pfl* in *B. coagulans***

M.S. Rhee*, L.O. Ingram and K.T. Shanmugam, University of Florida, Gainesville, FL

2-33 **Study of cell viability and morphology of *Zymomonas* in the presence of inhibitors**

Y.C. Chou*, M.A. Franden, P.T. Pienkos and M. Zhang, National Renewable Energy Laboratory, Golden, CO

2-34 **Engineering of yeast strains capable of broad substrate utilization in alcohol fermentation - Part I: Identification of founder strains and gene candidates**

P. Gujjari, J. Houseknecht, S.O. Suh and J. Zhou*, ATCC (American Type Culture Collection), Manassas, VA

2-35 **Rapid fermentation of glucose, xylose and cellobiose by the wood-boring beetle-associated yeast, *Spathaspora passalidarum***

T.M. Long*, Great Lakes Bioenergy Research Center, Madison, WI, J.R.H. Van Vleet, University of Wisconsin, Madison, WI, M.A. Caballero, UW-Madison, Madison, WI and T.W. Jeffries, USDA Forest Service, Madison, WI

2-36 **Evaluation of engineered xylose-fermenting industrial strains of *Saccharomyces cerevisiae* for improved ethanol production from lignocellulosic feedstocks**

R.E. Hector*, B.S. Dien and M.A. Cotta, United States Department of Agriculture, Agricultural Research Service, National Center for Agricultural Utilization Research, Peoria, IL

2-37 **The effect of raw glycerol discharged after biodiesel manufacturing in the xanthan gum production synthesized by *Xanthomonas* isolated in Brazil**

F.F. Padilha*, E. Reis, J. Cardoso and L. Cirino, Tiradentes University; Technology and Research Institute, Aracaju/SE, Brazil; J. Druzian, Universidade Federal da Bahia, Salvador/BA, Brazil; M.J.V. Fonseca, Universidade de São Paulo, Ribeirão Preto/SP, Brazil; C.P. Costa, Universidade Católica de Pelotas, Pelotas/RS, Brazil; R.D.L.R. Mariano, Universidade Federal Rural de Pernambuco, Recife/PE, Brazil

2-38 **Metabolic engineering of a novel thermophilic ethanologen *Geobacillus thermoglucosidasius* M10EXG for enhanced ethanol production**

C.L. Kozina*, A.S. Pawate, K.L. Sale and D.S. Reichmuth, Sandia National Laboratories, Livermore, CA; D. Joyner, Lawrence Berkeley National Laboratory, Berkeley, CA; T.C. Hazen, Lawrence Berkeley National Laboratory in conjunction with Joint BioEnergy Institute, Berkeley, CA; R. Sapra, Sandia National Laboratories in conjunction with Joint BioEnergy Institute, Livermore, CA

2-39 **Draft genome sequence, annotation and metabolic pathway reconstruction of the ethanol-tolerant thermophile *Geobacillus thermoglucosidasius* M10EXG**

A.S. Pawate*, K.L. Sale, C.L. Kozina, D.S. Reichmuth and R. Sapra, Sandia National Laboratories, Livermore, CA

2-40 **Heterologous expression of multiple cellulolytic enzymes in *Zymomonas mobilis***

J.G. Linger*, W.S. Adney, M. Zhang and A. Darzins, National Renewable Energy Laboratory, Golden, CO

2-41 **Characterization of biohydrogen production in metabolically engineered *Escherichia coli* strains**

J. Mathews* and G. Wang, University of Hawai'i Manoa, Honolulu, HI

2-42 **Engineering tolerance to spent sulfite liquor (SSL) by genome shuffling *Saccharomyces cerevisiae* leads to increased ethanologenic capacity**

D.J. Pinel*, F. D'aoust and V.J.J. Martin, Concordia University, Montreal, QC, Canada; H. Lee, University of Guelph, Guelph, ON, Canada

2-43 **Withdrawn**

2-44 **Production of xanthan gum from sisal juice and glycerol**

M.I. Campos* and J. Druzian, Universidade Federal da Bahia, Salvador/BA, Brazil; E. Reis and F.F. Padilha, Tiradentes University; Technology and Research Institute, Aracaju/SE, Brazil

2-45 **Engineering *E. coli* to digest and utilize cellulose and hemicellulose as sole carbon sources**

G. Bokinsky*, California Institute for Quantitative Biosciences, Berkeley, CA, S. Chhabra, Joint BioEnergy Institute, Emeryville, CA and J.D. Keasling, UC-Berkeley; Lawrence Berkeley National Laboratory, Emeryville, CA

2-46 **Yeast genes identified in HMF tolerance screen suggest link to other lignocellulosic derived inhibitors including furfural and vanillin**

Q. Li, S. Allen, S. Ghosh and S. Gorsich*, Central Michigan University, Mount Pleasant, MI

2-47 **Construction of yeast strains producing high concentration of ethanol from tapioca by using various genetic methods**

K. Kim, The University of Suwon, Hwaseong-si, South Korea

2-48 **Isolation of cellulose and agarose-degrading *Pseudoalteromonas* sp. NO3 from Sea squirt, *Halocynthia rorentzi***

D. Kim*, S.J. Jung, J.K. Song, T.S. Shin, M.J. Oh and K.R. Kim, Chonnam National University, Yeosu, South Korea; K.S. Baik, S.C. Park and C.N. Seong, Sunchon National University, Sunchon, South Korea; H.R. Kim, Pukyung National University, Busan, South Korea

2-49 **Construction of a reporter gene system for use in the ethanologen *Geobacillus thermoglucosidasius* SB2**

J. Bartosiak-Jentys* and D.J. Leak, Imperial College London, London, United Kingdom

2-50 **D-lactic acid production from xylose by a new bacterium found in thailand**

M. Boonmee and P. Yuvadetkun, Khon Kaen University, Khon Kaen, Thailand; V. Burapatana*, PTT Public Co. Ltd., Bangkok, Thailand

2-51 **Metabolic engineering of flocculent *Saccharomyces cerevisiae* with genome-integrated $NADP^+$-dependent xylitol dehydrogenase gene for ethanol production from xylose**

A. Matsushika*, H. Inoue and S. Sawayama, National Institute of Advanced Industrial Science and Technology (AIST), Hiroshima, Japan; S. Watanabe, T. Kodaki and K. Makino, Kyoto University, Kyoto, Japan

2-52 **The role of membrane phospholipids in xylose utilization**

J.M. Xia* and Y.J. Yuan, Tianjin University, Tianjin, China; M.W. Lao, V. Balan and B.E. Dale, Michigan State University, Lansing, MI

2-53 **Effect of lignocellulosic inhibitory compounds on the fermentative capacity of different yeast strains**

S. Ghatora*, M. Liu, L. Kumar, P. Chung, R. Chandra, A.A. Roos and J. Saddler, University of British Columbia, Vancouver, BC, Canada

2-54 **Riboflavin production during fermentation of genetically modified biobutanol-producing *Clostridium acetobutylicum***

X. Cai* and G. Bennett, Rice University, Houston, TX

2-55 **A proteomic analysis of granulation in liquid cultures of *Phanerochaete chrysosporium***

J.J. Diaz-Torres*, University of Puerto Rico, Mayaguez Campus, Mayagüez, PR and P. Ortiz-Bermúdez, University of Puerto Rico- Mayaguez Campus, Mayaguez, PR

2-56 **Phenotype microarray profiling of *Zymomonas mobilis* ZM4**

B.R. Bochner*, V. Gomez, M. Ziman and S. Montgomery, Biolog, Inc., Hayward, CA; S. Yang and S.D. Brown, Oak Ridge National Laboratory, Oak Ridge, TN

2-57 **Construction of a novel integration vector for expression of delta 6 desaturase gene in *Lipomyces kononenkoae***

M. Jiang*, X. Wan, P. Wang and Y. Zhang, Oil Crops Research Institute, CAAS, Wuhan, China

2-58 **Effects of S-adenosyl-l-methionine on membrane components and ethanol tolerance in *Saccharomyces cerevisiae***

S.W. Lee*, E.S. Choi and M.K. Oh, Korea University, Seoul, South Korea

2-59 **Development of two phase fermentation system for the stable and high butanol production using *Clostridium acetobutylicum* ATCC 824**

S.M. Lee*, M.O. Cho, Y. Um and B.I. Sang, Korea Institute of Science and Technology, Seoul, South Korea

2-60 **Enhanced production of 1,2-propanediol *Saccharomyces cerevisiae* by metabolic engineering and carbon source optimization**

J.Y. Jung*, E.S. Choi and M.K. Oh, Korea University, Seoul, South Korea

2-61 **Systems biology: The new frontier for bioenergy**

T.C. Hazen, Lawrence Berkeley National Laboratory, Berkeley, CA

2-62 **Role of efflux pumps in *Escherichia coli* solvent resistance**

M.J. Dunlop*, M. Hadi and H. Beller, Joint BioEnergy Institute, Emeryville, CA; P.D. Adams and A. Mukhopadhyay, Joint Bioenergy Institute, Emeryville, CA; J.D. Keasling, UC-Berkeley; Lawrence Berkeley National Laboratory, Emeryville, CA

2-63 **Harnessing genomic recombination to improve microbial metabolic phenotypes**

A.E. McKee*, J. Haliburton, V. Fok, M. Ouellete and S. Chhabra, Joint BioEnergy Institute, Emeryville, CA; J.D. Keasling, UC-Berkeley; Lawrence Berkeley National Laboratory, Emeryville, CA

2-64 **Glyceric acid production from raw glycerol by acetic acid bacteria**

H. Habe*, T. Fukuoka, D. Kitamoto, H. Yanagishita and K. Sakaki, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Ibaraki, Japan; Y. Shimada, M. Itagaki and K. Watanabe, Science University of Tokyo, Noda, Chiba, Japan

2-65 **Transcriptomic analysis of carbohydrate metabolism during ethanol fermentation by *Pichia stipitis***

W.H. Chen*, T.H. Lin, G.L. Guo and W.S. Hwang, Institute of Nuclear Energy Research, Taoyuan, Taiwan

2-66 **Development of recombinant yeast for L-arabinose fermentation**

A. Bera*, M. Sedlak, A. Khan and N. Ho, Purdue University, West Lafayette, IN

2-67 **Effect of Na^+, K^+, NH_4^+ and glycerol on the glucose/xylose co-fermentation by recombinant *S. cerevisiae* 424A(LNH-ST)**

H. Mohammad, N.S. Mosier, N. Ho and M. Sedlak*, Purdue University, West Lafayette, IN

2-68 **Metabolic analysis of the effect of acetic acid on the co-fermentation of glucose and xylose by *S. cerevisiae* 424A(LNH-ST)**

E. Casey*, M. Sedlak, N.W.Y. Ho, J. Adamec, A. Jannasch and N.S. Mosier, Purdue University, West Lafayette, IN

2-69 **Effect of soy-based glycerol concentration on 3-hydroxypropionaldehyde production by enteric species**

T.P. West, South Dakota State University, Brookings, SD

2-70 **Design of transcription factor-based in vivo biosensors for improved butanol production in *E. coli***

J.A. Dietrich*, UC-Berkeley; Joint BioEnergy Institute, Emeryville, CA; D.L. Shih and A. Chan, Joint BioEnergy Institute, Emeryville, CA; J.D. Keasling, UC-Berkeley and Lawrence Berkeley National Laboratory, Emeryville, CA

2-71 **Mixed sugars fermenting *Saccharomyces cerevisiae* strains with optimized pathway combination**

M. Bettiga*, B. Hahn-Hägerdal and M.F. Gorwa-Grauslund, Lund University, Lund, Sweden

2-72 **Withdrawn**

2-73 **Sugarcane platform for chemicals and next-gen biofuels**

R.F. Cascone, Nexant, Inc., White Plains, NY

2-74 **Harnessing the microbial fermentation of glycerol for the production of fuels and chemicals**

R. Gonzalez, Rice University, Houston, TX

2-75 **Microbial conversion of bio-oils to fuels and chemicals: A new biorefinery paradigm**

C. Dellomonaco* and R. Gonzalez, Rice University, Houston, TX; P. Campbell and C. Rivera, Glycos Biotechnologies Inc., Houston, TX

2-76 **Development and scale-up of xylose-rich hydrolysate fermentation process for lignocellulosic ethanol production**

T.H. Lin*, C.F. Huang, W.H. Chen and J.B. Wang, Institute of Nuclear Energy Research, Taoyuan, Taiwan

2-77 **Evaluation of the metabolic burden of recombinant cellulase expression by *Saccharomyces cerevisiae* in batch culture**

E. van Rensburg*, J.F. Gorgens, R. den Haan, D.C. la Grange and W.H. van Zyl, Stellenbosch University, South Africa

Biomass Pretreatment and Fractionation

3-07 **In-situ examination of biomass dissolution and cellulose regeneration enabling cellular level insight of ionic liquid pretreatment process**

S. Singh* and B. Simmons, Joint BioEnergy Institute, Physical Biosciences Division, Lawrence Berkeley National Laboratory, Emeryville, CA

3-08 **Efficiencies of designed xylanase combinations in releasing sugars from hemicellulose extract on mixed Northeast hardwood**

B.H. Um* and P. vanWalsum, University of Maine, Orono, ME

3-09 **Pretreatment of sweetgum (*Liquidambar styraciflua* L.) with dilute sulfuric acid**

C. Lau, J. Duke, E.M. Martin, E.C. Clausen, D.J. Carrier* and A.S. Engelberth, University of Arkansas, Fayetteville, AR; M. Pelkki, University of Arkansas, Monticello, Monticello, AR

3-10 **Chemical features of solid residues obtained from supercritical water treatment of lignocellulosics**

K.H. Kim*, I.Y. Eom and J.W. Choi, College of Agricultural and Life Science, Seoul National University, Seoul, South Korea; S.M. Lee and O.K. Lee, Korea Forest Research Institute, Seoul, South Korea

3-11 **Value prior to pulping: Extraction of hemicellulose from hardwood**

S.L. Walton*, A.R.P. van Heiningen and G.P. van Walsum, University of Maine, Orono, ME

3-12 **Bioethanol production from steam exploded forage sweet sorghum at high solid content**

I. Ballesteros, P. Manzanares*, M.J. Negro, J.M. Oliva, A. Gonzalez and M. Ballesteros, CIEMAT, Madrid, Spain

3-13 **Optimizing the saccharification of sugar cane bagasse using phosphoric acid and fungal cellulases**

C. Geddes*, University of Florida, FL and L.O. Ingram, University of Florida, Gainesville, FL

3-14 **Optimization of dilute acid hydrolysis of rapeseed straw as a pretreatment for conversion to ethanol**

T.S. Jeong, K.Y. Won and K.K. Oh*, Dankook University, Cheonan, South Korea; B.H. Um, Forest Bioproducts Researh Initiative, University of Maine, Orono, ME

3-15 **The effects of varying pretreatment chemicals on the enzymatic hydrolysis of organosolv pretreated mountain pine beetle killed lodgepole pine**

L.F. Del Rio*, R.P. Chandra and J.N. Sadler, University of British Columbia, BC, Canada

3-16 **The comparative analysis of lignin from steam and organosolv pretreated substrates**

S. Nakagame*, R.P. Chandra and J.N. Saddler, University of British Columbia, Vancouver, BC, Canada

3-17 **Application of Chemspeed AUTOPLANT® to Ammonia Fiber EXpansion (AFEX) pretreatment**

D.J. Marshall*, V. Balan, S. Leonardo and D.E. Bruce, Michigan State University, Lansing, MI

3-18 **Optimization of rice straw pretreatment by hydrothermal process**

A.Y. Wu*, W.H. Chen and W.S. Hwang, Institute of Nuclear Energy Research, Longtan, Taiwan

3-19 **Investigation of nitrogen-containing compounds produced during AFEX pretreatment of lignocellulosic biomass**

J.F. Humpula*, S. Chundawat, L. Sousa, V. Balan and B. Dale, Michigan State University, Lansing, MI; R. Vismeh and A.D. Jones, Michigan State University, East Lansing, MI; F. Lu and J. Ralph, University of Wisconsin - Madison, Madison, WI

3-20 **The optimum of enzymatic hydrolysis from pretreated rice straw by aqueous ammonia**

Y.C. Park*, K. Kim, H. Kim and J.S. Kim, Kyonggi University, Suwon, South Korea; K.K. Oh, Dankook University, Cheonan, South Korea

3-21 **Acid saccharification to ethanol production from red algae (*Gelidium amansii*)**

K. Kim*, Y.C. Park, H. Kim and J.S. Kim, Kyonggi University, Suwon, South Korea; K.K. Oh, Dankook University, Cheonan, South Korea

3-22 **Organosolv pretreatment of *Liriodendron tulipifera* with acid and alkali catalysts**

B.W. Koo*, N. Park, H. Yeo, S.Y. Lee, H.Y. Kim and I.G. Choi, Seoul National University, Seoul, South Korea; H. Kim, HaidongEokom Co,.Ltd, Seoul, South Korea

3-23 **Pretreatment of biomass by proton beam irradiation**

S.B. Kim*, J.H. Lee, H.Y. Shin, H.W. Lee and S.W. Kim, Korea University, Seoul, South Korea

3-24 **Two-step hot-compressed water treatment of woody biomass to enhance enzymatic digestibility of cellulose and hemicellulose**

H. Inoue*, T. Sakaki, S. Sawayama and T. Endo, National Institute of Advanced Industrial Science and Technology, Hiroshima, Japan

3-25 **Enzymatic hydrolysis and ethanol production from AFEX pretreated bagasse at high solid loading using recombinant ethanologens**

C. Krishnan*, Indian Institute of Technology Madras, Chennai, India; V. Balan, D. Marshall and B.E. Dale, Michigan State University, Lansing, MI

3-26 **Towards consolidated enzymatic biomass conversion**

T. Dale*, S. Iyer, G.L. Wagner, D.T. Fox, N.H. Pawley, K.D. Rector, G. Gnanakaran, P.J. Unkefer and P. Langan, Los Alamos National Laboratory, Los Alamos, NM; K.E. Hammel, United States Department of Agriculture Forest Products Laboratory, Madison, WI; D. Dunaway-Mariano, University of New Mexico, Albuquerque, NM

3-27 **Hemicellulose hydrolysis in South America lignocellulosic biomass by steam explosion**

C.A. Carrasco*, M. Galbe and G. Lidén, Lund University, Lund, Sweden; L.F. Quispe and D. Cuno, Instituto de Investigacion y Desarrollo de Procesos Quimicos, Bolivia; C. Roslander, Lund University, Sweden

3-28 **Alkaline pretreatment of recycle newsprint and its mill sludge**

L. Kang* and Y.Y. Lee, Auburn University, Auburn, AL

3-29 **Starch hydrolysis evaluation for the production of fermentable sugars**

A.E. Aurea Victoria, D.P. Enrique*, J.S. Cesar Agustin, V.D.T. Gustavo and R.M. Fabian, Unidad Profesional Interdisciplinaria de Biotecnologia del Instituto Politecnico Nacional, Mexico City, Mexico

3-30 **Ammonia recovery in AFEX biomass pretreatment systems**

T.J. Campbell*, D.T. Vu and F. Teymouri, MBI International, Lansing, MI

3-31 **Polysaccharide decomposition in hemp woody core induced by electron beam irradiation**

S.J. Shin* and N.S. Cho, Chunbuk National University, Cheongju, South Korea

3-32 **Chemical composition of the sugarcane bagasse**

W. Carvalho*, L. Canilha, P.F. Castro and L.D.F.O. Barbosa, Escola de Engenharia de Lorena, Lorena, Brazil

3-33 **The effect of wet oxidation pretreatment method on rape straw fibres for bioethanol production**

E. Arvaniti* and A.B. Thomsen, Risoe National Laboratory for Sustainable Energy DTU, Roskilde, Denmark

3-34 **Withdrawn**

3-35 **Compositional changes in sugarcane bagasse on low temperature, long-term ammonia treatment**

M. Kim*, G.A. DeQueiroz and D.F. Day, LSU Agricultural Center, St. Gabriel, LA

3-36 **Bioconversion of cellulose wastes using cellulolytic biofilms**

V. Jirku*, J. Hrdinova, T. Krulikovska, J. Masak and A. Cejkova, Institute of Chemical Technology, Prague, Czech Republic

3-37 **Biological conversion of municipal solid waste to ethanol**

M.A. Ebrik*, J. Shi, B. Yang and C.E. Wyman, University of California, Riverside, Riverside, CA

3-38 Activity and function of ionic liquids for lignocellulose dissolution and hydrolysis

P. Wolski, Univeristy of California, Berkeley, CA; S. Padmanabhan* and J. Pruasnitz, Energy Biosciences Institute, University of California Berkeley, Berkeley, CA; D.S. Clark, University of California, Berkeley, Berkeley, CA; H. Blanch, University of California, Berkeley, CA

3-39 Saccharification of ionic liquid pretreated biomass with different enzyme mixes

I.P. Samayam*, A. Coxe, J. Wheeler and S. Varanasi, University of Toledo, Toledo, OH; A.P. Dadi and C.A. Schall, University of Toledo, Toledo, OH

3-40 Biotransformation of coffee pulp/husk by ligniculous fungi

M.P. Sierra-Gomez* and P. Ortiz-Bermúdez, University of Puerto Rico- Mayaguez, Mayaguez, PR

3-41 Bioethanol production from plasma assisted pretreatment of wheat straw fermenting C5 and C6 sugars

N. Schultz*, Z. Kádár, F. Leipold, A.E. Jensen, T. Fernqvist and A.B. Thomsen, Risø National Laboratory for Sustainable Energy, Technical University of Denmark – DTU, Roskilde, Denmark

3-42 Effect of solvent extraction on pretreatment process of *Pinus rigida*

N. Park*, B.W. Koo, H. Yeo and I.G. Choi, Seoul National University, Seoul, South Korea; H. Kim, HaidongEokom Co,.Ltd, Seoul, South Korea

3-43 Development of a continuous AFEX process

J. Glassbrook, C.D. Nielson, J.J. Videto, T.J. Campbell, D. Senyk and F. Teymouri*, MBI International, Lansing, MI

3-44 Identification and quantitation of water extractives in sorghum

R.S. Sevcik*, Z. Hardie, R. Mowery and C.K. Chambliss, Baylor University, Waco, TX

3-45 Conversion of switchgrass to sugars and ethanol using dilute ammonium hydroxide pretreatment

B.S. Dien*, P.J. O'Byran, R. Hector, L.B. Iten and M.A. Cotta, USDA-ARS, Peoria, IL

3-46 Profiling of oligosaccharides and related degradation products generated by AFEX pretreatment of lignocellulosic biomass using LC/TOF-MS

R. Vismeh* and A.D. Jones, Michigan State University, East Lansing, MI; S. Chundawat, J.F. Humpula and B. Dale, Michigan State University, Lansing, MI

3-47 Hydrothermal pretreatment of switchgrass: Effect of temperature and potassium carbonate on enzymatic reactivity

S. Kumar*, U.D. Kothari, Y.Y. Lee and R.B. Gupta, Auburn University, Auburn, AL

3-48 Novel approach to prediction of hydrolysate fermentability based on chemometric modeling of spectroscopic data

N.S. Fard*, D.H. Rabbe, K.W. Busch and C.K. Chambliss, Baylor University, Waco, TX

3-49 Rice straw oxidation using hypochlorite-hydrogen peroxide for bioconversion to ethanol

H.C. Choi, D. Kim and H.K. Kang, Chonnam National University, Gwangju, South Korea; N.M. Kim* and J.M. Kim, Korean Minjok Leadership Academy, Kangwon-do, South Korea; G. Kim, UCSD, La Jolla, CA; D.F. Day, LSU Agricultural Center, St. Gabriel, LA

3-50 Investigating residence time distribution and effects on performance in continuous biomass pretreatment reactor designs

D.A. Sievers*, R.T. Elander, E.M. Kuhn, N.J. Nagle, M.P. Tucker and N.D. Weiss, National Renewable Energy Laboratory, Golden, CO

3-51 Inhibition effects of dilute-acid prehydrolyzates on enzymatic hydrolysis and SSCF of Solka Floc

U.D. Kothari* and Y.Y. Lee, Auburn University, Auburn, AL

3-52 Efficacy of lime and ammonium hydroxide for conditioning dilute acid pretreated corn stover hydrolysates

A. Mohagheghi*, G. McMillen, N. Dowe and D.J. Schell, National Renewable Energy Laboratory, Golden, CO

3-53 Residual calcium in neutralized hydrolysates can destroy HPLC columns

L.R. Madsen II, LSU AgCenter, St. Gabriel, LA

3-54 Evaluation of lignocellulose dissolution in ionic liquids as a pretreatment strategy for ethanol and lignin production

S. Karatzos*, W. Doherty and L. Edye, Queensland University of Technology, Brisbane, Australia

3-55 Mechanism of delignification in the ionic liquid tetradecyl(trihexyl)phosphonium chloride

S. Keskar*, W.O.S. Doherty and L.A. Edye, Queensland University of Technology, Brisbane, Australia

3-56 Ethanol production from sweet sorghum by a dilute ammonia solution

G.A. DeQueiroz*, D. Salvi, D. Robert and V. Bazan, Louisiana State University, Saint Gabriel, LA

3-57 Rapid analysis methods to predict component concentrations (liquor and solid) in a pretreated slurry stream

R.O. Ruiz, National Renewable Energy Laboratory, Golden, CO

3-58 Production of bioethanol in pilot-plant scale using dilute-acid hydrolysis of spruce

O. Wallberg, E. Joelsson, C. Roslander and M. Galbe*, Lund University, Lund, Sweden

3-59 Understanding the impact of corn stover compositional variability on pretreatment performance

N.D. Weiss, J. Farmer* and D.J. Schell, National Renewable Energy Laboratory, Golden, CO

3-60 Comparison of kinetics of xylose and lignin removal during hot water and dilute-acid pretreatment of corn stover using a continuous flow-through reactor

Y. Ji* and M.P. Tucker, National Renewable Energy Laboratory, Golden, CO; S. Viamajala, Utah State University, Logan, UT; M. Selig and T.B. Vinzant, National Renewable Energy Lab, Golden, CO

3-61 Effect of moisture content on the ensilage of sugar beet pulp and tomato pomace

Y. Zheng, M. Yates*, Y.S. Cheng, C. Yu, D. Todd, R. Zhang, J. VanderGheynst and B. Jenkins, University of California, Davis, Davis, CA

3-62 Leaching of food industry residues to improve feedstock quality and resource recovery

C. Yu*, B. Jenkins, J. VanderGheynst, R. Zhang, Y. Zheng and Y.S. Cheng, University of California, Davis, Davis, CA

3-63 Sugar beet pulp storage via ensilage: Effects of sugar yield upon enzymatic hydrolysis

Y. Zheng*, M. Yates, D. Yang, Y.S. Cheng, C.W. Yu, D. Todd, J. VanderGheynst, R. Zhang and B. Jenkins, University of California, Davis, Davis, CA

3-64 Optimization of enzyme cocktail for alkaline pretreated switchgrass

V.R. Pallapolu* and Y.Y. Lee, Auburn University, Auburn, AL

3-65 Pretreatment of seaweeds for production of chemical intermediates

G.T. Jeong*, S.H. Park, J.H. Park and D.H. Park, Chonnam National University, Gwangju, South Korea

3-66 A comparison of lime and sodium hydroxide pretreatment for delignification and enzymatic hydrolysis of rice straw

Y.S. Cheng*, J. VanderGheynst, Y. Zheng, R. Zhang and B. Jenkins, University of California, Davis, Davis, CA

3-67 Biodrying as a pretreatment of horticultural wastes

E.M. Silva-Rodríguez, F. Robles-Martínez, J.S. Aranda-Barradas and E. Durán-Páramo, Unidad Profesional Interdisciplinaria de Biotecnología del I.P.N., México D.F., Mexico; T. Espinosa-Solares*, West Virginia State University, Institute, WV; R. Bailón-Morales, Instituto Politécnico Nacional, México D.F., Mexico

3-68 Simplex optimization and mathematical modeling of wheat straw dilute acid hydrolysis

T. Fernandes, L.C. Duarte, F. Carvalheiro and F. Gírio*, INETI, Lisboa, Portugal

3-69 Biofuels production with cattails from constructed wetlands

B. Zhang*, K. Suda and L. Wang, North Carolina A & T State University, Greensboro, NC; A. Shahbazi, North Carolina A&T State University, Greensboro, NC

3-70 Integration of particles of different sizes in a hydrothermal process for the pre-treatment of agro-industrial residues such as wheat straw

H.A. Ruiz Leza, D.P. Silva, D.S. Ruzene, A.A. Vicente* and J.A. Teixeira, University of Minho, Braga, Portugal; A.R. Gonçalves, University of São Paulo, Lorena, Brazil

3-71 2-D NMR investigation of the effect of ionic liquid pretreatment on the chemical composition and structure of switchgrass

O.P. Cetinkol*, D.C. Dibble and B.M. Holmes, Joint BioEnergy Institute, Emeryville, CA; B.A. Simmons, Joint BioEnergy Institute, Physical Biosciences Division, Lawrence Berkeley National Laboratory, Emeryville, CA

3-72 Comparison of the enzymatic hydrolysis of ionic-liquid pretreated energy crops

B.M. Holmes* and A. Lockhart, Joint BioEnergy Institute, Emeryville, CA; B.A. Simmons, Sandia National Laboratories, Livermore, CA

3-73 A catalyzed wet-explosion of wheat straw

T. Hilstrøm*, H.R. Sørensen and B.K. Ahring, BioGasol, Ballerup, Denmark

3-74 Ethanol production from cashew apple bagasse: Improvement of enzymatic hydrolysis by microwave-assisted alkali pretreatment

T.H.S. Rodrigues* and L.R.B. Gonçalves, Universidade Federal do Ceará, Fortaleza, Brazil; M.V.P. Rocha and G.R. Macedo, Universidade Federal do Rio Grande do Norte, Natal, Brazil

3-75 Measurement of total lignin mass balance closure after dilute sulfuric acid pretreatment from herbaceous feedstocks

R. Katahira*, D.W. Templeton, D.J. Schell and M.F. Davis, National Renewable Energy Laboratory, Golden, CO

3-76 Optimization of the SPORL pretreatment of corn stover for ethanol production

S. Abylgaziyev* and X. Pan, University of Wisconsin, Madison, WI; J.Y. Zhu, USDA Forest Service, Forest Products Laboratory, Madison, WI; G. Wang, Tianjin University of Science and Technology, TianJian, China

3-77 Non-natural reactions to convert cellulosic biomass to fuels and chemicals

Q. Jing*, S. Duncan, W. Wafa AlDajani, A. Katona, D. Yu, J. Tewalt, J. Schilling, U. Tschirner and R. Kazlauskasa, University of Minnesota, Saint Paul, MN

3-78 Xylan hydrolysis during hot-water pretreatment of corn stover

A. Mittal*, M.E. Himmel and D.K. Johnson, National Renewable Energy Laboratory, Golden, CO

3-79 Method development for assessing feedstock reactivity using an automated solvent extractor

N.D. Weiss*, C.J. Scarlata and N.J. Nagle, National Renewable Energy Laboratory, Golden, CO

3-80 Method development to determine the acid concentration of acid impregnated biomass

E.M. Kuhn*, N.J. Nagle, N.D. Weiss and R.T. Elander, National Renewable Energy Laboratory, Golden, CO

3-81 Microbial pretreatment on corn stover by the white rot fungus *Ceriporiopsis subvermispora* for enzymatic digestibility

C. Wan* and Y. LI, The Ohio State University, Wooster, OH

3-82 Withdrawn

3-83 Weak acid hydrolysis of sugarcane bagasse for ethanol production

R. Boopathy, Nicholls State University, Thibodaux, LA

3-84 LHW pretreatment and enzymatic hydrolysis of rapeseed straw

M.J. Díaz, C. Cara, I. Romero, E. Ruiz, E. Castro* and M. Moya, University of Jaen, Jaen, Spain

3-85 **Monitoring process streams towards understanding ionic liquid pretreatment of switchgrass and corn stover**

R. Arora*, D. Haider, C. Manisseri, C. Li, B. Knierim, M. Auer, H.V. Scheller, B.A. Simmons and S. Singh, Joint BioEnergy Institute, Lawrence Berkeley National Laboratory, Emeryville, CA; K.P. Vogel, USDA-ARS, Lincoln, NE

3-86 **Can endoxylanase application in wet storage preserve dry matter and reduce pretreatment severity?**

W.A. Smith*, D.N. Thompson and V.S. Thompson, Idaho National Laboratory, Idaho Falls, ID

3-87 **Kinetic modeling of glucose reversion reactions**

H.M. Pilath, M.E. Himmel, M.R. Nimlos* and D.K. Johnson, National Renewable Energy Laboratory, Golden, CO

3-88 **Xylan hydrolysis kinetics**

H.M. Pilath*, M.R. Nimlos, D.K. Johnson and M.E. Himmel, National Renewable Energy Laboratory, Golden, CO

3-89 **Pretreatment of *Gelidium amansii* for the production of bioethanol**

Y.S. Kim*, S.W. Chae, D.H. Park and C. Sunwoo, Chonnam National University, Gwangju, South Korea

3-90 **Pretreatment of *P. densiflora* and *Solidago altissima* L. to produce bioethanol**

K.H. Kim*, Y.S. Kim, S.W. Chae, D.H. Park and C. Sunwoo, Chonnam National University, Gwangju, South Korea

3-91 **A novel pre-treatment method for the lignocellulose-to-ethanol route**

N. von Weymarn, VTT Technical Research Centre of Finland, Espoo, Finland

3-92 **Improved one-step steam pretreatment of softwood with time-dependent temperature profile for bioethanol production**

S. Monavari*, M. Galbe and G. Zacchi, Lund University, Lund, Sweden

3-93 **Bioconverting the nutrients in dairy manure for L-lactic acid production by *Rhizopus oryzae***

W. Yao*, J. Zhu and C. Miller, University of Minnesota, St Paul, MN; B. Sun, NorthEast Agricultural University, Harbin, China

3-94 **Integrated high throughput pretreatment and enzymatic hydrolysis in 96 well plates**

M. Studer, J.D. DeMartini*, H.L. McKenzie and C.E. Wyman, University of California, Riverside, Riverside, CA

3-95 **Effects of mixing on the enzymatic hydrolysis and simultaneous saccharification and fermentation of pretreated spruce**

M. Wiman*, B. Palmkvist and G. Lidén, Lund University, Lund, Sweden

3-96 **Distiller's dried grains with solubles (DDGS): An alternative cellulose fermentation media for biofuels production**

R. Islam*, N. Cicek, R. Sparling and D. Levin, University of Manitoba, Winnipeg, MB, Canada

3-97 **Biological pretreatments of corn stover with filamentous fungi**

D. Tanjore*, T.L. Richard and M.N. Marshall, Pennsylvania State University, University Park, PA

3-98 **Selecting microbial production hosts for lignocellulosic feedstock utilization**

J.W. Van Groenestijn*, M.J. Van der Werf, P.J. Punt and K. Rumbold, TNO, Zeist, Netherlands

3-99 **Consolidated Bioprocessing (CBP) of AFEX-pretreated corn stover using *Thermoanaerobacterium saccharolyticum* ALK2: A study case using AFEX-pretreated biomass as the self-sustained nutrient source for fermentation**

M.W. Lau*, V. Balan and B. Dale, Michigan State University, Lansing, MI; X. Shao, Thayer School of Engineering at Dartmouth College, Hanover, NH; L.R. Lynd, Dartmouth College, Hanover, NH; T. Llyod, Mascoma Corporation, Lebanon, NH

3-100 **Fractionation of corn stover using aqueous ammonia and hot water**

C.G. Yoo and T.H. Kim*, Iowa State University, Ames, IA

3-101 **A comparison of batch tube and microwave reactors for water-only and dilute acid pretreatment of corn stover**

J. Shi*, University of California at Riverside, Riverside, CA; Y. Pu and A.J. Ragauskas, Georgia Institute of Technology, Atlanta, GA; B. Yang and C.E. Wyman, University of California, Riverside, Riverside, CA

3-102 **Sugar yields from switchgrass for dilute acid and sulfur dioxide pretreatment and subsequent enzymatic hydrolysis**

J. Shi, T. Redmond*, M. Ebrik, B. Yang and C.E. Wyman, University of California at Riverside, Riverside, CA

3-103 **Progress toward automating biomass compositional analysis**

C.J. Scarlata*, J. Sluiter and D. Crocker, National Renewable Energy Laboratory, Golden, CO

3-104 **The effect of water on sugar reactions from ab initio calculations**

X. Qian* and H. Dong, Colorado State University, Fort Collins, CO; M.R. Nimlos, M.E. Himmel and D.K. Johnson, National Renewable Energy Laboratory, Golden, CO

3-105 **Recovery of sugars from ionic-liquid biomass liquor by solvent extraction**

T. Brennan* and B. Holmes, Joint BioEnergy Institute, Emeryville, CA

3-106 **Structural studies of enzymatic hydrolysis of cellulose by neutron scattering and reflectivity**

M. Kent*, Joint BioEnergy Institute, Emeryville, CA; J. Murton and E. Carles, Sandia National Laboratory; R. Hjelm, Los Alamos National Laboratory; B. Akgun, NIST; B.A. Simmons, Sandia National Laboratories, Livermore, CA; J. Browning and J. Ankner, Oak Ridge National Laboratory

3-107 **SPORL pretreatment for cellulose ethanol production – An update**

X. Pan*, L. Shuai and Q. Yang, University of Wisconsin, Madison, WI; J.Y. Zhu, USDA Forest Service, Forest Products Laboratory, Madison, WI

3-108 **Withdrawn**

3-109 **Evaluation of ensiling on biomass storage and bioconversion of corn stover**

N.J. Nagle*, N.D. Weiss and E.M. Kuhn, National Renewable Energy Laboratory, Golden, CO; G.L. Gresham, L.L. Petzke and M. Delwiche, Idaho National Laboratory, Idaho Falls, ID

3-110 **Understanding the effects of reactor design on glucose and xylose recovery from pretreatment and enzymatic hydrolysis**

H.L. McKenzie*, J.D. DeMartini, M. Studer and C.E. Wyman, University of California, Riverside, Riverside, CA

3-111 **Investigation on effect and mechanism of different surfactant applied in pretreatment of wheat straw**

Z. Li*, T. Zhang, S.S. Liao, X. Yu, M. Garcia-Perez and S. Chen, Washington State University, Pullman, WA

3-112 **Pretreatment of lignocellulosic biomass using ionic liquids for production of ethanol**

Thehazhnan K. Ponnaiyan*, Anantharam P. Dadi, Constance A. Schall, Jared L. Anderson and Sasidhar Varanasi, Chemical & Environmental Engineering, University of Toledo, Toledo, OH

Translational Genomics for Bioenergy Feedstocks and Microbes

4-07 **Starch to oil: Engineering an efficient biofuel currency**

D.M. Hayden* and K. Dehesh, University of California at Davis, Davis, CA; A. Ekman and S. Stymne, Swedish University of Agricultural Sciences, Alnarp, Sweden

4-08 **Random Shear BAC Cloning: An optimized genomic tool for improving biomass and bioenergy feedstocks**

D. Mead*, R. Ye, S. Jasinovica, R. Godiska and C.C. Wu, Lucigen, Middleton, WI

Microbial Science and Technology 2

6-07 **Simultaneous saccharification and fermentation of switchgrass with *Kluyveromyces marxianus* IMB 3 in pH controlled bioreactor**

B. Faga* and M.R. Wilkins, Oklahoma State University, Stillwater, OK; I.M. Banat, University of Ulster, Coleraine, United Kingdom

6-08 **Simultaneous saccharification and fermentation of xylan with *Kluyveromyces marxianus* IMB2**

M. Mueller* and M.R. Wilkins, Oklahoma State University, Stillwater, OK; I.M. Banat, University of Ulster, Coleraine, United Kingdom

6-09 **Effect the particle size on MFC maximum power generation, power longevity and coulombic efficiency**

F. Rezaei*, T.L. Richard and B.E. Logan, Pennsylvania State University, University Park, PA

6-10 **Media requirements for aerobic cultivation of *Saccharomyces cerevisiae* TMB 3400-F30-3 on softwood hydrolysate**

D.B. Hodge*, Michigan State University, East Lansing, MI; C. Häggström, K. Berglund and U. Rova, Luleå University of Technology, Luleå, Sweden; T. Brandberg, SEKAB E-Technology, Örnsköldsvik, Sweden

6-11 **Characterization of extremophilic cellulose-degrading bacteria from the deep subsurface of the Homestake gold mine, Lead, South Dakota, USA**

G. Rastogi, G. Muppidi, A. Adhikari and S. Bang, South Dakota School of Mines, Rapid City, SD; R. Gurram, L. Christopher and R.K. Sani*, South Dakota School of Mines and Technology, Rapid City, SD; K.M. Bischoff, USDA-ARS, Peoria, IL; S.R. Hughes, USDA-ARS, Peoria, IL; W. Apel, Idaho National Laboratory, Idaho Falls, ID

6-12 **Microbes derived from marine invertebrates and algae as a source of cellulolytic, chitinolyitic and lipolytic enzymes**

P. McCarthy*, K. Lewis, S. Ross and D. Harmody, Harbor Branch Oceanographic Institute at Florida Atlantic University, Fort Pierce, FL

6-13 **Optimization of lactic acid production by pelleted-form of *Rhizopus oryzae* in 3 L airlift bioreactor using response surface methodology**

T. Maneeboon and V. Kitpreechavanich*, Department of Microbiology, Faculty of Science, Kasetsart University, Bangkok, Thailand; W. Vanichsriratana, Department of Biotechnology, Faculty of Agro-Industry, Kasetsart University, Bangkok, Thailand; C. Promchaitaward, National Metal and Materials Technology Center, Pathumthani, Thailand

6-14 **Isolation and cultivation of cell wall decomposing bacteria from plant biomass decaying communities**

S. Taghavi, L.L. Li and D. Van der Lelie*, Brookhaven National Laboratory, Upton, NY; S.Y. Ding and M.E. Himmel, National Renewable Energy Laboratory, Golden, CO

6-15 **Ethanol bioproduction from sugarcane bagasse hemicellulosic hydrolysate**

L. Canilha*, W. Carvalho, M.D.G.A. Felipe and J.B. Almeida e Silva, Escola de Engenharia de Lorena, Lorena, Brazil; M. Giulietti, Instituto de Pesquisas Tecnológicas, São Paulo, Brazil

6-16 **Microbial contamination of biodiesel**

M.D. Vangsness*, L.L. Bowen, L.M. Brown, S.S. Mueller and L.M. Balster, University of Dayton Research Institute, Dayton, OH

6-17 **Cellulose degradation: Two potential novel cellulolytic bacteria able to produce thermostable cellulases**

Y. Liang*, Z. Feng and J. Yesuf, Southern Illinois University, Carbondale, IL

6-18 **Screening soil metagenomic libraries searching for novel lignin and cellulose degrading enzymes**

N. Figueroa Matias, University of Puerto Rico – Mayaguez, Anasco, PR

6-19 **Characterization and modeling of ethanol production from gluconate**

W. Wu* and Z. Fan, University of California, Davis, Davis, CA

6-20 **Defined co-culture approach for biohydrogen production**

A.A. Zeidan*, P. Rådström and E.W.J. van Niel, Lund University, Lund, Sweden

6-21 **Production of cellulases and hemicelulases by *Penicillium viridicatum* RFC3 on solid state fermentation**

R. Travaini, D. Silva, L.R. Do-Amaral and R. Da-Silva*, UNESP, Sao Jose do Rio Preto, Brazil; R.S.R. Leite, IBILCE-CSJRP/UNESP, São José do Preto, Brazil; G.E. Gomes, São Paulo State University-UNESP/IBILCE, São José do Rio Preto, Brazil

6-22 **Optimization process using experimental design for biosurfactant production**

C. Ferraz, Á.A. Araújo* and R.R. Souza, Universidade Federal de Sergipe, São Cristóvão, Brazil

6-23 **Screening of microbial fuel ethanol producers uding xylose from hydrolyzate obtained from dilute acid pretreated cashew apple bagasse**

M.V.P. Rocha* and G.D.R. Macedo, Universidade Federal do Rio Grande do Norte, Natal, Brazil; A.L.T.D. Jesus, T.L.D. Albuquerque, V.M.M. Melo and L.R.B. Gonçalves, Universidade Federal do Ceará, Fortaleza, Brazil

6-24 **Influence of different substrates on the production of a mutant glucoamylase in submerged fermentation**

F.C. Pavezzi*, A.A.J. Carneiro, D.A. Bocchini, E. Gomes and R. Da Silva, São Paulo State University – UNESP/IBILCE - Biochemistry and Applied Microbiology Laboratory, São José do Preto, Brazil

6-25 **Physiological characterization of a novel member of the genus *Caldicellulosiruptor* and identification of extracellular cellulolytic enzymes using multi-dimensional LC-MS/MS**

S.D. Hamilton-Brehm, A. Lochner, R.J. Giannone, J.J. Mosher, R.L. Hettich, M. Keller and J.G. Elkins*, Oak Ridge National Laboratory, Oak Ridge, TN

6-26 **Inhibition of growth of *Zymomonas mobilis* by model compounds found in lignocellulosic hydrolysates**

M.A. Franden*, P.T. Pienkos and M. Zhang, NREL, Golden, CO

6-27 **Fractionation of conditioned corn stover hydrolysates and inhibition of ethanologen growth and fermentation**

M.A. Franden, H.M. Pilath, A. Mohagheghi, E. Jennings, P.T. Pienkos and M. Zhang*, NREL, Golden, CO

6-28 **Fermentation of syngas produced from gasification of switchgrass by P11**

K.D. Ramachandriya, K. Patil* and M.R. Wilkins, Oklahoma State University, Stillwater, OK

6-29 **Ethanol production from sugarcane bagasse by *Zymomonas mobilis* using simultaneous saccharification and fermentation (SSF) process**

D. Silveira,* A.C. Camelo, K. Pedro, L. Carlos and N. Pereira
Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

Biorefinery Deployment

7-07 **Withdrawn**

7-08 **Withdrawn**

7-09 **MixAlco process: A biorefinery built on the carboxylate platform**

M.T. Holtzapple* and C.B. Granda, Texas A&M University, College Station, TX

Biofuels Logistics and Sustainability

8-07 **Uniform-format feedstock supply system for commodity-scale biomass intermediates**

C.T. Wright*, J.R. Hess and K.L. Kenney, Idaho National Laboratory, Idaho Falls, ID

8-08 **The effect of wet storage on the value of corn stover as a biofuel feedstock**

I.D. Darku*, T. Richard and M. Marshall, Pennsylvania State University, University Park, PA; L. Wendt and A. Ray, Idaho National Laboratory, Idaho Falls, ID

8-09 **Potential cost saving strategies for storage of wet biomass feedstocks**

M. Delwiche*, A. Ray and W.A. Smith, Idaho National Laboratory, Idaho Falls, ID

8-10 **Potential for biofuel and animal feed production from native prairie grasslands**

R.J. Garlock*, B. Bals, V. Balan and B.E. Dale, Michigan State University, East Lansing, MI

8-11 **The variability of biofuel feedstock availability and delivered price using GIS and the IBSAL dynamic model**

J.D. Stephen*, S. Sokhansanj and X. Bi, University of British Columbia, Vancouver, BC, Canada

8-12 **Industrial sustainability of Canada's forest based ethanol industry: Feedstock perspective**

E.K. Ackom*, P.N. McFarlane and J.N. Saddler, University of British Columbia, Vancouver, BC, Canada; W.E. Mabee, Queen's University, Kingston, **Canada**

8-13 **System analysis of integrated biorefineries**

L. Luo*, G. Huppes and E. van der Voet, Center of Environmental Sciences (CML), Leiden University, Leiden, Netherlands

8-14 **Barriers and opportunities for international bioenergy trade – An inventory by IEA Bioenergy Task 40**

H.M. Junginger* and A. Faaij, Utrecht University, Utrecht, Netherlands; S. Zarrilli, UNCTAD, Geneva, Switzerland

8-15 **Fuel algae cultivation in municipal wastewater: Some strategic lessons from *Chlorella minutissima***

A. Bhatnagar, S. Chinnasamy and K.C. Das, The University of Georgia, Athens, GA; M. Bhatnagar*, Maharshi Dayanand Saraswati University, Ajmer, India

Monday Morning, May 4

Invited Speaker Breakfast-Monday speakers

SoMa, 3rd Floor

7:15 AM-8:00 AM

Continental Breakfast (all registrants)

Grand Ballroom Foyer, 3rd Floor

Sponsored by Advance Bio LLC

7:15 AM-8:00 AM

Registration/Editor's Desk

Grand Ballroom Foyer, 3rd Floor

7:15 AM-5:00 PM

Tabletop Exhibits

Grand Ballroom Foyer, 3rd Floor

7:30 AM-10:30 AM

Session 3: Biomass Pretreatment and Fractionation

Chairs: M.T. Holtzapple, Texas A&M University, College Station, TX and C.A. Schall, The University of Toledo, Toledo, OH

Grand Ballroom A-B, 3rd Floor

8:00 AM **3-01** **On size reduction for woody biomass conversion**

J.Y. Zhu*, R. Gleisner, USDA Forest Service, Forest Products Laboratory, Madison, WI; G. Wang, Tianjin University of Science and Technology, TianJian, China; W. Zhu, South China University of Science and Technology, Guangzhou, China; X. Pan, University of Wisconsin, Madison, WI

8:30 AM **3-02** **Glucose and xylose yields from switchgrass for ammonia fiber expansion, ammonia recycle percolation, dilute sulfuric acid, hot water, lime, and sulfur dioxide pretreatments followed by enzymatic hydrolysis**

C. Wyman*, Center for Environmental Research and Technology, Riverside, CA, B.E. Dale, Michigan State University, E. Lansing, MI, R.T. Elander, National Renewable Energy Laboratory, Golden, CO, M.T. Holtzapple, Texas A&M University, College Station, TX, M.R. Ladisch, Purdue University, West Lafayette, IN; Y.Y. Lee, Auburn University, Auburn, AL, C. Mitchinson, Genencor, A Danisco Division, Palo Alto, CA and S. Thomas, Ceres, Inc., Thousand Oaks, CA

9:00 AM **3-03** **Sub- and super-critical water technology for biofuels: Switchgrass to ethanol, biocrude and hydrogen fuels**

S. Kumar, A. Byrd and R.B. Gupta*, Auburn University, Auburn, AL

9:30 AM **Break**

Sponsored by DNA2.0

10:00 AM **3-04** **The technical advantages and challenges of ionic liquid-based biomass pretreatments**

L.A. Edye*, W.O.S. Doherty, S. Karatzos and S. Keskar, Queensland University of Technology, Brisbane, Australia

10:30 AM **3-05** **Ultra-structural and physicochemical modifications within ammonia pretreated lignocellulosic cell walls that influence enzyme accessibility**

S. Chundawat*, R. Vismeh, J.F. Humpula, R. Garlock, A.D. Jones, V. Balan and B. Dale, Michigan State University, Lansing, MI; B.S. Donohoe and M.E. Himmel, National Renewable Energy Lab, Golden, CO; T. Elder, USDA-Forest Service, LA; P. Askeland, Michigan State University, East Lansing, MI; U. Agarwal, USDA-Forest Products Laboratory, Madison, WI; L.N. Sharma and K. Chambliss, Baylor University, Waco, TX

11:00 AM **3-06** **A novel biochemical platform for fuels and chemicals production from cellulosic biomass**

Z. Fan*, X. Xiong, W. Wu and R. Zhang, T. Kasuga, University of California, Davis, Davis, CA

Session 4: Translational Genomics for Bioenergy Feedstocks and Microbes

Chairs: A. Berry, University of California, Davis and D. Stalker, RMIT University Bundoora, Australia

Grand Ballroom C, 3rd Floor

8:00 AM **4-01** **Genetic dissection of bioenergy traits in sorghum**

W. Vermerris* and A. Saballos, University of Florida, Gainesville, FL; S. Murray and W. Rooney, Texas A&M University, College Station, TX; S. Kresovich, Cornell University, Ithaca, NY; J.F. Pedersen and S. Sattler, USDA-ARS, Lincoln, NE; Z. Xin, USDA-ARS, Lubbock, TX

8:30 AM **4-02** ***Acidothermus cellulolyticus*: From genome sequence to plant cell wall deconstruction**

R.D. Barabote*, J.V. Parales, R.E. Parales and A.M. Berry, University of California, Davis, CA

9:00 AM **4-03** **Community structure and functional diversity of thermophilic cellulolytic microbial consortia**

J. Izquierdo*, E. Barrett, P. Reed, M. Sizova and L. Lynd, Dartmouth College, Hanover, NH

9:30 AM **Break**

Sponsored by DNA2.0

10:00 AM **4-04** **Discovery of genes that mediate and regulate hemicellulose biosynthesis**

K. Keegstra*, M. Pauly, C. Wilkerson, D. Cavalier, J.C. Cocuron, J. Jensen, N. Thrower and Y. Wang, Michigan State University, East Lansing, MI

10:30 AM **4-05** **Mining the metatranscriptome of the rumen microbiota for feedstock-targeted glycosyl hydrolases**

M. Hess*, T. Zhang, S. Green-Tringe and E. Rubin, DOE Joint Genome Institute, Walnut Creek, CA; R. Mackie, University of Illinois at Urbana-Champaign, Urbana, IL

11:00 AM **4-06** **Discovery of switchgrass genes through genomics to improve biomass composition and conversion**

N.R. Apuya*, M. Ahyow, A. Pierson, T. Kruse, B. Hames, S. Thomas and R. Pennell, Ceres, Inc., Thousand Oaks, CA

LUNCH ON YOUR OWN

Monday Afternoon, May 4

Session 5: Enzyme Science and Technology 1

Chairs: L. Christopher, South Dakota School of Mines and Technology, Rapid City, SD and J. Kreps, Verenium Corp., San Diego, CA

Grand Ballroom A-B, 3rd Floor

1:00 PM **5-01** **Thermostable fungal lignocellulosic biomass saccharification enzyme cocktail**

M. Kumar, DSM Innovation Inc., Parsippany, NJ

1:30 PM **5-02** **Impact of solids loading on the economics of a lignocellulosic biomass to ethanol conversion process**

D. Humbird*, A. Mohagheghi, N. Dowe and D.J. Schell, National Renewable Energy Laboratory, Golden, CO

2:00 PM **5-03** **Substrate-based limitations in the enzymatic hydrolysis of cellulose: Crystallinity, reactivity and adsorption**

M. Hall*, P. Bansal, J. Lee, M. Realff and A.S. Bommarius, Georgia Institute of Technology, Atlanta, GA

2:30 PM **Break**

Sponsored by Synthetic Genomics

3:00 PM **5-04** **Characterization of novel bacterial expansins that promote enzymatic hydrolysis of plant cell wall polymers**

K.H. Kim*, H.J. Lee, E.S. Kim, I.J. Kim and I.G. Choi, Korea University, Seoul, South Korea

3:30 PM **5-05** **Industrial level production of enzymes by *Trichoderma reesei* for cellulosic bioethanol**

P.L. Bergquist*, S. Miyauchi and K.M.H. Nevalainen, Macquarie University, Sydney, Australia; V.S.J. T'eo, Applimex Systems Pty Ltd, Sydney, Australia

4:00 PM **5-06** **Use of palm kernel press cake for production of bioethanol and feed**

H. Jørgensen*, C. Felby and A.R. Sanadi, University of Copenhagen, Frederiksberg, Denmark; N.E.K. Lange and S. Ernst, Novozymes A/S, Bagsværd, Denmark

Session 6: Microbial Science and Technology 2

Chairs: D. van der Lelie, Brookhaven National Laboratory, Upton, NY and K. J. Prather, Massachusetts Institute of Technology, Cambridge, MA

Grand Ballroom C, 3rd Floor

1:00 PM **6-01** **Metagenomics for mining new deconstructive enzymes, exploring enzyme diversity and screening cellulolytic activities**

L.L. Li*, S. McCorkle, D.C. Monteleone, S. Taghavi and D. Van der Lelie, Brookhaven National Laboratory, Upton, NY; S.G. Tringe and T. Woyke, Joint Genome Institute; S.Y. Ding, National Renewable Energy Laboratory, Golden, CO; M.E. Himmel, National Renewable Energy Lab, Golden, CO

1:30 PM **6-02** **Cellulolytic extreme thermophiles and hyperthermophiles from terrestrial hot springs**

J.E. Graham and F.T. Robb*, University of Maryland, Baltimore, MD; P. Jayachandran, H.W. Blanch and D.S. Clark, University of California, Berkeley, Berkeley, CA; M. Clark, Energy Biosciences Institute, Berkeley, CA

2:00 PM **6-03** **Mining *Clostridium thermocellum* for enzymatically active carbohydrases**

P.J. Brumm*, C5-6 Technologies, Middleton, WI; B. Hochstein, J. Boyum, N. Magallanes, D. Desai, N. Hermersmann, A. Bettermann and D. Mead, Lucigen Corporation, Middleton, WI; P.J. Weimer, USDA-ARS, Madison, WI

2:30 PM **Break**

Sponsored by Synthetic Genomics

3:00 PM **6-04** **Genome shuffling of *Penicillium decumbens* to improve its cellulase production**

Y. Cheng, X. Song, Y. Qin and Y. Qu*, Shandong University, Jinan, China

3:30 PM **6-05** **Development and characterization of xylose-fermenting strains of *Saccharomyces cerevisiae* based on structure-based engineering of key metabolic enzymes**

B. Nidetzky*, M. Klimacek, S. Krahulec and B. Petschacher, Graz University of Technology, Graz, Austria

4:00 PM **6-06** **Understanding the relationship of toxic compounds in corn stover hydrolysates and their inhibitory effects on ethanologen growth and fermentation**

M. Zhang*, M.A. Franden, P.T. Pienkos, H.M. Pilath, E. Jennings, A. Mohagheghi, Y.C. Chou, N. Nagle and R. Elander, NREL, Golden, CO; C.K. Chambliss, Baylor University, Waco, TX

Monday Evening, May 4

Tabletop Exhibits

Grand Ballroom Foyer , 3rd Floor

5:00 PM - 8:00 PM

Poster Session 2/Reception

Chairs: T. Vander Wall, NREL, Golden, CO, M. Santa-Maria and E. Tozzi, Univ. of California, Davis, CA

Sessions 5, 9-12

InterContinental Ballroom, 5th Floor

Pacific Terrace Foyer, 4th Floor

6:00 PM-9:00 PM

Enzyme Science and Technology

5-07 **Limiting factors of enzymatic hydrolysis of lignocellulosic biomass at high solids loadings**

J. Shi, M. Ebrik, B. Yang and C.E. Wyman, University of California at Riverside, Riverside, CA; J. Shen*, Shandong University, Jinan, China

5-08 **Cellulolytic enzyme production and response to pH and temperature by *Trichoderma reesei***

L. Lehmann* and T. Hobley, Technical University of Denmark, Kgs. Lyngby, Denmark; L. Olsson, Chalmers University of Technology, Göteborg, Sweden; S.M. Stocks and H.S. Jørgensen, Novozymes, Bagsvaerd, Denmark

5-09 **Microfluidic glycan assays for cellulosic biomass**

R. Bharadwaj*, Joint BioEnergy Institute, Lawrence Berkeley National Laboratory, Emeryville, CA; K. Chen, S. Datta and B.M. Holmes, Joint BioEnergy Institute, Emeryville, CA; R. Sapra, Sandia National Laboratories, Livermore, CA; A. Singh, Joint Bioenergy Institute, Emeryville, CA

5-10 **Discovering novel protein families for biomass deconstruction: A domain-based functional classification**

H. Roh, K.H. Kim and I.G. Choi*, Korea University, Seoul, South Korea

5-11 **Effect of the carbon source consumption rate on cellulase production by *Trichoderma reesei* in fed-batch cultures**

F. Ben Chaabane*, C. Cohen, S. Prigent, B. Chaussepied, A. Margeot and F. Monot, IFP, Rueil-Malmaison, France

5-12 **Beta-glucosidase and its effect on lignocellulosic biomass hydrolysis**

M. Fujdala*, R. Caldwell, R. Chin, B. Kelemen and E. Larenas, Genencor a Division of Danisco, Palo Alto, CA; P. Ntarima and K. Piens, Ghent University, Gent, Belgium

5-13 **Improving cellulase compositions for lignocellulose hydrolysis**

E. Vlasenko, Novozymes, Inc., Davis, CA

5-14 **Cellulase production, enzymatic hydrolysis and ethanol production on steam-pretreated spruce using *Trichoderma atroviride* mutants**

K. Kovacs* and G. Zacchi, Lund University, Lund, Sweden; G. Szakacs, Budapest University of Technology and Economics, Budapest, Hungary

5-15 **Analysis of the white rot degradome by mass spectrometry identifies small molecules correlated with lignin degradation**

N.H. Pawley*, M. Teshima, C.J. Unkefer, P.A. Langan and P.J. Unkefer, Los Alamos National Laboratory, Los Alamos, NM; K.E. Hammel, USDA Forest Products Laboratory, Madison, WI

5-16 **Cloning of cellulase and regulation factor genes in *Penicillium decumbens* and their expression profile analysis in glucose-repressed and cellulose-induced culture conditions**

K. Zheng, X. Wei, G. Liu, Y. Qin, X. Sun, M. Chen and Y. Qu*, Shandong University, Jinan, China

5-17 **The potential of agro-industrial residues for production of holocellulases from filamentous fungi**

F.G. de Siqueira, E.G. de Siqueira and E.X.F. Filho*, University of Brasília, Brasília, Brazil; L.R. Batista, Federal University of Lavras, Lavras, Brazil

5-18 ***Pichia pastoris* as host for the expression of lignocellulolytic enzymes: Expression of *Trichoderma reesei* cellobiohydrolase II (Cel6A) as a model case**

K. Flicker* and A. Glieder, Graz University of Technology and Research Centre Applied Biocatalysis, Graz, Austria; A. Mellitzer, Graz University of Technology, Graz, Austria; R. Weis, VTU Technology GmbH, Grambach, Austria

5-19 **Multi-mode spectroscopic High Throughput Screening (HTS) of phenols and monolignols**

K.E. Achyuthan*, Joint BioEnergy Institute (JBEI), Emeryville, CA; B.A. Simmons, Sandia National Laboratories, Livermore, CA; P.D. Adams and A. Singh, Joint Bioenergy Institute, Emeryville, CA

5-20 **The effect of xylooligomers on enzymatic hydrolysis of cellulose and pretreated corn stover**

Q. Qing*, B. Yang and C. Wyman, Center for Environmental Research and Technology, Riverside, CA

5-21 **Fundamentals of enzymatic hydrolysis of cellulose through a restart approach**

J. Shi, B. Yang* and C.E. Wyman, University of California, Riverside, Riverside, CA

5-22 **Aspartic protease from *Trichoderma reesei*: Crystal structure and statistical coupling analysis**

I. Polikarpov*, A.S. Nascimento and S. Krauchenco, Instituto de Fisica de São Carlos (IFSC), Universidade de São Paulo (USP), São Carlos, SP, Brazil; A. Golubev, Petersburg Nuclear Physics Institute, St. Petersburg, Russia; A. Gustchina and A. Wlodawer, National Cancer Institute, Frederick, MD

5-23 **Directed evolution of a thermophilic beta-glucosidase for cellulosic bioethanol production**

P.L. Bergquist* and E.M. Hardiman, Macquarie University, Sydney, Australia; M.D. Gibbs and R.A. Reeves, Applimex Systems Pty Ltd, North Ryde, Australia

5-24 **High throughput mass spectrometry based enzymatic assays for biofuels development**

T. Northern* and C. Petzold, Joint BioEnergy Institute, Emeryville, CA; J.C. Lee, National Health Sciences Institute, Taipei, Taiwan; B.A. Simmons, Sandia National Laboratories, Livermore, CA; P.D. Adams and A. Singh, Joint Bioenergy Institute, Emeryville, CA

5-25 **Second generation bioethanol from sugarcane bagasse: SSF operative conditions and flowsheeting implementation**

S. Macrelli* and G. Zacchi, Lund University, Lund, Sweden

5-26 **Enhancement of cellulase production from kraft paper mill sludge by *Trichoderma Reesei* Rut C-30**

W. Wang*, L. Kang and Y.Y. Lee, Auburn University, Auburn, AL

5-27 **Production of biodiesel via enzymatic ethanolysis of sunflower and soybean oils: Modeling**

F.L.P. Pessoa, P.W. Falcão* and S.P.D. Magalhães, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

5-28 **A cellulase related dehydrogenase of *Hypocrea jecorina* (*Trichoderma reesei*) specifically responsive to soluble inducing compounds**

A. Schuster*, C.P. Kubicek and M. Schmoll, Vienna University of Technology, Vienna, Austria

5-29 **Mechanistic study of enzymatic cellulolysis inhibitions**

A. Tejirian*, Novozymes, Davis, CA and F. Xu, Novozymes North America, Franklinton, NC

5-30 **The effect of substrate properties on enzyme adsorption during hydrolysis of ethanol organosolv pretreated hardwoods and softwoods: Potential for enzyme recycling**

A.A. Roos*, R. Chandra, L. Del Rio, A. Pribowo, S. Ghatora and J. Saddler, University of British Columbia, Vancouver, BC, Canada; X. Pan, University of Wisconsin, Madison, WI

5-31 **JBEI Computational Biology Core**

D. Chivian*, J. Bates and A. Arkin, Joint BioEnergy Institute, Emeryville, CA; P. Dehal, M. Joachimiak, K. Keller, M. Price and J. Baumohl, Virtual Institute for Microbial Stress and Survival, Berkeley, CA

5-32 **Cellulase enzymatic complex production in solid state fermentation using tropical Amazon agro industrial wastes**

A.A. De Araujo*, UFS, São Cristovão, SE, Brazil; C. Amarante and L. Lourenço, UFPA, Belém; R. Bergamasco, Universidade Estadual de Maringá, Maringá, Brazil

5-33 **Immobilization and stabilization of xylanase by multipoint covalent attachment on glyoxyl agarose support**

A. Manrich, P.W. Tardioli, W.S. Adriano and R.L.C. Giordano*, Universidade Federal de São Carlos, São Carlos, SP, Brazil

5-34 **Effect of ammonia pretreatment on switchgrass for production of cellulase using Trichoderma reesei Rut C-30**

A. Jain* and D.T.H. Walker, Clemson University, Clemson, SC

5-35 **Conversion of *Thermobifida fusca* free exoglucanases into cellulosomal components: Comparative impact on cellulose-degrading activity**

J. Caspi*, Genencor, a Danisco division, Palo Alto, CA; D.C. Irwin, Y. Li and D. Wilson, Cornell University, Ithaca, NY; R. Lamed, Tel Aviv University, Tel Aviv, Israel; H.P. Fierobe, CNRS, Marseille, France; E.A. Bayer, Weizmann Institute of Science, Rehovot, Israel

5-36 **Biodiesel synthesis from babassu oil catalysed by immobilized lipases on poly-(hydroxybutyrate)**

A.A. Mendes, R.C. Giordano* and R.L.C. Giordano, Universidade Federal de São Carlos, São Carlos, SP, Brazil; H.F.D. Castro, University of São Paulo, Lorena - SP, Brazil

5-37 **Isolation and biochemical characterization of novel esterases for transesterification of hemicellulose and production of value-added fiber**

L. Wang* and E. Master, University of Toronto, Toronto, ON, Canada

5-38 **Biochemical characterization and performance testing of family 48 exocellulases**

R. Brunecky*, L.E. Taylor, V.V. Lunin, M. Alahuhta, J.O. Baker, Q. Xu, M.E. Himmel and W.S. Adney, National Renewable Energy Laboratory, Golden, CO; D. Wilson, Cornell University, Ithaca, NY

5-39 **Characterization of cellulases and hemicellulases produced by *Thermoascus aurantiacus* in hydrolysis experiments**

J.R. Monte and M. Brienzo, Engineering School of Lorena, Lorena, Brazil; A. Milagres*, Escola de Engenharia de Lorena EEL/USP, Lorena, Brazil

5-40 **A study of cellulases production by filamentous fungi strains using experimental design**

M.C.T. Damaso and S. Couri*, Embrapa Agroindústria de Alimentos, Rio de Janeiro, Brazil; A.C.P.D. Oliveira, Universidade Estadual do Norte Fluminense, Campos dos Goytacazes, Brazil; A.D.F. Ferreira, Universidade Federal Rural do Rio de Janeiro, Seropédica, Brazil

5-41 **Enzyme characterization for hydrolysis of lignocellulosic biomass and their bonversion to ethanol**

S. Le*, M. Li and C. Mitchinson, Genencor, A Danisco Division, Palo Alto, CA

5-42 **Pipeline for novel biomass degrading enzymes**

D. Mead, B. Hochstein, J. Boyum, N. Magallanes, C. Brumm, D. Desai, S. Vande Zande, A. Bettermann, E. Steinmetz, R. Godiska, K. Gowda and P. Brumm*, Lucigen, Middleton, WI

5-43 **Coarse-grained modeling of cellulose 1β identifies processive movement of family 1 carbohydrate-binding modules on a broken-chain surface**

L. Bu*, G.T. Beckham, M.F. Crowley, C.H. Chang, J.F. Matthews, Y.J. Bomble, W.S. Adney, M.E. Himmel and M.R. Nimlos, National Renewable Energy Laboratory, Golden, CO

5-44 **Comparison of the hydrolytic activity of different cellulase to develop simultaneous saccharification and fermentation process of pretreated rice straw**

D.C. Hsu*, G.L. Guo, S.H. Chou and W.S. Hwang, Institute of Nuclear Energy Research, Taoyuan, Taiwan

5-45 **Investigating the expression, secretion and enzymatic activities of the cellulolytic machinery of the filamentous ascomycete fungus, *Neurospora crassa***

J. Sun*, C. Tian, W. Wang, N.L. Glass and M.A. Marletta, University of California, Berkeley, CA; W. Beeson and J. Cate, University of California, Berkeley

5-46 **Cellulase enzymes production from rice straw pretreated without sulfuric acid by *Acremonium cellulolyticus***

A. Hideno*, H. Inoue, K. Tsukahara, X. Fang, T. Endo and S. Sawayama, National Institute of Advanced Industrial Science and Technology, Kure, Japan

5-47 **The potential of ethanol production from the organic fraction of Municipal Solid Wastes**

M. Ballesteros*, I. Ballesteros, M.J. Negro, J.M. Oliva, F. Saez and P. Manzanares, CIEMAT, Madrid, Spain

5-48 **Determination of product inhibition of CBH1, CBH2 and EG1 using a novel cellulase activity assay**

F. Du*, E. Wolger, T. Kaper and B. Kelemen, Genencor, A Danisco Division, Palo Alto, CA

5-49 **The improved cellulosome: Computational modeling to minisomes**

Y.J. Bomble*, M.F. Crowley, Q. Xu, M.R. Nimlos, S.Y. Ding and M.E. Himmel, National Renewable Energy Laboratory, Golden, CO; J. Xu, M. Saharay and J.C. Smith, Oak Ridge National Laboratory, Oakridge, TN; H. Guo, University of Tennessee, Knoxville, TN; J.W. Brady and D. Wilson, Cornell University, Ithaca, NY

5-50 **The use of calorimetry to monitor inhibition during biomass hydrolysis**

C. Bohlin* and P. Westh, Roskilde University, Roskilde, Denmark; K. Borch, Novozymes, Bagsvaerd, Denmark

5-51 **Thermochemical screening of cellulolytic enzymes for second generation bioethanol production**

L. Murphy* and P. Westh, Roskilde University, Roskilde, Denmark; K. Borch, Novozymes, Bagsvaerd, Denmark

5-52 **Enzymatic hydrolysis of lignocellulosic biomass: modeling and simulation of CSTR's in series**

A. Gonzàlez Quiroga*, A.C.D. Costa and R. Maciel Filho, State University of Campinas, Campinas, Brazil

5-53 **Understanding dynamics of cellulase adsorption on AFEX treated corn stover during the course of enzymatic hydrolysis**

D. Gao*, S. Chundawat, C. Krishnan, V. Balan and B. Dale, Michigan State University, Lansing, MI

5-54 **Induction and repression of ß-xylanase by different strains of *Thermomyces lanuginosus***

K. Khucharoenphaisan* and V. Kitpreechavanich, Kasetsart University, Bangkok, Thailand; K. Ratanakhanokchai, King Mongkut's University of Technology Thonburi, Bangkok, Thailand; S. Tokuyama, Shizuoka University, Shizuoka, Japan

5-55 **Optimization of extracellular catalase production from *Aspergillus phoenicis* K30 by Plackett-Burman design and linear regression using date flour as single carbon source and purification of the enzyme**

N. Kacem Chaouche, Sr.*, Z. Merahi and L. Dehimat, Département de Biochimie – Microbiologie, Faculté des Sciences de la Nature et de la Vie, Université Mentouri de Constantine, Constantine, Algeria; J. Destain and Ph. Thonart, Centre Wallon de Biologie Industrielle, Faculté Universitaire des Sciences Agronomiques, Gembloux, Belgium; A. Zaatri, Laboratoire des Applications de la Technologie Avancée, Université Mentouri de Constantine, Constantine, Algeria; T. Haddoum and J.P.Wathelet, Unité de Chimie Générale et Organique, Faculté Universitaire des Sciences Agronomiques, Gembloux, Belgium

5-56 **Dose response modelling of enzymatic biomass hydrolysis**

J. Frickmann, Novozymes North America, Inc, Franklinton, NC

5-57 **Hemicellulolytic enzymes from the maize endophyte *Acremonium zeae***

K.M. Bischoff*, D.B. Jordan and J.O. Rich, USDA-ARS-NCAUR, Peoria, IL; S.T. de Rezende, Universidade Federal de Vicosa, Minas Gerais, Brazil

5-58 ***Saccharophagus degradans* 2-40 utilizes a novel group of processive endoglucanases to degrade cellulose**

B.J. Watson*, H. Zhang, Y.H. Moon, A.G. Longmire and S.W. Hutcheson, University of Maryland, College Park, MD

5-59 **Kinetics and synergy of *Trichoderma reesei* cellulases on ionic liquid pretreated Avicel and *Miscanthus giganticus***

J. Fox, H.A. Chokhawala, C. Dana, D. Nadler, H.W. Blanch and D.S. Clark*, University of California, Berkeley, Berkeley, CA; P. Wolski, Univeristy of California, Berkeley, CA

5-60 **Cost driver influences on a biomass to ethanol process model**

B. Emme*, J. Mogensen, M. Hershkowitz and P. Iyer, Novozymes North America, Inc., Franklinton, NC

5-61 **Characterization of the specific activities and hydrolytic properties of the cell-wall degrading enzymes produced by *Trichoderma reesei* RUT C30 on different carbon sources**

B. Sipos*, Z. Benkő and K. Réczey, Budapest University of Technology and Economics, Budapest, Hungary; L. Viikari, University of Helsinki, Helsinki, Finland; M. Siika-aho, VTT Technical Research Centre of Finland, Espoo, Finland

5-62 **Packet bed reactor running on babassu oil and glycerol to produce monoglycerides by enzymatic route using immobilized *Burkholderia cepacia* lipase**

L. de Freitas, H.F. de Castro and G.M. Zanin*, University of São Paulo , Lorena São Paulo, Brazil

5-63 **Withdrawn**

5-64 Evaluation of oxalate decarboxylase and oxalate oxidase for industrial applications

P. Cassland,(1) A. Sjode,(2), S. Winestrand,*(2), L.J. Jonsson(3) and N.-O. Nilvebrant2) (1) Novozymes, Bagsvaerd, Denmark; (2) Karlstad Unversity, Karlstad, Sweden; (3) Umea University, Umea, Sweden

5-65 The effects of high solids conditions on specific enzymatic activities involved in the saccharification of lignocellulosic biomass

M.J. Selig*, C.M. Roche, M. Resch, R. Brunecky, M. Zucarello and S.R. Decker, National Renewable Energy Laboratory, Golden, CO

5-66 Effects of analysis error on the results of enzymatic hydrolysis of acid pretreated bagasse

C. Liu* and C. Mitchinson, Genencor, A Danisco Division, Palo Alto, CA

5-67 Production and characterization of recombinant cell wall-active enzymes from *Aspergillus nidulans* for use in plant biomass utilization

P. Vasu, A. Ratti and B.J. Savary*, Arkansas State University, State University, AR; A. Mort, Oklahoma State University, Stillwater, OK

5-68 Withdrawn

5-69 Changes in *Pleurotus ostreatus* laccase isoenzyme pattern in cocultivation with *Trichoderma viride*

M.A. Contreras-Ordónez*, L. Serrano and E. Galindo, Instituto Biotecnologia UNAM, Mexico City, Mexico

5-70 Analysis of cellulase hyper-producing mutants derived from the fungus *Trichoderma reesei* QM9414

T. Fujii*, K. Murakami and S. Sawayama, National Institute of Advanced Industrial Science and Technology, Kure, Japan

5-71 A thermodynamic study of the carbohydrate binding modules (CBMs) from *Trichoderma reesei* cellobiohydrolase I and II

G.T. Beckham*, W.S. Adney, J.F. Matthews, L. Bu, M.F. Crowley, M.E. Himmel and M.R. Nimlos, National Renewable Energy Laboratory, Golden, CO

5-72 Enzymatic conversion of butyric acid to butyl butyrate in a packed bed reactor

J.H. An, Korea Institute of Science and Technology, Y.H. Kim, Kwangwoon University, Seoul, South Korea and B.I. Sang*, Korea Institute of Science and Technology, Seoul, South Korea

5-73 Raffinose and lactose induce α-galactosidase and β-galactosidase activity from *Lactobacillus reuteri*

A. Alazzeh* and S. Ibrahim, North Carolina A&T State University, Greensboro, NC; A. Shahbazi, North Carolina A&T State University, Greensboro, NC; A. AbuGhazaleh, Southern Illinois University Carbondale, Carbondale, IL

5-74 Insights into the structural basis for the thermostability of a glycosyl hydrolase family 12 endoglucanase from *Acidothermus cellulolyticus*

V.V. Lunin*, G.T. Beckham, W.S. Adney, Q. Xu, L.E. Taylor, E.P. Knoshaug, S.Y. Ding and M.E. Himmel, National Renewable Energy Laboratory, Golden, CO

5-75 Detection of a dihidro-dihydroxy-naphthalendiol dehydrogenase in a strain of *Mucor circinelloides* isolated from petroleum-contaminated soil

R.L. Camacho, A. Durón-Castellanos, K. García-Belmonte and R. Zazueta-Sandoval*, University of Guanajuato, Guanajuato, Mexico

5-76 Desorption of CBH1 from BMCC substrate is a function of enzymatic activity

Z. Ye* and R.E. Berson, University of Louisville, Louisville, KY; A. Lane, JG Brown Cancer Center and University of Louisville, Louisville, KY

5-77 Screening and production study of xylanase producer microorganisms from the Brazilian Cerrado

H.F. Alves-Prado*, FE-CIS/UNESP, Ilha Solteira, Brazil; F.C. Pavezzi, R.S.R. Leite and R. DaSilva, IBILCE-CSJRP/UNESP, São José do Preto, Brazil

5-78 Development of continuous process on biodiesel production by immobilizeed and co-immobilized lipases

J.H. Lee*, S.B. Kim and S.W. Kim*, Korea University, Seoul, South Korea; C. Park, Kwangwoon University, Seoul, South Korea

5-79 Adsorption of cellulases on cellulolytic enzyme lignin from Lodgepole pine

M. Tu*, Auburn University, Auburn, AL and J. Saddler, University of British Columbia, Vancouver, BC, Canada

5-80 Porcine pancreatic lipase purification on poly(ethylene glycol)-potassium phosphate aqueous two-phase system

R.L. Souza, University Tiradentes, Aracaju, Brazil; G.M. Zanin, Universidade Estadual de Maringa, Maringa, Brazil; M.W.N. Lobão, Universidade Tiradentes, Aracaju, Brazil; C.M.F. Soares and A.S. Lima*, Universidade Tiradentes, Aracaju, Brazil

5-81 Production and characterization of a thermostable cellulase from *Geobacillus* sp

C.M. Lo, G. Muppidi, R.K. Sani and L.P. Christopher*, South Dakota School of Mines and Technology, Rapid City, SD

5-82 Biochemical characterization of bacterial and fungal hemicellulases and heterologous expression *in planta*

Y.L.A. Tsai*, T. Canam, M. Campbell and E. Master, University of Toronto, Toronto, ON, Canada

5-83 Nitrogen source optimization for cellulase production by *Penicillium funiculosum* using experimental planning

R.N. Maeda, M.M.P.D. Silva and N. Pereira Jr*, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

5-84 Development of a high solids enzymatic saccharification method for lignocellulosic biomass

C.M. Roche*, G.A. McMillen and J.J. Stickel, National Renewable Energy Laboratory, Golden, CO

5-85 Over-expression and purification of *Trichoderma reesei* glycosyl hydrolases in Pichia and in *T. reesei*

J.S. Scott-Craig*, G. Banerjee, M.S. Borrusch, S. Nagendran and J.D. Walton, Michigan State University, East Lansing, MI

5-86 **Production of hemicellulolytic enzymes by a thermophilic *Aspergillus* strain isolated from sugarcane bagasse**

M.M.S. Moretti, D.A. Bocchini*, R. Da Silva, E. Gomes and L. Sette, São Paulo State University - UNESP/ IBILCE - Biochemistry and Applied Microbiology Laboratory, Sao Jose do Rio Preto, Brazil

5-87 **Production of cellulolytic enzymes by fungi *Acrophialophora nainiana* and *Ceratocystis paradoxa* using different carbon sources**

R.R.O. Barros*, R.A. Oliveira, L.M.F. Gottschalk and E.P.S. Bon, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

5-88 **Directed evolution of hyperthemophilic endoglucanase, Cel5A, from *Thermotoga maritma* MSB8**

Z. Chen*, J.I. Park, S. Datta, H.M. Tran, H. Liu, D. Tullman-Ercek, R. Bharadwaj, K.E. Achyuthan, B. Holmes, S. Chhabra and M. Hadi, Joint BioEnergy Institute, Emeryville, CA; A. Singh, Joint Bioenergy Institute, Emeryville, CA; B.A. Simmons and R. Sapra, Sandia National Laboratories, Livermore, CA

5-89 **Towards the development of cellulases compatible with ionic liquid pretreatment for saccharification of cellulosic biomass**

S. Datta*, B.M. Holmes, Z. Chen, D.C. Dibble and M. Hadi, Joint BioEnergy Institute, Emeryville, CA; H.W. Blanch, University of California, Berkeley, Berkeley, CA; B.A. Simmons and R. Sapra, Sandia National Laboratories, Livermore, CA

5-90 **Effect of cellulose-binding module choice on catalytic activity of cellulases**

D. Tullman-Ercek*, S. Datta, D.C. Dibble, M. Hadi, M. Kent and S. Singh, Joint BioEnergy Institute, Emeryville, CA; B.A. Simmons and R. Sapra, Sandia National Laboratories, Livermore, CA

5-91 **Probing the function of N-terminal Ig domain in the crystal structure of endoglucanase Cel9A from the thermoacidophilic *Alicyclobacillus acidocaldarius* using computational modeling**

H. Liu* and J.H. Pereira, Joint BioEnergy Institute, Emeryville, CA; K. Sale, Sandia National Laboratories; P.D. Adams, Joint Bioenergy Institute, Emeryville, CA; B.A. Simmons and R. Sapra, Sandia National Laboratories, Livermore, CA

5-92 **Enzyme engineering of glycoside hydrolase-5 endoglucanases enzymes for consolidated bioprocessing**

J.I. Park*, M. Hadi and S. Chhabra, Joint BioEnergy Institute, Emeryville, CA; B.A. Simmons and R. Sapra, Sandia National Laboratories, Livermore, CA

5-93 **Monitoring the rheological properties of pretreated biomass in high-consistency enzymatic liquefaction**

N. Szijártó* and L. Viikari, University of Helsinki, Helsinki, Finland; M. Siika-aho, Technical Research Centre of Finland, Espoo, Finland

5-94 **Agarases for red algae biomass deconstruction and saccharification**

S. Lee*, S. Kim, H.T. Kim, K.H. Kim and I.G. Choi, Korea University, Seoul, South Korea

5-95 **Synergistic enhancement of enzymatic hydrolysis of sugar cane bagasse by *Trichoderma* and *Aspergillus* cellulases and xilanases enzyme pools**

L.M.F. Gottschalk*, R.A. Oliveira, R.R.O. Barros, H.S. Reis and E.P.S. Bon, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

5-96 **Comparative production and characterization of cellulolitic enzymes from thermophilic fungi *Thermoascus aurantiacus* CBMAI756 and *Thermomyces lanuginosus***

L.R. Do-Amaral, R. Travaini, T.O.P. Pinto and R. Da-Siliva*, UNESP, Sao Jose do Rio Preto, Brazil; G.E. Gomes, São Paulo State University-UNESP/IBILCE, São José do Rio Preto, Brazil

5-98 **Induction of α-and β-galactosidases in *Lactobacillus reuteri* by different metal ions**

A. Alazzeh, S. Ibrahim*, D. Song and A. Shahbazi, North Carolina A&T State University, Greensboro, NC; A. AbuGhazaleh, Southern Illinois University Carbondale, Carbondale, IL

5-99 **Optimization of lipase extraction conditions obtained by solid-state fermentation**

J.N. Silva*, Instituto de Química, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil; M.L.E. Gutarra and D.M.G. Freire, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil

5-100 **Effect of high-temperature enzymatic pretreatment on saccharification of acid-pretreated corn stover**

H. Ding* and E. Vlasenko, Novozymes, Davis, CA

5-101 **Biochemical characterization of cellobiohydrolases from different GH families**

R. Benyamino* and H. Ding, Novozymes, Davis, CA

5-102 **Production by solid-state fermentation and structural modeling of a lipase from *Aspergillus parasiticus***

L.C. Cortás and D.M.G. Freire, Instituto de Química, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil; V.F. Soares, B.C.P. Santos, M.L.E. Gutarra* and R.V. Almeida, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil

5-103 **Synthetic genes + design of experiment = BioEngineering**

B. Leavy-Young, DNA2.0, Menlo Park, CA

5-104 **Biochemical comparison of a gycosyl hydrolase family 7 library**

L.E. Taylor*, T.A. Vander Wall, J. Baker, M.E. Himmel and W.S. Adney, National Renewable Energy Laboratory, Golden, CO

5-105 **Probing the effects of glycosylation on the flexibility of the *Trichoderma reesei* cellobiohydrolase I linker peptide with fluorescence resonance energy transfer and molecular simulation**

J.M. Yarbrough*, G.T. Beckham, J.F. Matthews, W.S. Adney, S.Y. Ding and M.E. Himmel, National Renewable Energy Laboratory, Golden, CO

Bioprocessing and Separations Technology

9-07 **Withdrawn**

9-08 **Membrane based extraction of acetic acid and other compounds from dilute acid pretreated lignocellulosic hydrolysates**

D.L. Grzenia*, and R. Wickramasinghe, Colorado State University, Fort Collins, CO; D.J. Schell, National Renewable Energy Laboratory, Golden, CO

9-09 **Microbial fuel cells for removal of fermentation inhibitors from biorefinery recycle water**

A.P. Borole*, J.R. Mielenz, C. Hamilton and T.A. Vishnivetskaya, Oak Ridge National Laboratory, Oak Ridge, TN

9-10 **Biodiesel from canola oil using a 1:1 mole mixture of methanol and ethanol**

H. Joshi* and T. Walker, Clemson University, Clemson, SC; B.R. Moser, United States Department of Agriculture, Agricultural Research Service, Peoria, IL

9-11 **Application of ceramic membranes to recover of high value hemicellulose from alkaline peroxide pretreated wheat straw**

H. Lin*, R. Gustafson, R. Bura and S. Ewanick, University of Washington, Seattle, WA

9-12 **Simple process for production of ethanol from soybean hulls while maintaining protein value**

J.R. Mielenz*, Oak Ridge National Laboratory, Oak Ridge, TN, J.S. Bardsley, Dartmouth College, Hanover, NH and C. Wyman, University of California, Riverside, Riverside, CA

9-13 **Non-sterile fermentation of bioethanol**

J. Larsen and L. Thirup*, Inbicon, Fredericia, Denmark

9-14 **Techno-economic evaluation of an integrated biological hydrogen and biogas (BioHythane) production process**

M. Ljunggren* and G. Zacchi, Lund University, Lund, Sweden

9-15 **Oil accumulation from waste via heterotrophic/mixotrophic *Chlorella vulgaris* and *Chlorella protothecoides***

T. Heredia*, University of Puerto Rico, Mayagüez, PR and B. Hu, University of Puerto Rico Mayaguez, Mayaguez, PR

9-16 **Optimisation of lipase production by *Citrobacter freundii***

A. Innani, University of Newcastle, Newcastle upon Tyne, United Kingdom

9-17 **A simultaneous isomerization and fermentation (SIF) process for efficient co-fermentation of hexose and pentose sugars**

D. Yuan*, S. Varanasi and P. Relue, University of Toledo, Toledo, OH

9-18 **Investigating the changing rheology of high-solids biomass slurries during enzymatic saccharification**

J.S. Knutsen* and M.W. Liberatore, Colorado School of Mines, Golden, CO; J.J. Stickel, C.J. Dibble and C.M. Roche, National Renewable Energy Laboratory, Golden, CO

9-19 **Simulation and optimization of an extractive fermentation process for bioethanol production**

R.R.D. Andrade*, E.C. Rivera, R. Maciel Filho, F. Maugeri and A.C. da Costa, University of Campinas-UNICAMP, Campinas, Brazil; D.I.P. Atala, Center of Sugarcane Technology (CTC), Piracicaba, Brazil

9-20 **A criterion for selecting renewable energy processes**

E. Searcy* and P.C. Flynn, University of Alberta, Edmonton, AB, Canada

9-21 **Integration of fermentation and crystallization for fumaric acid production**

C.A. Roa Engel*, A.J.J. Straathof, W.M. van Gulik and L.A.M. van der Wielen, Delft University of Technology, Delft, Netherlands

9-22 **High silica zeolites as an alternative to amine based adsorbents in succinic acid recovery**

C. Efe*, A.J.J. Straathof and L.A.M. van der Wielen, Delft University of Technology, Delft, Netherlands

9-23 **Withdrawn**

9-24 **Reaction kinetics and selective product removal during high-solids enzymatic saccharification**

B.T. Smith*, J.S. Knutsen and R.H. Davis, University of Colorado at Boulder, Boulder, CO

9-25 **A new low capital process to convert municipal solid waste fiber into ethanol**

B. Levie* and J. Gao, Catchlight Energy LLC, Federal Way, WA; J. Mayovsky and K. Chundakkadu, Weyerhaeuser, Federal Way, WA

9-26 **Compositional analysis for the 21st century: High throughput methods to evaluate lignocellulosic biomass for bioethanol production**

S. Ewanick*, R. Bura, R. Gustafson and B. Marquardt, University of Washington, Seattle, WA

9-27 **Separation of algae cells from the solution using cationic polymers combined with ferric chloride**

Q. Kong*, M. Min, L. Li, M. Fuad, Y. Li, B. Martinez, P. Chen and R. Ruan, University of Minnesota, St. Paul, MN

9-28 **Biodiesel production from integration between reaction and separation system: Reactive distillation process**

N. De Lima da Silva*, State University of Campinas, Campinas, Brazil, M.R. Wolf Maciel, State University of Campinas (UNICAMP), Campinas-SP, Brazil, C.M.G. Santander, State University of Campinas, São Paulo, BC, Brazil, R. Maciel Filho, State University of Campinas, SAO Paulo, Brazil and C.B. Batistella, State University of Campinas - UNICAMP, Campinas, Brazil

9-29 **Biodiesel production from castor oils: Optimization of alkaline ethanolysis and scale up**

N. De Lima da Silva, State University of Campinas, Campinas, Brazil, C.B. Batistella, State University of Campinas - UNICAMP, Campinas, Brazil, M.R. Wolf Maciel*, State University of Campinas (UNICAMP), Campinas-SP, Brazil and R. Maciel Filho, State University of Campinas, SAO Paulo, Brazil

9-30 **Ethanol production from sorghum grain (*Sorghum bicolor* [L.] Moench): Optimization of the enzymatic hydrolysis and evaluation of the hydrolysate fermentability**

C.A. Barcelos*, R.N. Maeda, G.J. Vargas Betancur, D.S. Andrade and N. Pereira, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

9-31 **An intelligent system for multivariate on-line monitoring of a continuous flash fermentation process**

E.C. Rivera*, D. Ibraim Pires Atala, A. Carvalho da Costa and R. Maciel Filho, University of Campinas-UNICAMP, Campinas, Brazil

9-32 **Understanding mass transfer in lignocellulosic ethanol using whole cell biocatalyst**

K.S. Khare*, S. Ryu and M.N. Karim, Texas Tech University, Lubbock, TX

9-33 **Influence of nutrients supplementation on ethanol batch production rates**

A.M.D. Santos, J.N.D. Vasconcelos* and R.M.R.G. Almeida, Federal University of Alagoas, Maceio, Brazil

9-34 **One step cellulosic ethanol production: Modeling of enzymatic hydrolysis of cellulose by whole-cell biocatalyst**

S. Ryu* and M.N. Karim, Texas Tech University, Lubbock, TX

9-35 **Bioethanol production from germinated grain by inherent enzymes**

Z. Kádár*, A.D. Christensen, M.H. Thomsen and A.B. Thomsen, Risø National Laboratory for Sustainable Energy, Technical University of Denmark – DTU, Roskilde, Denmark

9-36 **The $K_L a$ influence on ethanol production by *Pichia stipitis***

J.P.A. Silva and I.C. Roberto, Engineering College of Lorena, University of São Paulo, Lorena, Brazil; S.I. Mussatto* and J.A. Teixeira, University of Minho, Braga, Portugal

9-37 **Ethanol production by fermentation using immobilized cells of *Saccharomyces cerevisae* in cashew apple bagasse**

A. Pacheco, D.R. Gondim and L.R.B. Goncalves*, Universidade Federal do Ceará, Fortaleza, Brazil

9-38 **Modeling temperature variations in a pilot plant thermophilic anaerobic digester**

S. Valle-Guadarrama and F.R. Ramírez-Arpide, Universidad Autónoma Chapingo, Chapingo, Edo de México, Mexico; T. Espinosa-Solares* and M. Domaschko, West Virginia State University, Institute, WV; I.L. López-Cruz, Universidad Autónoma Chapingo, Chapingo, Mexico; J. Bombardiere, Consultant for Enviro Control Ltd., Monmouth, United Kingdom

9-39 **Light regime characterization in an airlift photobioreactor for production of microalgae with high starch content**

B.D. Fernandes*, G.M. Dragone, J.A. Teixeira and A.A. Vicente, Universidade do Minho, Braga, Portugal

9-40 **Production of lactic acid from sucrose: Strain selection, fermentation and kinetic modeling**

B.H. Lunelli*, R.R.D. Andrade, M.R. Wolf-Maciel and R. Maciel Filho, State University of Campinas, Campinas - SP, Brazil; D.I.P. Atala, Center of Sugarcane Technology (CTC), Piracicaba, Brazil

9-41 **Synthesis of biodiesel from non-edible oil and solid catalyst**

J.H. Park*, G.T. Jeong, S.H. Park and D.H. Park, Chonnam National University, Gwangju, South Korea

9-42 **Production of acetone-butanol-ethanol (ABE) by direct fermentation of cassava using *Clostridium saccharoperbutylacetonicum* N1-4**

V.H. Thang*, G. Kobayashi and K. Kanda, Saga University, Saga, Japan

9-43 **Immobilization of nitrifier for nitrogen removal**

S.H. Park*, J.H. Park, G.T. Jeong and D.H. Park, Chonnam National University, Gwangju, South Korea; S.H. Bhang and E.T. Lim, Taerim Industry Co., Ltd, Jeonnam, South Korea

9-44 **Statistical optimization of 1,3-propanediol fermentation using the engineered strain of *Klebsiella pneumoniae***

B.R. Oh* and D.H. Park, Chonnam National University, Gwangju, South Korea; M.Y. Seo, J.W. Seo and C.H. Kim, KRIBB, Jeonbuk, South Korea

9-45 **Characterization of α-amylases for the removal filter cake on petroleum wall**

N. Kyaw, R.F. Mesquita, E. Kameda and M.A.Z. Coelho, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil; M.A.P. Langone*, Rio de Janeiro State University, Rio de Janeiro, Brazil

9-46 **Microalgae production of lipids and starches for bio-fuel production**

B.B. Elmore* and J. Liu, Mississippi State University, Mississippi State, MS

9-47 **Mixed culture acidogenic fermentation and acid extraction from pre-pulping wood extract**

R. Baddam, G.P. van Walsum*, A. Abdulrahman, A. van Heiningen and B.H. Um, University of Maine, Orono, ME

9-48 **Evaluation of ammonium hydroxide for conditioning dilute acid pretreated corn stover**

E.W. Jennings*, N.D. Farmer and D.J. Schell, National Renewable Energy Laboratory, Golden, CO

9-49 **Improvements to the analysis for triglycerides, diglycerides and monoglycerides by liquid chromatography by using 2.2-µm C18 columns with alternative solvent systems**

M.L. Tracy, X. Liu and L. Lopez*, Dionex Corp., Sunnyvale, CA

9-50 **Development of jatropha oil extraction from biodiesel feedstocks using accelerated solvent extraction**

L. Lopez*, Dionex Corporation, Sunnyvale, CA; P. Thepsithar, Y. Zhang, Z. Zhang and R. Yan, Institute of Environmental Science and Engineering, Singapore, Singapore

9-51 **Possible co-products from sweetgum (*Liquidambar styraciflua* L.)**

C. Lau, J. Duke, E.M. Martin*, D.J. Carrier and E. Clausen, University of Arkansas, Fayetteville, Fayetteville, AR

9-52 **Micellar enhanced detoxification and extractive fermentation in surfactant systems for biofuel production**

B. Wang* and H. Feng, Energy Biosciences Institute, University of Illinois at Urbana-Champaign, Urbana, IL

9-53 **Improvement of high-solid slurries mixing for enzymatic hydrolysis of pretreated rice straw**

S.H. Chou*, H.C. Huang, D.C. Hsu and G.L. Guo, Institute of Nuclear Energy Research, Taoyuan, Taiwan

9-54 **Continuous saccharification and fermentation of dilute acid pretreated corn stover and Avicel**

S. Brethauer*, M.H. Studer and C.E. Wyman, University of California, Riverside, Riverside, CA

9-55 **Quantifying livestock feed value of AFEX-Treated DDGS and subsequent biorefinery byproducts**

K. Rosentrater*, USDA, Brookings, SD, F. Teymouri, MBI International, Lansing, MI and K. Kalscheur, South Dakota State University, Brookings, SD

9-56 **Chitooligomers production by *Metarhizium anisopliae***

C.F. Assis*, N.K. Araújo, G.R. Macedo and E.S. Santos, Universidade Federal do Rio Grande do Norte, Natal, Brazil

9-57 **Medium composition effect on the expression of lack antigen of *Leishmania chagasi***

M.R. Vaz*, S.S. Andrade, D.R. Martins, E.S. Santos and G.R. Macedo, Universidade Federal do Rio Grande do Norte, Natal, Brazil

9-58 **Continuous bioethanol production in immobilized cells fermentor coupled with a pervaporation system**

I. De Bari*, D. Cuna and F. Liuzzi, ENEA, Rotondella (MT), Italy; V. Stillo and V. Calabrò, Università della Calabria, Arcavacata di Rende (CS), Italy

9-59 **Performances of *Lactobacillus brevis* for producing lactic acid from hydrolysate of lignocellulosics**

W. Guo, Y. Li and W. Jia, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China; S. Chen*, Washington State University, Pullman, WA

9-60 **High-throughput techniques for microalgal biofuels feedstock analysis: Rapid fatty acid fingerprinting using gas chromatography**

E. Wolfrum*, L. Laurens and D. Crocker, National Renewable Energy Laboratory, Golden, CO

9-61 **Determination of monosaccharides in acid hydrolyzed biocrop feedstock using HPAE-PAD**

V.P. Hanko, L.L. Lopez* and J.S. Rohrer, Dionex Corporation, Sunnyvale, CA

9-62 **Ethanol from xylose using an immobilized enzyme recirculation reactor**

C.C. Beatty*, S.J. Potochnik and C. Khavari, Trillium FiberFuels, Inc, Corvallis, OR; V.T. Remcho, Oregon State University, Corvallis, OR

9-63 **Profiling 32 low molecular mass organic acids in biomass by ion chromatography mass spectrometry**

L. Wang* and W.C. Schnute, Dionex Corporation, Sunnyvale, CA

9-64 **Analysis of carbohydrates in microalgal biomass samples with HPAEC-MS**

T. Zheng*, R. Slingsby, S. Rao and L. Lopez, Dionex Corporation, Sunnyvale, CA

9-65 **Ethanol production from food wastes**

Y.S. Hong*, S.J. Park, J.H. Kim and H.H. Yoon, Kyungwon University, Sungnam, South Korea; J.W. Lee, Sogang University, Seoul, South Korea

9-66 **Batch equilibrium and kinetic studies – Biosorption of uranium by *Sargassum filipendula* biomass**

J.I.R. Silva, IRD/CNEN, Rio de Janeiro, Brazil, A.C.D.M. Ferreira, IRD/CNEN, Rio de Janeiro and A.C.A. da Costa*, Universidade do Estado do Rio de Janeiro - Instituto de Quimica, Rio de Janeiro, Brazil

9-67 **Foaming tannin from a cellulose-tannin solution**

R.D. Tanner* and J. Mikhail, Vanderbilt University, Nashville, TN

9-68 **Production of fuel ethanol from softwood at high dry matter content**

K. Hoyer*, M. Galbe and G. Zacchi, Lund University, Lund, Sweden

9-69 **Evaluation of sweet sorghum for ethanol production**

C.H. Chung*, M. Kim and D. Day, LSU Agricultural Center, St. Gabriel, LA; K.J. Han, LSU Agricultural Center, Franklinton, LA

9-70 **Effect of higher culture temperature on the metabolism of *Escherichia coli***

C.M.M. Hasan, Kyushu Istitute of Technology (KIT), Japan, Iizuka, Fakuoka, Japan

9-71 **Extraction and identification of julibroside saponins from the bark of *Albizia julibrissin***

A.S. Engelberth*, D.J. Carrier and E.C. Clausen, University of Arkansas, Fayetteville, AR

9-72 **Optimization of biodiesel production by fungus cells immobilized in fibrous supports**

J.P. Chen* and G.H. Lin, Chang Gung University, Taoyuan, Taiwan

9-73 **Stabilization and delivery of *Chlorella vulgaris* in water-in-oil emulsions**

H. Scher*, H. Guo, Y.S. Cheng and J. VanderGheynst, University of California, Davis, Davis, CA

9-74 **Eco-ethanol production from lignocellulosics with hot-compressed water treatment followed by acetic acid fermentation and hydrogenolysis**

S. Saka*, N. Phaiboonsilpa, Y. Nakamura, S. Masuda, X. Lu, K. Yamauchi, H. Miyafuji and H. Kawamoto, Graduate School of Energy Science, Kyoto University, Kyoto, Japan

Emerging Biofuels and Chemicals

11-07 Biohydrogen production converted from lignocellulose: a novel source and approach from wood-feeding termites

J. Sun* and Y. Cao, Mississippi State University, Poplarville, MS; J. Rodriguez, Mississippi state University, Starkville, MS

11-08 High production of 1,3-propanediol from crude glycerol using suspended and immobilized *Klebsiella pneumoniae*

S.A. Jun, C. Moon, B.I. Sang and Y. Um, Korea Institute of Science and Technology, Seoul, South Korea; C.H. Kang*, INWOO corporation, Seoul, South Korea

11-09 Production of acetone-butanol from wheat straw hemicellulose hydrolyzates

R. Marchal, B. Clément, M. Ropars and F. Monot*, IFP, Rueil-Malmaison, France

11-10 Characteristics of biodemulsifier produced by one strain of *Alcaligenes* sp. S-XJ-1 and its application in emulsion destabilization

X.F. Huang*, J. Liu and L.J. Lu, Tongji University, Shanghai, China

11-11 Application of high-throughput methods to chemical transformations of renewable feedstocks

J.C. Yoder, Symyx Technologies, Sunnyvale, CA

11-12 Benefits of real-time analytical monitoring of fermentation processes during development, scale-up and production

L. McDermott, M. Koslin, N. Wright* and M. Ranc, Hamilton Sundstrand - Applied Instrument Technologies, Pomona, CA

11-13 Withdrawn

11-14 Production and toxicity of γ-decalactone and 4-hydroxydecanoic acid from *R. aurantiaca*

M. Alchihab*, J. Destain, M. Aguedo, M. Masson, J.P. Wathelet and P. Thonart, Gembloux Agricultural University, Gembloux, Belgium

11-15 Development of value added products from hydrolyzed Lignin

S. Mani*, University of Georgia, Athens, GA, V. Balan, Michigan State University, East Lansing, MI and B.E. Dale, Michigan State University, E. Lansing, MI

11-16 Growth media optimization for polyhydroxyalkanoate and hydrogen coproduction from *Rhodospirillum rubrum* cultured on synthesis gas

D.C. Chipman*, D.W. Choi and R.C. Brown, Iowa State University, Ames, IA

11-17 Improvement of coenzyme Q_{10} production by *ispB* knockout and dxs overexpression in recombinant *Escherichia coli* expressing *Agrobacterium tumefaciens* decaprenyl diphosphate synthase

J.H. Choi*, S.R. Han, Y.J. Lee, Y.C. Park and J.H. Seo, Seoul National University, Seoul, South Korea; D.H. Kweon, School of Biotechnology and Bioengineering, Sungkyunkwan University, Suwon, South Korea; Y.W. Ryu, Ajou university, Suwon, South Korea

11-18 Effects of overexpression of endogenous *ALD6*, *ACS1* and *Kluyveromyces lactis* GPD1 on xylitol production in recombinant *Saccharomyces cerevisiae*

E.J. Oh*, W.C. Park, J.W. Kim, Y.C. Park and J.H. Seo, Seoul National University, Seoul, South Korea; Y.W. Ryu, Ajou university, Suwon, South Korea

11-19 Biodiesel and H_2 production from CO_2 by sequential use of microorganisms in bioreactors

S.A. Markov*, D. Danielson, N. Patel, T. Bisquera, M. Murphy, R. Willingham and L. Holliday, Austin Peay State University, Clarksville, TN

11-20 Effect of biodiesel-derived raw glycerol on 1,3-propanediol production by different microorganisms

C. Moon, B.I. Sang and Y. Um*, Korea Institute of Science and Technology, Seoul, South Korea; S.W. Kim, Korea University, Seoul, South Korea

11-21 The use of alternative pathways in 1-butanol production from *Escherichia coli*

N.A. Crowhurst* and D.J. Leak, Imperial College London, London, United Kingdom

11-22 Building a future in renewable industrial chemicals

P. Smith, Archer Daniels Midland Research, Decatur, IL

11-23 Thermodynamic models for solid-liquid equilibrium of xylose in water and water-ethanol mixtures

E.A. Martinez*, M. Giulietti, M.M. Uematsu and S. Derenzo, Institute of Technological Research, São Paulo, Brazil; J.B. Almeida e Silva, Engineering College of Lorena-University of São Paulo, Lorena, Brazil

11-24 Engineering of *Saccharomyces cerevisiae* metabolism for high-level and energy-independent production of cytosolic acetyl-CoA

C. Weber*, J. Duvnjak and E. Boles, Goethe-University Frankfurt, Frankfurt, Germany; G. Festel, Butalco GmbH, Huenenberg, Switzerland

11-25 Hydrogen production from COSLIF-treated cellulosic feedstocks

S. Harvey* and A. E., USA Army RDECOM, ECBC, Aberdeen Proving Ground, MD; P. Zhang, Virginia Tech University, Blacksburg, VA

11-26 Approach for pentitol production from acid-pretreated rice straw hydrolysate by an adapted *Pichia stipitis*

C.F. Huang*, W.H. Chen, W.H. Chen and J.B. Wang, Institute of Nuclear Energy Research, Taoyuan, Taiwan

11-27 Withdrawn

11-28 Influence of nutrients on C_{50}-carotenoids production by *Haloferax mediterranei* ATCC 33500

N.W. Su*, C.J. Fang and M.H. Lee, National Taiwan University, Taipei, Taiwan

11-29 Reactor perspectives in indoor cultivation of phototrophic algae at large scale

R.K. Bajpai*, B. Benson and M.E. Zappi, University of Louisiana at Lafayette, Lafayette, LA

11-30 Analysis of hydrocarbons produced by marine microalga, *Scenedesmus* sp. JPCC GA0024

M. Matsumoto*, Technology Development Center, Wakamatsu Research Institute, Kitakyusyu, Japan; Y. Maeda, T. Tanaka and T. Matsunaga, Tokyo University of Agriculture and Technology, Koganei, Japan

11-31 Maximizing algal growth in batch reactors through sequential change in light intensity

S. Wahal*, S. Viamajala and B. Barney, Utah State University, Logan, UT

11-32 Evaluation of biofuel potential through wastewater treatment using algae

E.W. Griffiths*, S. Viamajala, R. Thompson, J. Jones and R. Sims, Utah State University, Logan, UT; I. Hamud, Logan City, Logan, UT

Biomass Recalcitrance

12-07 Understanding natural paradigms for plant cell wall deconstruction: Community dynamics and structure in decaying poplar wood pile

H. Wei*, J. Baker, M. Tucker and S.Y. Ding, National Renewable Energy Laboratory, Golden, CO

12-08 Identify molecular structural features of biomass recalcitrance Using advanced imaging techniques

S.Y. Ding, National Renewable Energy Laboratory, Golden, CO

12-09 Chemical imaging of lignin in plant cell walls using CARS microscopy

Y. Zeng*, Y.S. Liu, M.E. Himmel and S.Y. Ding, National Renewable Energy Laboratory, Golden, CO; X.S. Xie, Harvard University, Cambridge, MA; F. Chen and R.A. Dixon, Samuel Roberts Noble Foundation, Ardmore, OK

12-10 Probing structural and chemical properties of cellulose with multi-scale theoretical methods

G. Gnanakaran*, T. Shen and P. Langan, Los Alamos National Labs, Los Alamos, NM; A. French and G. Johnson, Southern Regional Research Center, USDA, New Orleans, LA

12-11 Biomass compositional analysis method errors

D.W. Templeton*, C.J. Scarlata, J. Sluiter, E.S. Fisk, C. Payne and E. Wolfrum, National Renewable Energy Laboratory, Golden, CO

12-12 Rapid conversion analysis of switchgrass feedstocks using NIR spectroscopy

T. Kruse*, A. Alexiades, G. East, B.R. Hames and S.R. Thomas, Ceres, Inc., Thousand Oaks, CA

12-13 Molecular mechanics simulations of cellulose microfibrils

J.F. Matthews*, M.E. Himmel and M.F. Crowley, National Renewable Energy Laboratory, Golden, CO; J.W. Brady, Cornell University, Ithaca, NY

12-14 Changes in cellulose molecular weight during biomass pretreatment

S. Park*, R. Katahira, S. Black, M.E. Himmel and D.K. Johnson, National Renewable Energy Laboratory, Golden, CO

12-15 Microscopic evaluation of plant cell wall structure of ensiled corn stover by correlative microscopy

B. Donohoe*, T. Haas, N. Weiss, N. Nick, S.Y. Ding and M. Himmel, National Renewable Energy Laboratory, Golden, CO

12-16 How pretreatment can overcome the natural recalcitrance of biomass to cellulase hydrolysis

D.K. Johnson*, W.S. Adney, R. Brunecky, S.Y. Ding, B.S. Donohoe, T.B. Vinzant and M.E. Himmel, National Renewable Energy Laboratory, Golden, CO

12-17 Compositional analysis of lignin in bioenergy crops and de-polymerization via pretreatment

C. Manisseri*, C. Li, A.M. Smith, R. Arora, P. Benke, R. Bharadwaj, H.V. Scheller, B.A. Simmons and S. Singh, Joint BioEnergy Institute, Physical Biosciences Division, Lawrence Berkeley National Laboratory, Emeryville, CA; F. Zendejas, Sandia National Laboratories, Livermore, CA; K.P. Vogel, USDA-ARS, Lincoln, NE

12-18 A comparative study of dilute acid and ionic liquid pretreatment of biomass and model lignocellulosics

C. Li*, R. Arora, C. Manisseri, B.A. Simmons and S. Singh, Joint BioEnergy Institute, Physical Biosciences Division, Lawrence Berkeley National Laboratory, Emeryville, CA; K.P. Vogel, USDA-ARS, Lincoln, NE

12-19 Molecular dynamics simulations of cellulose-solvent interactions

M.S. Skaf*, State University of Campinas - UNICAMP, Campinas, Brazil and O. El-Seoud, University of Sao Paulo, Sao Paulo, Brazil

12-20 Application of biological pretreatment to improve thermal and chemical processes releasing sugars and bioproducts from wheat straw

D. Singh*, X. Yu and S. Chen, Center for Bioproducts and Bioenergy, Pullman, WA

12-21 Structural changes in lignin and cellulose resulting from the two-step dilute acid pretreatment of Loblolly pine

P. Sannigrahi* and A.J. Ragauskas, Georgia Institute of Technology, Atlanta, GA; S.J. Miller, Chevron Energy Technology Company, Richmond, CA

12-22 Enzymes loading optimization in the hydrolysis of sugarcane bagasse – A comparison between bagasse pretreatment with lime and alkaline hydrogen peroxide

S.C. Rabelo*, L.L.G. Fuentes, D.R. Garcia, R. Maciel Filho and A.C. Costa, State University of Campinas, Campinas, Brazil

12-23 Alkaline hydrogen peroxide pretreatment of sugarcane bagasse for enzymatic hydrolysis: The influence of temperature and pretreatment time on delignification and sugars yield

D.R. Garcia*, S.C. Rabelo, L.L.G. Fuentes, R. Maciel Filho and A.C. Costa, State University of Campinas, Campinas, Brazil

12-24 Optimization and kinetics of lime pretreatment of sugarcane bagasse to enhance enzymatic hydrolysis

L.L.G. Fuentes*, D.R. Garcia, S.C. Rabelo, R. Maciel Filho and A.C. Costa, State University of Campinas, Campinas, Brazil

12-25 Parallel plate processing for high throughput pretreatment and enzymatic saccharification of lignocellulosic materials

M.J. Selig, M.P. Tucker, R. Brunecky, M.E. Himmel and S.R. Decker*, National Renewable Energy Laboratory, Golden, CO

12-26 **Structural analysis of steam pretreated spruce after enzymatic hydrolysis**

A. Várnai*, M. Peura, R. Serimaa and L. Viikari, University of Helsinki, Helsinki, Finland

12-27 **Synergism of a bacterial expansin, BsEXLX1 with the catalytic doamin endo-β-1,4-glucanase in cellulose hydrolysis**

E.S. Kim*, I.J. Kim, H.J. Lee, I.G. Choi and K.H. Kim, Korea University, Seoul, South Korea

12-28 **Functional analysis of a bacterial expansin from *Hahella chejunsis* for promoting hydrolysis of xylan**

H.J. Lee*, I.G. Choi and K.H. Kim, Korea University, Seoul, South Korea

12-29 **Using carbohydrate-binding module as molecular probe to map biomass polysaccharides**

Y. Luo*, Q. Xu, Y.S. Liu, Y. Zeng, M.E. Himmel and S.Y. Ding, National Renewable Energy Laboratory, Golden, CO

12-30 **Rates and yields of cellulosic ethanol from maize silage with effect of Brown Midrib mutations**

Y. Kim, M.R. Ladisch and N. Mosier*, Purdue University, West Lafayette, IN; D.W. Lickfeldt and K. Armstrong, Dow AgroSciences, Indianapolis, IN

Tuesday Morning, May 5

Invited Speaker Breakfast-Tuesday speakers

SoMa, 3rd Floor

7:15 AM-8:00 AM

Continental Breakfast (all registrants)

Grand Ballroom Foyer, 3rd Floor

7:15 AM-8:00 AM

Tabletop Exhibits

Grand Ballroom Foyer, 3rd Floor

7:30 AM-10:30 AM

Session 7: Biorefinery Deployment

Chairs: A. Bratis, NREL and M. Klembara, US Department of Energy, Washington, DC

Grand Ballroom A-B, 3rd Floor

8:00 AM **7-01** **Commercialization of Second Generation Biofuels: An Independent Engineer's View**

Doug Dudgeon, Harris Group, Seattle, Washington

8:30 AM **7-02** **Successful commercialization of second generation**

J. Huttner, DuPont Danisco Cellulosic Ethanol, Itasca, IL

9:00 AM **7-03** **Development and deployment of consolidated bioprocessing for production of ethanol**

M. Ladisch*, J. Flatt, A. Belcher, J. van Rooyen, M.S. Sivasubramanian and D. Dimasi, Mascoma Corporation, Boston, MA; L.R. Lynd, Dartmouth College, Hanover, NH; C. Wyman, Univ. of California, Riverside, CA; D.A. Hogsett, Mascoma Corporation, Lebanon, NH; J. Draeger, Frontier Renewable Resources, LLC; Y. Kim, E.A. Ximenes and N. Mosier, Purdue University, West Lafayette, IN

9:30 AM **Break**

Sponsored by DSM

10:00 AM **7-04** **Demonstration plant scale production of lignocellulosic ethanol**

K. Gray, Verenium Corp, San Diego, CA

10:30 AM **7-05** **Ethanol from wheat straw – A reality in Denmark from November 2009**

M. Persson, Inbicon A/S, Fredericia,

11:00 AM **7-06** **Third generation biofuels from lignocellulosic biomass materials**

B.K. Ahring*, WSU, Richland, WA, P. Westermann, AAU-Ballerup, Ballerup, Denmark; M.J. Mikkelsen, BioGasol ApS, DK-2750 Ballerup, Denmark; J.E. Holladay, J. White, D. Elliot, S. Jones and R. Orth, Pacific Northwest National Lab/DOE, Richland, WA

Session 8: Biofuels logistics and sustainability

Chairs: J.R. Hess, Idaho National Laboratory, Idaho Falls, ID and W. Mabee, Queen's University, Canada

Grand Ballroom C, 3rd Floor

8:00 AM **8-01** **An up-to-date overview and comparison of sustainability certification schemes for biofuels**

A. Faaij* and J. van Dam, Utrecht University, Utrecht, Netherlands

8:30 AM **8-02** **International trade in lignocellulose biomass**

P.P. Schouwenberg, Duferco Energy International, Lugano, Switzerland

9:00 AM **8-03** **Food and fuel: Investigation into integrating animal feed production with cellulosic ethanol from switchgrass**

B.D. Bals* M. Allen and B. Dale, Michigan State University, East Lansing, MI

9:30 AM **Break**

Sponsored by DSM

10:00 AM **8-04** **Sourcing stover: Results from the ISU Integrated Corn Stover Feedstock Supply Systems Project**

Rob Anex*1, Kenneth J. Moore1, Stuart Birrell1, Kendall R. Lamkey Lamkey1, M. Paul Scott1, K. Mark Bryden1, Tom L. Richard2, Kevin J. Shinners3, James Coors3, Richard E. Muck4 and Liz Marshall5, (1)Iowa State University, Ames, IA, (2)Pennsylvania State University, University Park, PA, (3) University of Wisconsin, Madison, WI, (4)U.S. Dairy Forage Research Center, USDA-ARS, Madison, WI, (5)World Resources Institute

10:30 AM **8-05** **A Life Cycle Impact Assessment framework for characterizing human health benefits and impacts from emerging biofuels**

A.B. Lobscheid and T.E. McKone*, Energy Biosciences Institute and Lawrence Berkeley National Laboratory, Berkeley, CA

11:00 AM **8-06** **Process technology and integration options for a sustainable biorefinery**

C.M. Alles*, R.E. Jenkins, J.P. Ginn, B.M. Vrana and R.W. Sylvester, DuPont Engineering Research & Technology, Wilmington, DE; S.M. Hennesey, DuPont Central Research and Development, Wilmington, DE

Tuesday Afternoon, May 5

FREE AFTERNOON

Organizing Committee Meeting

Sutter, 5th Floor

12:15 PM-1:30 PM

Special Topic 1: International Commercialization of 2nd Generation Biofuels

Cosponsored by IEA Bioenergy Tasks 39 and 40.

Chairs: J. Saddler, University of British Columbia, Vancouver, BC, Canada and J.D. McMillan, National Renewable Energy Laboratory, Golden, CO

Grand Ballroom C, 3rd Floor

7:00 PM **ST1-01** **One million tons of biomass per year: Feedstock management for large scale BTL plants**

M. Deutmeyer,* CHOREN Biomass GmbH, Hamburg, Germany and C. Kiener, CHOREN Industries GmbH, Freiberg, Germany

7:15 PM **ST1-02** **POET update on Project LIBERTY**

J. Kwiatkowski, POET Research, Sioux Falls, SD

7:30 PM **ST1-03** **Commercialization of Biomass Ethanol at Abengoa Bioenergy**

Quang Nguyen, Abengoa Bioenergy, Chesterfield, MO

7:45 PM **ST1-04** **Lignocelulosic ethanol-breaking through the barriers**

C. Lauridsen* and H. Ren, Novozmes China, Beijing, China; C.C. Fuglsang, Novozmes, Inc., Davis, CA and Novozymes A/S, Bagsværd, Denmark

8:00 PM **ST1-05** **Why greenhouse gas balances are good for business**

T. Sidwell, British Sugar Group, Peterborough, UK

8:15 PM **ST1-06** **Deployment at scale: A grand challenge for advanced biofuels**

J.D. Newman, Amyris, Emeryville, CA

8:30 PM **ST1-07** **Global feasibility of large-scale biofuel production**

Lee R. Lynd*, Dartmouth College, Hanover, NH, Nathanael Greene, Natural Resources Defense Council; Tom Richard, Pennsylvania State University, State College, PA; Andre Faaij, Utrecht University, Utrecht, Netherlands; Jon Foley, University of Minnesota; Jose Goldemberg, University of Sao Paulo, Sao Paulo, Brazil; Reinhold Mann, PETRONAS Renewable Energy Laboratory, Kuala Lumpur, Malaysia; Patricia Osseweijer, Delft University of Technology, Delft, Netherlands; and W. H. van Zyl, University of Stellenbosch, Stellenbosch, South Africa

Special Topic 2: Development and Commercialization of Algal-based Biofuels

Chairs: A. Darzins, National Renewable Energy Laboratory, Golden, CO and S. Viamajala, Utah State University, Logan, UT

Grand Ballroom A-B, 3rd Floor

7:00 PM **ST2-01** **Overview of algal biofuels: From cell biology to biotechnology**

Q. Hu* and M. Sommerfeld, Arizona State University, Tempe, AZ;

7:20 PM **ST2-02** **Biofuels from microalgae: Biochemistry and regulation of triacylglycerol accumulation in the model *Chlamydomonas reinhardtii***

C. Benning*, E.R. Moellering, R. Miller, X. Li and A. Vieler, Michigan State University, East Lansing, MI

7:40 PM **ST2-03** **Strain development of non-model photosynthetic microbes for biofuel production**

B. Vick, Aurora Biofuels, Alameda, CA

8:00 PM **ST2-04** **Low-cost photobioreactor technology: Promise, progress and challenges**

B. Willson, Colorado State University/Solix Biofuels, Ft. Collins, CO

8:20 PM **ST2-05** **Feedstock-flexible renewable oil production from heterotropohic algae using a proven, scalable system**

H.F. Dillon, Solazyme, Inc., South San Francisco, CA

Wednesday Morning, May 6

Invited Speaker Breakfast-Wednesday speakers

SoMa, 3rd Floor

7:15 AM-8:00 AM

Continental Breakfast (all registrants)

Grand Ballroom Foyer, 3rd Floor

7:15 AM-8:00 AM

Session 9: Bioprocessing and Separations Technology

Chairs: L.P. Walker, Cornell University, Ithaca, NY and V. Kochergin, Louisiana State University AgCenter, Baton Rouge, LA

Grand Ballroom C, 3rd Floor

8:00 AM **9-01** **Assessment of potential fermentation inhibitors in wet cake cellulosic hydrolysate**
N. Mosier*, R. Hendrickson, Y. Kim and M.R. Ladisch, Purdue University, West Lafayette, IN

8:30 AM **9-02** **Conversion of municipal solid waste into bioenergy**
J.W. Jensen*, C. Felby and H. Jørgensen, University of Copenhagen, Frederiksberg, Denmark; N. Nørholm and G. Rønsch, DONG Energy A/S, Fredericia, Denmark

9:00 AM **9-03** **Evaluation of target efficiencies for solid-liquid separation steps in biofuels production**
V. Kochergin* and K. Miller, Louisiana State University AgCenter, Baton Rouge, LA

9:30 AM **Break**

10:00 AM **9-04** **Reactive separations for esterification and purification of individual organic acids from mixed solutions**
A.K. Kolah*, A. Orjuela, C.T. Lira and D.J. Miller, Michigan State University, East Lansing, MI

10:30 AM **9-05** **Process development of integrated cellulose- and starch-based ethanol production**
B. Erdei*, M. Galbe and G. Zacchi, Lunds University, Lund, Sweden

11:00 AM **9-06** **Fluorescence resonance energy transfer sensors for quantitative monitoring of pentose and disaccharide accumulation in bacteria**
T. Kaper*, Genencor, A Danisco Division, Palo Alto, CA, I. Lager, Lund University, Lund, Sweden, L.L. Looger, Howard Hughes Medical Institute, Ashburn, VA, D. Chermak, Carnegie Institution, Stanford and W.B. Frommer, Carnegie Institution, Stanford, CA

Session 10: Enzyme Science and Technology 2

Chairs: E. Larenas, Genencor a Division of Danisco, Palo Alto, CA and J. Brainard, NREL

Grand Ballroom A-B, 3rd Floor

8:00 AM **10-01** **Enzymatic synergy examined using an engineered complex of cellulosomal enzymes from *Clostridium thermocellum***
C. Paavola*, S. Reinsch, S. Bhattacharya, E. Almeida and J. Trent, NASA Ames Research Center, Moffett Field, CA; S. Mitsuzawa, University of California, Santa Cruz, Santa Cruz, CA; S. Chan, H. Kagawa and Y. Li, SETI Institute, Mountain View, CA; O. Marcu and N. Dvorochkin, National Space Grant Foundation, Moffett Field, CA

8:30 AM **10-02** **The three-dimensional structure of an intact glucoamylase gives insight on how substrate is directed towards the active site**
H. Hansson*, M. Sandgren and S. Karkehabadi, Swedish University of Agrucultural Sciences, Uppsala, Sweden; R. Bott, M. Saldajeno, W. Cuevas, D. Ward and M. Scheffers, Genencor - A Danisco Division, Palo Alto, CA; W. Aehle, Genencor - A Danisco Division, Leiden, Netherlands

9:00 AM **10-03** **Computational estimates of free energy profiles of cellodextrin motion in Cel7A reaction tunnel**
M.F. Crowley*, G.T. Beckham and M.E. Himmel, National Renewable Energy Laboratory, Golden, CO

9:30 AM **Break**

10:00 AM **10-04** **A family of thermostable fungal cellulases created by structure-guided recombination**
P. Heinzelman*, C.D. Snow, I. Wu and F.H. Arnold, Caltech, Pasadena, CA; A. Villabolos, S. Govindarajan and J. Minshull, DNA2.0 Inc., Menlo Park, CA

10:30 AM **10-05** **Engineering cellulases on their natural substrates by directed evolution**
W. Liu, X. Zhang and Z. Zhang, Virginia Tech, Blacksburg, VA; P. Zhang*, Virginia Tech University, Blacksburg, VA

11:00 AM **10-06** **Developing improved thermostable cellulases: High-throughput cellulolytic assays and protein engineering strategies**
H.A. Chokhawala and T.W. Kim, Energy Biosciences Institute, Berkeley, CA; C. Dana, D. Nadler, H.W. Blanch and D.S. Clark*, University of California, Berkeley, Berkeley, CA

LUNCH ON YOUR OWN

Wednesday Afternoon, May 6

Session 11: Emerging Biofuels and Chemicals

Chairs: S. del Cardayre, LS9, Inc., South San Francisco, CA and D. Cameron, Piper Jaffray, Minneapolis, MN

Grand Ballroom C, 3rd Floor

1:00 PM **11-01** **Hydrocarbon fuels from plant biomass**
D.A. Glassner*, M. Peters and P.R. Gruber, Gevo, Inc., Englewood, CO

1:30 PM **11-02** **Renewable Petroleum™ products and technologies: Engineering microbial fatty acid metabolism for fuel and chemical production**
S. del Cardayre, LS9, Inc., South San Francisco, CA

2:00 PM **11-03** **High-titer production of hydroxyvalerates and 4-valerolactone from levulinate in *Pseudomonas putida***
C.H. Martin* and K. Jones Prather, Massachusetts Institute of Technology, Cambridge, MA

2:30 PM **Break**

3:00 PM **11-04** **Sustainable chemicals through biotechnology: 1,4-butanediol**
M.J. Burk, Genomatica, Inc., San Diego, CA

3:30 PM **11-05** **Rapid optimization of microorganisms for the cost superior production of chemicals and fuels**

M.D. Lynch, OPX Biotechnologies, Inc., Boulder, CO

4:00 PM **11-06** **Production of terpene based biofuels in *S. cerevisiae***

P.P. Peralta-Yahya*, Joint BioEnergy Institute, Emeryville, CA and J.D. Keasling, UC-Berkeley; Lawrence Berkeley National Laboratory, Emeryville, CA

Session 12: Biomass Recalcitrance

Chairs: C. Somerville, University of California, Berkeley, CA and L. Viikari, University of Helsinki, Helsinki, Finland

Grand Ballroom A-B, 3rd Floor

1:00 PM **12-01** **Identification of desirable traits in *Miscanthus* to enhance total sugar yields in biological conversion**

T. Zhang*, B. Yang and C.E. Wyman, University of California Riverside, Riverside, CA; F. Zhou and J. Zhang, Mendel Biotechnology Inc, Hayward, CA

1:30 PM **12-02** **Elucidation of alfalfa lignin structures on gene down-regulation**

Y. Pu* and A. Ragauskas, Georgia Institute of Technology, Atlanta, GA; F. Chen and R.A. Dixon, Samuel Roberts Noble Foundation, Ardmore, OK; M. Davis, National Renewable Energy Laboratory, Golden, CO; B.H. Davison, Oak Ridge National Laboratory, Oak Ridge, TN

2:00 PM **12-03** **Small-scale enzymatic conversion screens to assist in the development of improved energy crop varieties**

T. Kruse*, A. Alexiades, G. East, B.R. Hames and S.R. Thomas, Ceres, Inc., Thousand Oaks, CA

2:30 PM **Break**

3:00 PM **12-04** **Effects of chemical pretreatment on enzymatic hydrolysis of lignocellulose observed by AFM**

H. Liu, South China University of Technology, Guangzhou, China; W. Zhu, South China University of Science and Technology, Guangzhou, China; J.Y. Zhu*, USDA Forest Service, Forest Products Laboratory, Madison; WI and S. Fu, South China University of Technology, Guangzhou, China

3:30 PM **12-05** **Understanding ionic liquid pretreatment of lignocellulosic biomasses**

S. Singh*, R. Arora, C. Manisseri, C. Li, H.V. Scheller and B.A. Simmons, Joint BioEnergy Institute, Lawrence Berkeley National Laboratory, Emeryville, CA; K.P. Vogel, USDA-ARS, Lincoln, NE

4:00 PM **12-06** **Single molecule tracking of carbohydrate-binding modules bound to cellulose crystals**

Y.S. Liu*, Y. Zeng, Y. Luo, Q. Xu, M.E. Himmel and S.Y. Ding, National Renewable Energy Laboratory, Golden, CO; S. Smith, South Dakota School of Mines and Technology, Rapid City, SD

Wednesday Evening, May 6

Pre-banquet Reception

Grand Ballroom Foyer, 3rd Floor

6:00 PM-7:00 PM

Annual Banquet and Award Presentations

Generously sponsored by Novozymes

Grand Ballroom, 3rd Floor

7:00 PM-10:00 PM

Banquet Speaker: Jennie Hunter-Cevera, President, University of Maryland Biotechnology Institute, Rockville, MD

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32nd Symposium in Clearwater Beach, Florida
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» Hilton Clearwater Beach

Oral Presentations

Oral Presentation 1-01

The French initiative on renewable carbon for green chemistry and bioenergies

P. Colonna*[1], F. Houllier[1], A. Kammoun[1], X. Montagne[2] and C. Sales[3]
(1)INRA, Paris, France
(2)IFP, Rueil-Malmaison, France
(3)CIRAD, Montpellier, France
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The European Union plans to cut its carbon emissions by 20% while raising renewable sources up to 20% of total energy use by 2020. Simultaneously, chemical industry aims at raising the share of renewable carbon up to 17% in the broad family of chemicals and materials by 2017.

Plant biomass has therefore a great potential to become a major alternative source to fossil carbon.

The foresight workshop « Which plants and sustainable production systems for biomass in the future? », launched in April 2008 aims at characterizing annual and perennial plants, micro-algae and biomass production systems that would meet the needs and requirements of new bioenergy and green chemistry chains. Sustainability being a key issue, the workshop also integrates environmental, social and economic dimensions.

The foresight workshop federates 20 French bodies: public research or higher education organizations, professional unions, private companies and associations which play a leading role in their respective area.

The set of experts who participate to the workshop includes specialists from many disciplines (e.g. plant physiology and genetics, biotechnologies, agronomy, ecology, economics and social sciences).

This workshop articulates 3 interrelated approaches: a reverse engineering approach that starts from the needs expressed by different industries; the exploration and optimization of production systems based on relevant plant and algal species, including biorefinery as well as green and white biotechnologies; the assessment of the environmental, territorial and economic performances of these systems.
The presentation will outline the first results before the final deliverables scheduled for Spring 2010.

Oral Presentation 1-02

Plant growth promoting microorganisms allow for sustainable growth and increased biomass production of poplar on marginal soils

D. van der Lelie*, S. Monchy, L. Newman and S. Taghavi
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Looking at the drivers behind a biofuel economy it is clear that once the problem of the cost efficient decomposition of lignocellulosic biomass has been solved, the sustainable production of lignocellulosic biomass will become the major critical success factor.

Poplar is considered as a model tree species for bioenergy feedstock production. Plants live in close association with symbiotic microorganisms. We showed that specific endophytic bacteria had a beneficial effect on the development and growth of poplar on marginal soils, resulting in up to 50%-80% increase in biomass production.

Short term beneficial effects of plant growth promoting microorganisms result in improved plant establishment on marginal soils. These effects include accelerated root development resulting in better access to nutrients and water, and consequently a faster initial growth, which will allow the plants to out compete weeds for available resources, thus resulting in less need to apply herbicides. Long term beneficial effects of plant growth promoting microorganisms will result in improved plant growth, health and survival, leading to economically sustainable feedstock production. This can be obtained by counteracting stress responses caused by drought and contamination, protection against pathogens via competition for available resources, and by assisting the plant's defense response against pathogenic invasions.The genomes of four plant growth promoting endophytic bacteria were sequenced and genome annotation and "omics" approaches were used to better understand their synergistic interactions with poplar. This basic knowledge will be further exploited to improve plant establishment and sustainable bioenergy feedstock production on marginal, non-agricultural land.

Oral Presentation 1-03

Plants begetting plants: Lignocellulose saccharification by plant-expressed cellulases

J.D. Nichols*, B. Link, S. Miles, M. Kim, B. Ember, S. Arellano and P. Oeller
Syngenta Biotechnology, Inc., Research Triangle Park, NC
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Significant conversion of recalcitrant cellulosic biomass to fermentable sugars requires, at a minimum, *endo*-glucanase (EC 3.2.1.4) and *exo*-glucanase (EC 3.2.1.91) activities to reduce the insoluble cellulose chains to soluble cellobiose units which are subsequently hydrolyzed to glucose by β-glucosidase (EC 3.2.1.21). A major obstacle to the development of a commercially viable cellulosic ethanol industry is the cost associated with vast quantity of cellulase required. At ~50mg enzyme/gram cellulose, the current level of microbially-expressed enzymes required for efficient degradation of lignocellulosic biomass makes microbial expression an economically untenable means for enzyme production. While efforts to address this problem have primarily focused upon engineering more efficient cellulases and maximizing fungal expression, utilizing plants to produce and deliver the enzymes offers a convenient and cost effective alternative. Syngenta has pioneered the concept of *in planta* expression of enzymes and traits focused on the biofuels industry. Indeed, Syngenta is the only company to date to have taken a plant-expressed biofuels trait, our corn-expressed amylase for dry grind ethanol production, into the US regulatory system. We have successfully generated transgenic crop plants, including maize and tobacco, expressing active bacterial and fungal cellulases at high levels. This paper will outline the characterization of *in planta*-expressed microbial cellobiohydrolases (CBH I and II) and *endo*-glucanases and will demonstrate the capacity of these enzymes to function in defined enzyme cocktails for the degradation of lignocellulosic biomass.

Oral Presentation 1-04

Transgenic Expression of Endoglucanase and Xylanase Genes Increases Tobacco Digestibility and Biomass Conversion

K.L. Pappan*[1], D. Corredor[1], B. Gerdes[1], D.A. Lee[2], S.A. Yelundur[2], X. Wu[3] and D. Wang[3]
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(2)Edenspace Systems Corporation, Chantilly, VA
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pappan@edenspace.com

The objective of this project was to test the processing performance of transgenic feedstocks expressing cell wall hydrolyzing enzymes and to identify ways to exploit the properties of these plants during biorefining to improve conversion efficiency. Transgenic tobacco lines expressing an endoglucanase, E1, from *Acidothermus cellulolyticus* and a xylananse, Xyn Z, from *Clostridium thermocellum* as single enzymes or transgenic tobacco expressing both enzymes were tested using dilute acid pretreatment and enzyme hydrolysis, as well as *in vitro* dry matter digestibility analysis, to evaluate their performance as value-added cellulosic ethanol feedstocks. Compared to wild-type tobacco, transgenic lines displayed greater digestibility and glucan conversion when biomass was digested with commercial enzyme cocktails. These properties were further enhanced by incubating the slurried biomass at moderate temperatures prior to hydrolysis with commercial enzyme cocktails. These results demonstrate that transgenic crop feedstocks have potential to improve the efficiency and lower the cost of existing bioprocessing regimes by reducing enzyme loading, and point to the possibility of further modifying bioprocessing to exploit the properties of transgenic feedstocks. This project was supported by grant 0810640 from the National Science Foundation.

Oral Presentation 1-05

Enhanced bioprocessing of maize cell wall mutants

W. Vermerris*[1], H.M. Caicedo[1], N.S. Mosier[2] and M.R. Ladisch[2]
(1)University of Florida, Gainesville, FL
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Modification of lignin subunit composition can significantly increase the yield of fermentable sugars from maize stover. The *brown midrib1* (*bm1*) and *bm3* mutations each increase the yield of glucose per gram dry stover by 50% relative to the wild-type control (inbred A619). When combined in a near-isogenic *bm1-bm3* double mutant, the two mutations act in an additive manner, resulting in a doubling of the yield of glucose. Even though there is no apparent increase in cellulose content, based on kinetic studies both the rate of hydrolysis and the overall yield of glucose increase as a result of the mutations. We are investigating the basis of the enhanced hydrolysis in these *bm* mutants by assaying the adsorbance of cellulases to stover, using recombinant proteins consisting of the cellulose binding module (CBM) isolated from *Trichoderma reesei* endoglucanases labeled with green-fluorescent protein (GFP). Because of lignin autofluorescence, this approach can not be performed *in situ*, but instead has to rely on a fluorescence subtraction assay. We have also shown that biomass from these mutants yields high levels of fermentable sugars under less severe pretreatment conditions compared to biomass from wild-type control plants. The more efficient cell wall deconstruction in these mutant can thus be viewed as genetic pretreatment. The combined data from these experiments will be of value for the design of plant cell wall composition in such a way that agronomic properties and biomass conversion are optimally balanced.

Oral Presentation 1-06

Impact of divergent selection on the abundance and activities of lignin biosynthetic enzymes in switchgrass, and characterization of recombinant switchgrass CAD and COMT proteins

A.J. Saathoff*[1], N.A. Palmer[1], C. Tobias[2], P. Twigg[3], S.E. Sattler[1], E.J. Haas[4], R.B. Mitchell[1], K.P. Vogel[1] and G. Sarath[1]
(1)USDA-ARS, Lincoln, NE
(2)USDA, Agricultural Research Service, Albany, CA
(3)University of Nebraska, Kearney, Kearney, NE
(4)Creighton University, Omaha, NE
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The relative composition of lignin monomers in cell walls provides key information on the integrated functions of the underlying biosynthetic machinery, as well as a useful window into the efficacy of broad or narrow selection criteria for improvement of plants with more optimal biomass quality. Here, we evaluated a number of switchgrass genotypes by thioacidolysis for lignin composition and content, and by biochemical characterization of protein levels and activities of select enzymes in internode extracts. The data indicated that divergent selection of switchgrass for digestibility resulted in changing the ratios of G to S lignins in plants, and impacted the relative levels of cinnamyl alcohol dehydrogenase (CAD) and caffeic-acid-O-methyl transferase (COMT) proteins, but levels of caffeoyl-CoA-O-methyl transferase (CCoAOMT) were unchanged. Enzyme activity data generally mirrored protein level data. These findings suggest that (i) enzymes required for lignin biosynthesis in switchgrass can be differentially affected by broad selection for digestibility; and (ii) discovery of the mechanisms controlling the endogenous levels of these proteins could uncover novel markers and lead to accelerated improvement of switchgrass via traditional breeding. We have also cloned and initiated biochemical characterization of recombinant switchgrass and sorghum CAD and COMT proteins. Recombinant grass CADs displayed greater substrate preference for sinapyl aldehyde and sinapyl alcohol when compared to coniferyl derivatives. There was essentially no activity against caffeoyl alcohol. Initial modeling of sorghum CAD suggested that observed changes in specific amino acid residues in monocot CADs relative to dicot CADs could account for changes in substrate specificity.

Oral Presentation 1-06A

Functional genomic analysis of plant biomass deconstruction by extremely thermophilic, cellulolytic bacteria in pure and co-culture

D.L. Lewis*[1], S. Blumer-Schuette[1], I. Ozdemir[1], A.L. VanFossen[1], I. Kataeva[2], S.J. Yang[2], M.W.W. Adams[2] and R.M. Kelly[1]
(1)North Carolina State University, Raleigh, NC
(2)University of Georgia, Athens, GA
dllewis2@ncsu.edu

The breakdown of lignocellulosic biomass to fermentable sugars remains a key challenge in the production of biofuels, such as hydrogen and ethanol. To this end, microbial consortia need to be considered so that natural synergistic contributions to biomass deconstruction can be used advantageously. To develop such consortia, an understanding of the relevant molecular microbial ecology of the constituent organisms is critically important. In our lab, functional genomics approaches are being used to explore interspecies interactions between extremely thermophilic bacteria that have the capacity to degrade lignocellulosic biomass. Two gram-positive, oligotrophic, fermentative anaerobes, with growth T_{opt} of ~75°C, *Caldicellulosiruptor saccharolyticus* (Csac) and *Anaerocellum thermophilum* (Athe), are being investigated as model cellulolytic extreme thermophiles. Although 16S rRNA phylogeny suggests that these two bacteria are closely related, genome sequence analysis revealed that Athe contains almost 700 ORFs not present in Csac, while Csac has over 600 ORFs missing from Athe. A key objective is to determine the physiological and ecological significance of genome sequence differences as this relates to biomass deconstruction. Using whole genome oligonucleotide microarrays, both pure and co-cultures of *C. saccharolyticus* and *A. thermophilum* were monitored at various stages of growth on monosaccharides, polysaccharides and plant biomass substrates. Operons, regulons, and key protein-encoding ORFs responsive to specific substrates, growth conditions and interspecies interactions were identified. The results illustrate how strategic use of transcriptional response analysis can be a powerful tool for examining microbial biomass deconstruction by pure and co-cultures capable of consolidated bioprocessing.

Oral Presentation 2-01

One-step Cellulosic Ethanol: Can We Really Do This?

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A whole-cell biocatalyst system was developed in our research laboratories to directly produce ethanol from cellulose in a single step; initially we used a model amorphous cellulose (Phosphoric acid swollen cellulose, PASC). This whole cell biocatalyst was constructed with LY01, which is one of the most developed ethanologenic *Escherichia coli* strains, as a host cell. The cellulase genes, celCCA, celCCE and β-glucosidase, were prepared from the mesophilic strain *Clostridium cellulolyticum*. To enhance the stability and activity of the cellulosic enzymes, these enzymes were co-displayed with the anchor protein PgsA on the surface of the host cell. For inducing the synergism of enzymes, this recombinant cellulolytic microorganism co-expressed endoglucanase, cellobiohydrolase, and β-glucosidase, simultaneously. The saccharification product, monosaccharides, can be uptaken immediately by the host cell and produce ethanol so that the inhibition of the catalytic activity of enzymes due to high substrate (sugars) concentration, can be effectively minimized. In this research, we also applied the whole-cell biocatalyst system in a bioethanol production process with the lignocellulosic biomass as a substrate. With the enzymatic hydrolysis of natural biomass, there are several factors, which determine the hydrolysis rate, e.g. crystallinity, degree of polymerization, particle size, pore volume, and accessible surface area. Since cellulose hydrolysis occurs on the surface of cellulose, we especially focus on the relationship between particle size of cellulose and hydrolytic rate in whole-cell biocatalyst system. The results are very promising.

Oral Presentation 2-02

Genetically engineering yeast for CO2 capture during ethanol fermentation

Z. Dai*, K. Panther, S. Baker, J. Magnuson and L. Lasure
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Biomass represents an abundant carbon-neutral renewable resource for the production of bioenergy and biomaterials. Bio-ethanol is one of the predominant bio-fuels, which is produced mainly by yeast (saccharomyces cerevisiae) fermentation. During ethanol fermentation, more than forty percent of the carbons are released into the atmosphere as carbon dioxide. The ability to capture part of the CO2 released during fermentation will provide an alternative way to improve ethanol productivity per unit of biomass used. To achieve this objective, we explored the feasibility of expressing cyanobacterial photosynthetic enzymes in fermentative yeast to capture CO2. The genes for ribulose bisphophate carboxylase and phosphoribulokinase were isolated from cyanobacterium Synechococcus sp. and were heterologously expressed in the yeast under the control of the yeast actin, pgk1 or adh1 promoters. RNA and protein blotting analyses confirmed that both genes were properly expressed in S. cerevisiae. Codon optimization of both genes significantly improved protein accumulation in the yeast. The 14C labeling analysis demonstrated that ribulose bisphosphate carboxylase was active in the yeast cells though the activity was low. The effects of both enzymes on yeast ethanol production were also examined in culture media supplemented with a xylose-xylulose mixture. Some improvement was observed. Further improvement of this CO2 capturing process is on-going.

Oral Presentation 2-03

Production of a xylose utilizing Zymomonas mobilis strain for ethanol production from high concentrations of mixed sugars

P. Viitanen*[1], C. Mc Cutchen[1], M. Emptage[1], P. Caimi[1], M. Zhang[2], Y.C. Chou[2] and M.A. Franden[2]
(1)DuPont Co., Wilmington, DE
(2)NREL, Golden, CO
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Maximizing yield of ethanol from C5 utilizing micro organisms requires both high rates of sugar utilization and minimizing the production of by products that detract from carbon yield to ethanol. *Zymomonas mobilis* that has been engineered to utilize xylose by way of the pathway through xylose isomerase and xylulose kinase to the endogenous sugar phosphate pathway produces xylitol and xylitol phosphate as byproducts. Xylitol production results in loss of ethanol yield and xylitol phosphate is a general metabolic inhibitor as a dead end phosphate sink. In order to correct these deficiencies in xylose utilizing *Z. mobilis*, the pathway to xylitol and xylitol phosphate was determined and the gene for the enzyme at the head of the pathway was inactivated to produce a strain that has better fermentation properties and a higher ethanol yield. Effective means for achieving osmotic balance in high initial sugar fermentations was also established for the mutant and parent strain.

Oral Presentation 2-04

Construction of pentose fermenting industrial Saccharomyces cerevisiae strains expressing a bacterial xylose isomerase

E. Boles*, D. Brat and B. Wiedemann
Goethe-University Frankfurt, Frankfurt, Germany
e.boles@bio.uni-frankfurt.de

We have cloned and successfully expressed a prokaryotic xylose isomerase with high activity in the yeast *Saccharomyces cerevisiae*. The corresponding gene was isolated from the anaerobic bacterium *Clostridium phytofermentans*. The enzyme has only very limited sequence similarities to the xylose isomerases from *Piromyces* and *Thermus thermophilus* which up to now were the only xylose isomerases which could be expressed in yeast in a functional form. Activity and kinetics of the new enzyme are comparable to the *Piromyces* xylose isomerase. However, it is far less inhibited by xylitol, which typically is produced by yeast cells during xylose fermentations. We have expressed a codon-optimized version of the gene in industrial yeast strains. Evolutionary engineering enabled the strains to ferment xylose efficiently.

Additionally, we have also integrated genes of a bacterial arabinose pathway into the yeast strains together with an arabinose-transporter gene from *Pichia stipitis*. Codon-optimization of the heterologous genes considerably improved pentose fermentations. To this end, we have obtained industrial yeast strains able to produce ethanol from the main pentose sugars, xylose and arabinose, present in lignocellulosic biomass.

Oral Presentation 2-05

Development of a Robust Yeast Biocatalyst for Low pH Lactic Acid and Cellulosic Ethanol Fermentation.

Pirkko Suominen[1], Dan Beacom[1], Tom McMullin[1], Arlene Fosmer[1], Chris Miller[1], Brian Rush[1], Jon Veldhouse[1], Gary Folkert[1], Liz Dierickx[1], Ken Finley[1], Beth Mastel[1], Holly Jessen[2], Josh Lundorff[2], Ana Negrete-Raymond[2] and Jian Yi[2]
(1)Biotechnology Development Center, Cargill, Minnetonka, MN,
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Common characteristics are required for an economically viable biocatalyst for cellulosic ethanol and lactic acid production for commodity applications like Poly Lactic Acid (PLA). These include high yield, fast fermentation, robust growth in simple media, and tolerance to organic acids at low pH. Cargill started with non-conventional yeast naturally possessing some of these characteristics and successfully developed it to efficiently produce a new end product (lactic acid) or to ferment new sugars (pentoses). Replacing yeast's ethanol pathway with lactic acid pathway was straight forward. Development of a strain capable of producing polymer grade lactic acid at commercially interesting titer, yield and productivity required concerted utilization of genome wide tools, targeted modifications, evolution and classical mutagenesis. The developed strain and low pH fermentation process offers considerable cost savings over conventional lactic acid processes.

Cargill has previously demonstrated efficient fermentation of xylose to ethanol in yeast (USPatentApp 10/554887). We are now combining our xylose fermentation technology into the acid tolerant yeast first developed for lactic acid production. Goals have been set for ethanol production from mixed sugars (dextrose, mannose, xylose, and arabinose) in the presence of 10 g/L acetate at 40oC and at a pH less than 5.0. Under these conditions Cargill host can utilize 80 g/l of dextrose and 80 g/l of mannose in less than 36 hours, producing ~ 70 g/l ethanol. A xylose utilization pathway has been engineered into this host and efficient fermentation of xylose to ethanol demonstrated both in defined medium and in hydrolyzate.

Oral Presentation 2-06

Metabolic engineering of Saccharomyces cerevisiae for the production of n-butanol

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Background
Increasing energy costs and environmental concerns have motivated engineering microbes for the production of "second generation" biofuels that have better properties than ethanol.

Results & Conclusions
Saccharomyces cerevisiae was engineered with an n-butanol biosynthetic pathway, in which isozymes from a number of different organisms (S. cerevisiae, Escherichia coli, Clostridium beijerinckii, and Ralstonia eutropha) were substituted for the Clostridial enzymes and their effect on n-butanol production was compared. By choosing the appropriate isozymes, we were able to improve production of n-butanol ten-fold to 2.5 mg/L. The most productive strains harbored the C. beijerinckii 3-hydroxybutyryl-CoA dehydrogenase, which uses NADH as a co-factor, rather than the R. eutropha isozyme, which uses NADPH, and the acetoacetyl-CoA transferase from S. cerevisiae or E. coli rather than that from R. eutropha. Surprisingly, expression of the genes encoding the butyryl-CoA dehydrogenase from C. beijerinckii (bcd and etfAB) did not improve butanol production significantly as previously reported in E. coli. Using metabolite analysis, we were able to determine which steps in the n-butanol biosynthetic pathway were the most problematic and ripe for future improvement.

Oral Presentation 2-06A

Integration of genomics and bioinformatics to identify genetic differences in an ethanol tolerant Clostridium thermocellum ATCC27405 strain

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Clostridium thermocellum is a gram-positive, anaerobic, thermophilic bacterium that can ferment cellulose at one of the highest growth rates directly to ethanol via a large extracellular enzyme complex termed the cellulosome. *C. thermocellum* is a candidate industrial biocatalyst for future lignocellulosic fuel production. The metabolic byproducts of fermentation can inhibit fermentation performance and lignocellulosic biomass pretreatment processes also produce a variety of inhibitory chemicals that can adversely affect the fermentation. Limited information is available on the mechanisms and responses of *C. thermocellum* to different inhibitors. The genetic differences between wild-type *C. thermocellum* and an ethanol tolerant mutant have been identified through microarray based comparative genome sequencing and 454-pyrosequencing. We detected more than 400 differences in the ethanol tolerant mutant compared to the *C. thermocellum* wild-type strain. The resequencing data were in agreement with published membrane proteomic data and identified new mutations in key genes such as alcohol dehydrogenase. Bioinformatics analyses identified 16 mutational hot-spots in the ethanol tolerant strain, with 7 out of 16 related to cellulose degradation and likely accounted for the strain's decreased growth on cellulose. Further work to identify and verify important loci and physiological changes conferring tolerance to inhibitors will assist in the development of industrial strains for consolidated bioprocessing (CBP) of lignocellulosic biomass and therefore reduce biofuel production costs.

Oral Presentation 3-01

On Size Reduction for Woody Biomass Conversion

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Physical size reduction through mechanical means is a necessary step in bioconversion of woody biomass to increase the surface accessible to enzymes to achieve satisfactory cellulose conversion efficiency. Unfortunately, reducing wood size to typical substrate scale of millimeter requires a significant amount of electric-mechanical energy. It is estimated that the energy consumed in size reduction can be 10-30% of the total biomass ethanol energy produced using current technology.

In this study, we used various fiber fractionations, different chemical pretreatments, and mechanical milling (size reduction) processes to produce wood substrates with varied physical sizes, chemical structures and physical properties. We demonstrated a wet imaging technique to determine two dimensions of these woody fibrous substrates. The measured two dimensions were used to estimate the substrate specific surface by using a cylinder model for individual fibers. The determined substrate specific surface was related to the enzymatic hydrolysis cellulose conversion of the substrate. We also compared the effectiveness of different chemical pretreatments applied directly to wood chips (~2x3x0.5 cm) on reducing size-reduction energy consumption and enhancing enzymatic saccharification, so that the efficiencies of different chemical pretreatments and size-reduction processes can be compared objectively. It was found that Chemical pretreatment affects not only cellulose conversion efficiency, but also post-pretreatment size-reduction energy consumption and liquefaction of substrates during high solids enzymatic hydrolysis. The SPORL pretreatment process that we developed is the most efficient with cellulose conversion of over 90% and post-pretreatment wood chip size-reduction energy consumption of about 30 Wh/kg.

Oral Presentation 3-02

Glucose and xylose yields from switchgrass for ammonia fiber expansion, ammonia recycle percolation, dilute sulfuric acid, hot water, lime, and sulfur dioxide pretreatments followed by enzymatic hydrolysis

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Switchgrass promises to become a major resource for making fuels and chemicals by cellulose conversion technologies. However, it must be pretreated to realize reasonable yields of sugars by enzymatic hydrolysis, but pretreatment is expensive and strongly influences cost and performance of other operations. The Biomass Refining Consortium for Applied Fundamentals and Innovation (CAFI) was formed in 2000 to develop the first comparative data of sugar yields from leading pretreatment operations followed by enzymatic hydrolysis of resulting solids. The CAFI Team achieved high sugar yields from corn stover for all pretreatments but found much greater variations in performance for poplar wood with changes in pretreatment technologies and biomass source. In this project, pretreatment by ammonia fiber expansion (AFEX), ammonia recycle percolation (ARP), dilute acid, hot water, lime, and sulfur dioxide steam explosion were applied to shared sources of switchgrass, and the same enzymes, experimental protocols, and material balance approaches were employed by all the members of the team. Three types of switchgrass, Alamo, Shawnee, and Dacotah, were evaluated from different locations and harvest times to determine whether these factors influence glucose and xylose yields from the combined operations of pretreatment and enzymatic hydrolysis. Comparisons will be reported for sugar yields from pretreatment alone (Stage 1), enzymatic hydrolysis (Stage 2), and the two combined over a range of enzyme mass loadings and formulations for each pretreatment approach. These results should help select pretreatment technologies for commercial operations and define new directions to improve plants, enzymes, and pretreatment technologies.

Oral Presentation 3-03

Sub- and super-critical water technology for biofuels: Switchgrass to ethanol, biocrude and hydrogen fuels

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Sub- and super-critical water (critical point: 374 °C, 221 bar) provide a novel reaction medium for the efficient conversion of lignocellulosic biomass to usable liquid and gas fuels. Recently, this medium has attracted much attention as a non-toxic, environmentally benign, inexpensive and tunable reaction medium for conducting ionic/free radical reactions. Dielectric constant of water near the critical point decreases considerably, which enhances the solubility of organic compounds. Almost complete conversion of crystalline cellulose (>90%) to water-soluble products above 330 °C in a short residence time (3-5 s) was possible, and high yield (65-67%) of hydrolysis products (glucose and oligomers) was achieved in subcritical water (335 - 354 °C) (Kumar and Gupta, Ind. Eng. Chem. Res., 2008).

Subcritical water was used for the pretreatment of switchgrass in a flow through reactor in temperature range 150 to 180 °C and pressure 35 to 136 bar. The process mainly removed hemicelluloses causing structural changes, which improved the accessibility to enzymes to cellulose. This pretreatment method can be effectively used for ethanol production.

At a higher temperature, subcritical water converts biomass to biocrude, a mixture of oxygenated hydrocarbons. Liquefaction of switchgrass for biocrude production in subcritical water (230-260 °C) was studied. More than 80% of switchgrass was solubilized in only 20 minutes.

At even higher temperature, supercritical water can effectively convert carbohydrates into hydrogen fuel (Byrd, Pant, and, Chem. Res., 2007). Biocrude produced from switchgrass liquefaction was reformed in supercritical water. The gaseous products contained mainly hydrogen and CO_2.

Oral Presentation 3-04

The technical advantages and challenges of ionic liquid-based biomass pretreatments

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The dissolution and derivitization of cellulose in Ionic liquids (ILs) has been demonstrated at laboratory scale. The use of ILs to totally dissolve lignocellulosics and to selectively dissolve lignin in biomass have also been reported. Proposed methods of IL and biomass recovery based on anti-solvent addition complete descriptions of novel closed-loop IL-based biomass pretreatments that have advantages over more conventional processes. For example, the IL-based processes are relatively rapid and are conducted at atmospheric pressure. Furthermore, cellulosic fractions recovered from IL-based pretreatments are more amenable to enzymatic hydrolysis than those recovered from other pretreatments. However, there remain several technical and economic barriers to the use of IL processes in industrial settings. The technical advantages and challenges of ionic liquid-based biomass pretreatments are described.

Oral Presentation 3-05

Ultra-structural and physicochemical modifications within ammonia pretreated lignocellulosic cell walls that influence enzyme accessibility

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The development of an economically viable and environmentally sustainable bio-based chemical industry has been impeded due to the native recalcitrance of lignocellulosics to chemical and biological processing. Lower severity ammonia based pretreatments (e.g. AFEX) and minimizing enzyme usage could help reduce processing costs. However, unlike other pretreatments AFEX does not extract lignin and hemicellulose into separate liquid fractions. Instead, AFEX enhances enzymatic digestibility through certain ultra-structural and chemical modifications within the cell wall that are currently not well understood.

An important goal of this research was to identify the major ultra-structural and chemical modifications incorporated within lignocellulosic cell walls during AFEX using several microscopic, spectroscopic and spectrometric techniques. High resolution microscopic (SEM, TEM) and 3D-EM-Tomographic studies indicate an ultra-structural alteration of AFEX treated cell walls via formation of a nanoporous tunnel-like network. Closer analysis (via ESCA, AFM and confocal fluorescence microscopy) of outer cell wall surfaces shows heterogeneous deposits rich in AFEX cell wall extractives. Raman spectral data indicates conversion of cellulose I to III is intricately dependent on AFEX pretreatment conditions. More than 45 degradation products have been quantified using LC-MS/MS and GC-MS. Some of the major degradation products include organic acids, aromatics, phenolic acids and amides.

A fundamental understanding of physicochemical modifications incorporated within lignocellulosic cell walls during pretreatment and its effect on enzyme accessibility are critical to further advancements in reducing cell wall recalcitrance to bioprocessing. This understanding would be critical to re-engineer plant cell walls, hydrolytic enzymes and ethanologenic microbes amenable for cellulosic biorefineries.

Oral Presentation 3-06

A novel biochemical platform for fuels and chemicals production from cellulosic biomass

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One significant obstacle impeding the large scale production of fuels and chemicals from cellulosic biomass is the lack of a low cost processing technology. The conventional biochemical platform for biorefinery involves four distinct steps: pretreatment, enzymatic hydrolysis, fermentation, and product recovery. Sugars are produced as the reactive intermediate for the subsequent fermentation. Steps involved with overcoming the recalcitrance of cellulosic biomass (pretreatment and enzymatic hydrolysis) are the two most costly steps in the whole process. Here we propose a novel biochemical platform for fuels and chemical production that will replace the two most costly steps in the conventional platform with a single biological step. Cellulolytic microorganism(s) that can secrete all the enzymes needed to hydrolyze cellulose and hemicellulose in spite of the presence of lignin will be modified to convert most of the carbohydrate contained in the cellulosic biomass to sugar aldonates. In a second step, sugar aldonates will be utilized as the carbon source to produce ethanol and other products. The new platform can potentially lower the cost of cellulosic bioprocessing substantially. Factors contributed to the cost reduction include elimination of the high capital cost and high operating cost associated with thermo-chemical pretreatment process, consolidation of the process, and reduction of the product recovery cost due to higher ethanol concentrations produced in the fermentation step. Feasibility study has demonstrated that sugar aldonates can be produced from modified *Neurospora crassa* under simulated conditions; and sugar aldonates can be converted to ethanol at high efficiency and at high yields by fermentation.

Oral Presentation 4-01

Genetic dissection of bioenergy traits in sorghum

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Sorghum has a number of characteristics that make it a very attractive biomass crop for ethanol production: low water and fertilizer requirements, tolerance to heat and drought, high biomass yield, and great genetic diversity. Two traits of particular interest are the sweet sorghum trait, which results in the accumulation of fermentable sugars in the juice of the stems, and the *brown midrib* (*bmr*) trait, which changes the color and the chemical composition of the vascular tissue, and results in higher yields of fermentable sugars obtained after enzymatic saccharification of the lignocellulosic biomass. The genetic basis of these traits, however, is poorly understood and impedes the full exploitation of sorghum as a bioenergy crop. High throughput expression profiling using 454-sequencing is being applied to identify the gene(s) underlying a recently mapped quantitative trait locus (QTL) for stem sugar concentration. In addition, we are developing a population of recombinant inbred lines to map QTL for juice volume. To identify novel genes affecting cell wall composition, we are using a sorghum TILLING population to identify mutants, including *bmr* mutants, with improved saccharification properties. The *Brown midrib* genes from the most promising mutants are being cloned using a candidate-gene approach. This approach recently resulted in the identification of *Bmr6* as the gene encoding cinnamyl alcohol dehydrogenase2. These combined approaches will enable the development of sorghums that offer maximum flexibility for the production of food, feed, fiber and fuel. Funding from the US Department of Energy for this project (DE-FG02-07ER64458) is gratefully acknowledged.

Oral Presentation 4-02

***Acidothermus cellulolyticus*: From genome sequence to plant cell wall deconstruction**

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The 2.4 Mb genome of the cellulose-degrading thermophile, Acidothermus cellulolyticus 11B, was completely sequenced by the Joint Genome Institute (DOE) in 2006. The genome offers useful insights into the lignocellulose deconstruction capabilities of the organism. The A. cellulolyticus genome revealed several new secreted glycoside hydrolases and carbohydrate esterases, indicating a diverse biomass-degrading enzyme repertoire, and significantly elevating the industrial value of this organism. A sizeable fraction of these hydrolytic enzymes break down plant and fungal cell walls. Findings from functional enzyme studies and insights into the evolutionary and functional implications of the A. cellulolyticus cellulolytic and xylanolytic capabilities will be presented.

Oral Presentation 4-03

Community structure and functional diversity of thermophilic cellulolytic microbial consortia

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In nature, cellulose degradation is performed by complex microbial communities in synergistic fashion. The enrichment in mixed cultures from nature of new microbial consortia with high cellulolytic activity is essential in the identification of novel organisms, novel metabolic capabilities and novel functions that will enhance our fundamental understanding of how the potential benefits of CBP (consolidated bioprocessing) can be realized at the industrial scale. In this work we identify the key players in cellulolytic enrichment cultures with thermophilic compost as an inoculum. Community structure and functional diversity have been characterized with clone libraries targeting both the 16S rRNA gene and glycosyl hydrolase gene fragments from mixed microbial cultures. Our studies have revealed varying levels of diversity and community composition, with a narrower range of novel and very specific clostridial cellulose degraders playing the main functional role in these cultures.

Oral Presentation 4-04

Discovery of genes that mediate and regulate hemicellulose biosynthesis

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One attractive strategy for identifying the required genes is to perform expression profiling during periods of rapid hemicellulose deposition. Many plants produce large quantities of specific polysaccharides as storage polymers in developing seeds. Earlier studies of these seed systems has been used to identify proteins involved in mannan and xyloglucan biosynthesis. We are extending these earlier studies by using 454 sequencing technology to perform deep EST sequencing at various stages of seed development during and just before rapid synthesis of mannan (Fenugreek-*Trigonella foenum-graecum*), xyloglucan (Nasturtium-*Tropaeolum majus*), or arabinoxylan (Psyllium-*Plantago ovata*). Analysis of the sequences obtained has confirmed the expression of genes known to be involved in the biosynthesis of these polysaccharides. In addition, a number of other genes have emerged as strong candidates for involvement in the production of these polysaccharides or in regulation of these pathways. Among the candidates that have been identified are putative sugar nucleotide biosynthetic enzymes, putative sugar nucleotide transporters, putative and known glycosyltransferases and glycan synthases, proteins of unknown function, and transcription factors. Many of the genes have homologs that are expressed in developing wood or in other plant tissues where secondary wall synthesis is occurring rapidly, providing support for the hypothesis that the same genes are involved in depositing these polymers in secondary cell walls. Promising examples from each class of candidate genes have been selected for detailed functional analysis. Selected examples from each polysaccharide will be presented on the poster.

Oral Presentation 4-05

Mining the metatranscriptome of the rumen microbiota for feedstock-targeted glycosyl hydrolases

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Stable and highly active cellulolytic enzymes are essential for the efficient conversion of lignocellulosic biomass into fermentable sugars. Natural cellulolytic systems such as the bovine rumen are known to harbor fibrolytic microbes and represent promising sources of enzymes for biomass degradation. In the project presented here, pyrosequencing has been employed to identify feedstock-targeted enzymes within the transcriptome of rumen microbial communities.

Switchgrass and alfalfa were incubated for 72 hr in the bovine rumen and nucleic acids were extracted from the fiber-associated microbial communities. Based on 16S rRNA sequencing, the microbial community tightly associated with switchgrass differed significantly from that associated with alfalfa, suggesting that distinct sets of organisms are involved in degrading each of these two feedstocks.

Expression profile of the switchgrass-associated organisms was determined by 454-pyrosequencing. We identified 85 highly expressed putative glycosyl hydrolases and 201 unique glycosyl hydrolase transcripts. ~4,000 genes without assigned function were highly expressed and some of them might encode truly novel proteins involved in biomass degradation. We will expand our analysis to expression profiles of rumen microbial communities associated with other biofuel crops.

The results obtained in the course of our project indicate that the fiber-bound microbes are indeed a rich source of putative cellulolytic enzymes that might be useful for large-scale biofuel production. Currently we are developing techniques to capture the full-length sequence of selected transcripts from rumen community DNA. Expressing the recombinant proteins and subjecting them to detailed physicochemical characterization will allow us to verify the sequence-based annotation of the transcript tags.

Oral Presentation 4-06

Discovery of Switchgrass Genes through Genomics to Improve Biomass Composition and Conversion

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The ability to manipulate the relative proportions of cell wall components is critical to improving a bioenergy crop like switchgrass. An essential step towards this goal is to understand the genetic components involved in cell wall biosynthesis. To address this problem, we are using genomics tools to discover and select genes from switchgrass. We are using two parallel approaches in the selection process: (1) identification of differentially expressed genes through gene expression analysis utilizing GRASS chip technology, and (2) misexpression analysis in Arabidopsis to evaluate the potential effects of identified genes on cell wall composition & conversion and on biomass accumulation. In this poster, we will present preliminary data to demonstrate the effectiveness of our selection efforts to identify candidate genes that can be used to transform and manipulate switchgrass to improve conversion efficiency to biofuels.

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Oral Presentation 5-01

Thermostable fungal lignocellulosic biomass saccharification enzyme cocktail

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Lignocellulosic biomass is the most abundant, least expensive renewable natural biological resource for the production of biobased products and bioenergy is important for the sustainable development of human civilization in 21st century.

For making the fermentable sugars from lignocellulosic biomass, a reduction in cellulase production cost, an improvement in cellulase performance, and an increase in sugar yields are all vital to reduce the processing costs of biorefineries. Improvements in specific cellulase activities for non-complexed cellulase mixtures can be implemented through cellulase engineering based on rational design or directed evolution for each cellulase component enzyme, as well as on the reconstitution of cellulase components. In this presentation, we will update on DSM efforts on developing thermostable enzyme cocktail for saccharification of lignocellulosic biomass.

Oral Presentation 5-02

Impact of solids loading on the economics of a lignocellulosic biomass to ethanol conversion process

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Technoeconomic modeling describes the impact of performance tradeoffs on process economics and helps guide research efforts. A rigorous model in Aspen Plus was used to compute material and energy balances for a biomass-to-ethanol conversion process using dilute acid pretreatment of corn stover, separate enzymatic cellulose hydrolysis and fermentation, and ethanol distillation. Subsequent economic analysis determined the minimum ethanol selling price (MESP) for the process, assuming nth-plant equipment and operating costs. To understand the cost impact of solids loading in the hydrolysis step, a correlation of cellulose conversion as a function of enzyme and solids loading was developed from bench-scale enzymatic hydrolysis experiments on pretreated corn stover slurries. Higher solids processing should show more favorable economics, since stream volumes are reduced and less energy is required to separate the product from water. However, using the correlation it was found that the MESP rises at high solids loading due to a drop in cellulose conversion yields. For assumed enzyme costs of $10-$15/kg protein, the minimum MESP was between 15-20% total solids. For projected costs of $2.50-$5/kg, the minimum MESP occurred at 25% total solids, and was relatively flat from 15-25%. Only in the ideal case where conversion was independent of solids loading did the MESP decrease monotonically from 5 to 30% total solids. These results indicate the economic benefit of processing at higher solids loading, but highlight the need to develop enzymes that maintain conversion yields at high solids.

Oral Presentation 5-03

Substrate-based Limitations in the Enzymatic Hydrolysis of Cellulose: Crystallinity, Reactivity and Adsorption

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The enzymatic hydrolysis of crystalline cellulose encounters various limitations that are both substrate- and enzyme-related. They directly impact the rate of the reaction and lead to its dramatic slowdown, observed especially at high degrees of conversion. Although the initial crystallinity of cellulose plays a major role in determining the rate of hydrolysis, it was shown not to evolve over the time of conversion by cellulases, implying other reasons for the decrease in rate. Using increasing concentration of phosphoric acid to generate acid-swollen Avicel, samples with intermediate crystallinity indexes were obtained and their subsequent enzymatic hydrolysis gave a clearer overview of the relevance of the initial degree of crystallinity on reaction rate. Change in adsorption capacity and decrease in reactivity along conversion were also confirmed to be involved. Reactivity (measured as of glucose production rate from restart experiments) experienced a serious drop already after 5% conversion, supporting the hypothesis that the cellulose surface has been modified by the action of the enzymes. Both X-ray diffraction and solid state CP/MAS 13C-NMR were employed and gave insight into molecular changes occurring with cellulose along the conversion. The (021) face was shown to be converted first by pure cellobiohydrolase. Strategies to improve the overall reaction rate will be presented.

Oral Presentation 5-04

Characterization of novel bacterial expansins that promote enzymatic hydrolysis of plant cell wall polymers

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Plan cell wall polymers, which are considered to be the most abundant renewable resource on earth, are mainly composed of cellulose, hemicellulose and lignin. Due to the recalcitrance of cellulose itself and the protective barriers of lignin, the enzymatic hydrolysis of cellulose and hemicellulose to obtain sugars has long been a challenge. In the process of cell growth, a plant cell wall protein, expansin, is found to be involved in inducing extension of cell wall without hydrolysis. In our study, the functions of novel expansins from bacterial sources, which are the structural homologs of maize expansin, EXPB1, were targeted and elucidated for the first time in this area (Kim, E. S. et al., J. Biotechnol. 136S: S426, 2008; H. J. Lee et al., J. Biotechnol. 136S: S343, 2008; Kim, E. S. et al., Biotechnol. Bioeng. In press) since expansins only from eukaryotic sources such as plants, animals or fungi were so far functionally characterized. Many eucarytic expansins were found, but none of their overexpression in microorganisms has been successful yet. The bacterial expansins expressed in a soluble form in the present work showed binding and weakening activities towards cellulose or xylan and also exhibited significant synergistic activity with enzymes in the hydrolysis of cellulose or xylan. These findings might have opened a door to the possible applications of bacterial expansins in effective enzymatic conversion of lignocellulosic biomass into sugars.

Oral Presentation 5-05

Industrial level production of enzymes by Trichoderma reesei for cellulosic bioethanol

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Efficient expression is the key for economically-viable bulk production of enzymes. Filamentous fungi naturally secrete large amounts of proteins into the growth medium and are commonly used for the large-scale manufacture of proteins, particularly industrial enzymes (1). However, although other fungal proteins are efficiently expressed, expression of gene products from other organisms such as thermophilic bacteria, is subject to a number of bottlenecks that reduce yield. Following a proteomic analysis of the Trichoderma secretome, we constructed four expression vectors that utilise either the cbh2 or egl2 promoters. Thermophilic xylanases are of particular interest for efficient hydrolysis of the hemicellulosic component of woody materials into fermentable pentose sugars (2). The xynB gene encoding xylanase B from the thermophilic bacterium Dictyoglomus thermophilum has been inserted into the vectors for heterologous expression as a model system. This enzyme is particularly effective in the hydrolysis of both soluble and insoluble xylan in hemicellulose. The codon usage of the xynB gene has been modified for expression in T. reesei. Expression of xynB from the pEG2-cbmlin and pCBH2-sigpro vectors was found to be greater than that using the EG2-sigpro and CBH2-cbmlin constructions, based on zymogram analysis and liquid enzyme activity assays. We will discuss the ability of the new promoter constructions to drive xylanase production as influenced by the structural differences in the expression cassette motifs and their contribution to improved yields under fermentor conditions (1) Nevalainen et al (2005), Trends Biotechnol. 23: 468 (2) Viikari et al (2007), Adv. Biochem. Eng. Biotech. 108: 121

Oral Presentation 5-06

Use of palm kernel press cake for production of bioethanol and feed

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Palm kernel press cake (PKC) is a residue from palm oil extraction, composed mainly of polysaccharides from cell-wall material, of which the carbohydrate mannan is the most predominant. Due to increased demand for plant oils globally there has been a tremendous increase in palm oil production in recent years. In Indonesia and Malaysia large amounts of palm kernels are processed at centralised plants, which make PKC an ideal feedstock for further processing into e.g. bioethanol or other biorefinery processes. At present the PKC is only used for feeding purpose, but due to the high content of mannan, it cannot be included in high amounts in broiler diets.

Mannose can readily be fermented to ethanol by normal yeast Saccharomyces cerevisiae. The first step in the conversion of PKC is then an efficient hydrolysis of polymers, mainly mannan, into fermentable monomers. The present study examines the pretreatment requirements and especially the enzymatic hydrolysis of polysaccharides from the cell-wall material present in PKC by various enzyme preparations in order to achieve high amounts of free monosaccharides. The process has been tested at high solids concentrations and it was possible to operate at up to 50% DM. The resulting hydrolysates were easily fermented by Saccharomyces cerevisiae with high ethanol yields. Various process configurations (SHF and SSF) were also tested. In addition, the processing of PKC resulted in a new feed product with increased protein content, which could be beneficial for the feeding value of this new residue.

Oral Presentation 6-01

Metagenomics for mining new deconstructive enzymes, exploring enzyme diversity and screening cellulolytic activities

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Plant biomass is the most abundant biopolymer on earth and has long been recognized as a potential source of mixed sugars for bioenergy production. Our goals are to understand the diversity and metabolic capabilities of the complex microbial communities, and to exploit their dynamics for converting plant biomass into feedstock for biofuels production. Metagenomics allows the discovery of new enzymes from microbial communities, especially from organisms that are unknown or have never been cultivated. From an anaerobic microbial community actively decaying poplar biomass, metagenomic DNA was isolated and microbial species distribution was investigated via 16S and 18S rRNA sequencing. Saccharomycetes composed the major group among the Eukaryotes, and Clostridiales composed the major group among the Bacteria. No major population of Archaea was found in this microbial community. Using the 454-GS-FLX Titanium pyrosequencing, approximately 580Mbp metagenomic DNA was sequenced. Preliminary blastx searches identified approximately 4,000 glycosyl hydrolase homologues. Five candidates were selected for further investigation based on homology to enzyme families of interest (families 5, 9, 48, and 51 representing cellulase, hemicellulase, and xylanase activities) and quality of sequences. Full-length open reading frames were obtained using inverse PCR and DNA walking, and gene cloning is presently in process. A lambda-based expression library of one isolated strain from the community was also constructed and enzyme activity screening is in process. Our metagenomic studies successfully provided insight into the microbial community composition as well as a resource of diverse, community-encoded glycosyl hydrolases, for mining new deconstructive enzymes and screening cellulolytic activities.

Oral Presentation 6-02

Cellulolytic Extreme Thermophiles and Hyperthermophiles from Terrestrial Hot Springs

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Extremely thermophilic microorganisms are good candidates to provide highly stable, active enzymes for cellulose deconstruction. Elevated operating temperatures may also be beneficial in fermentations producing biofuels. To that end, cellulose-degrading microorganisms from hot springs in Nevada and Northern California were enriched on anaerobic medium containing ground (80 mm diameter particles) Miscanthus sinensis as the sole carbon source. Enrichments were performed at temperatures between 70°C and 90°C. Organisms growing in the enrichments were identified by 16S RNA clone libraries generated from PCR amplification of 16S coding sequences from DNA extracted from the enrichments, or 454 sequencing with bar-coded primers from the same DNA. Enrichments at 70°C contained a variety of thermophilic bacteria related to strains known to be cellulolytic as well as some that were not closely related to any characterized strain and may represent new genera of cellulolytic organisms. In order to verify that cellulolytic organisms were present in miscanthus enrichments, secondary enrichments were made using Whatman #1 and #3 filter paper as the carbon source and a portion of the primary enrichments as the inoculum. At 90°C the dominant microorganisms present in miscanthus or filter paper enrichments were archaeal, establishing a role for archaea as cellulose degrading organisms and as potential targets for the identification of new cellulases. Cellulase assays were performed to determine the activity of free and bound cellulases in each of the enrichment cultures. The media constituents were refined to promote accelerated cellulose degradation, and pure cultures, including archaeal species, were isolated from the enrichments.

Oral Presentation 6-03

Mining Clostridium thermocellum for Enzymatically Active Carbohydrases

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The efficient hydrolysis of biomass to 5 carbon and 6 carbon sugars is limited by the lack of affordable, high specific activity enzymes. Screening of genomic and metagenoic libraries for new biomass-degrading enzymes has had only limited success. We examined a number of screening strategies using Clostridium thermocellum (Cth) as a target-rich model organism to validate the efficiency of capturing carbohydrases that may prove useful for biomass degradation. The Cth genome has been sequenced and is predicted to have genes for over 60 potential biomass-degrading enzymes associated with the cellulosome, and another 18 enzymes that are noncellulosomal. Two different cloning systems were used for gene expression and two different screening methods were utilized for identification of positive clones. A comparison of the methods showed large differences both in the total number of positive clones identified as well as large differences in the total number of different enzymes captured. This poster also describes the gene products that were captured by the individual screens.

Oral Presentation 6-04

Genome shuffling of Penicillium decumbens to improve its cellulase production

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Genome shuffling is an efficient approach for the rapid improvement of important industrial microorganisms. The cellulase production of P. decumbens was improved by genome shuffling of an industrial catabolite-repression-resistant strain JU-A10 with its mutants. The mutants were obtained by UV-EMS mutation or N+ ions implanting of JU-A10, and prepared for protoplasts fusion. Six improved fusant strains were selected as parents for the second genome shuffling. Three fusants, GS2-15, GS2-21 and GS2-22, were selected based on their capacity to show clear hydrolysis halo on the two-layer plate containing 2% glucose and 5% ball-milled microcrystalline cellulose. The fusants showed 100%, 109% and 94% increase in filter paper activity, respectively. The cellulase production of the fusants on various substrates, such as corn stover, wheat straw, bagasse and the corncob residue from xylitol production, were studied. It was obvious that the three fusants could produce abundant cellulase much earlier than the parental strain JU-A10, the maximum volumetric productivity of GS2-15, GS2-21 and GS2-22 was 92.15, 102.63, and 92.35 FPU/L/h respectively when fermented with the corncob residue at 44 h, which was 117%, 142%, 118% higher than that of JU-A10 (42.44 FPU/L/h at 90 h). Higher glucose yield from the corncob residue were also observed by using the fermented broth of the fusants as crude cellulase. The improved cellulase production of the fusants was proposed to be mainly due to their increased growth rates and enhanced secretion of extracellular proteins.

Oral Presentation 6-05

Development and characterization of xylose-fermenting strains of Saccharomyces cerevisiae based on structure-based engineering of key metabolic enzymes

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Metabolic engineering of Saccharomyces cerevisiae for xylose fermentation has often relied on insertion of a heterologous pathway consisting of NAD(P)H-dependent xylose reductase (XR) and NAD-dependent xylitol dehydrogenase (XDH). Low ethanol yield and formation of fermentation by-products such as xylitol and glycerol seen for many of the strains constructed in this way have been ascribed to incomplete coenzyme recycling in the steps catalyzed by XR and XDH. We have used structure-guided engineering of Candida tenuis XR and Galactocandida mastotermitis XDH to obtain enzyme pairs that display well matched utilization of NAD(H) and NADP(H). Yeast strains producing XR and XDH variants that show altered coenzyme selectivity exhibit notably improved fermentation capabilities as compared to the reference strain expressing the genes for the wild-type enzymes.

Oral Presentation 6-06

Understanding the Relationship of Toxic Compounds in Corn Stover Hydrolysates and Their Inhibitory Effects on Ethanologen Growth and Fermentation

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Overcoming the effects of hydrolysate toxicity after pretreatment and/or enzymatic hydrolysis is a key technical barrier in the biochemical conversion process for biomass feedstocks to ethanol. Yet, the complexity of the hydrolysate toxicity phenomena and the lack of systematic studies and tools surrounding this issue has prevented us from fully understanding relationships involving toxic compounds in hydrolysates, their relative inhibitory effects on ethanologen growth and fermentation, and the impact of various conditioning approaches to effectively mitigate these inhibitory effects. We conducted systematic studies to analyze chemical composition of the hydrolysates and developed quantitative, high throughput biological growth assays to obtain the inhibitory kinetics for individual compounds, along with correlation of growth and fermentation performance in conditioned diluted acid corn stover hydrolysates and hydrolysate fractions. These key findings provide important insights for understanding hydrolysate toxicity and provide guidance for potential process development in both pretreatment and hydrolysate conditioning operations, along with potential future strain improvement and tolerance strategies. The tools that have been developed can also be more broadly applied to other feedstock and pretreatment process situations as well as other ethanologens.

Oral Presentation 7-01

Commercialization of Second Generation Biofuels: An Independent Engineer's View

Doug Dudgeon, Harris Group, Seattle, Washington

The renewable fuels industry, which experienced steady progress through first generation biofuels such as corn based ethanol and oil seed based biodiesel has slowed significantly in the commercialization of second generation technologies including cellulosic ethanol and algae based biodiesel. While the base technologies are promising, there are structural issues that go beyond technology development which impede implementation. The purpose of this presentation is to provide a detailed background on the way large scale renewable energy technology is financed and implemented, outlining a roadmap for stakeholders bringing these technologies to market.

A brief overview of the factors that allowed first generation biofuels to grow rapidly will be covered and contrasted with the factors that are limiting next generation technologies. A review of the capital cost of emerging biofuel technologies will be provided, along with a summary of the engineering approach and construction contracting options, and how they can impact the ability to gain financing. Common forms of equity and project financing will be reviewed. Finally, the topics will be summarized with a roadmap of how large, commercial scale emerging biofuel projects can be moved forward. This presentation comes from direct experience by the author in taking an emerging technology from pilot scale to a full size commercial facility.

Oral Presentation 7-02

Successful commercialization of second generation biorefineries

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Successful commercialization of second generation biorefineries will require navigating significant technical, commercial and supply chain challenges. This presentation will review the technical challenges and report on progress to date on the integration and optimization of technologies from parent companies and others. It will also report on the innovations in business models and collaborations that will mitigate the commercial risk of pioneer deployment.

Oral Presentation 7-03

Development and Deployment of Consolidated Bioprocessing for Production of Ethanol

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Consolidated Bioprocessing ("CBP") employs single microorganism that simultaneously generates sugars and produces ethanol from wood and other forms of pretreated lignocellulosic biomass. Pretreatment opens up the structure of wood by disrupting the lignin seal and exposing cellulosic plant cell wall components. This enables CBP microorganisms to access the cellulosic constituents, hydrolyze them, and produce ethanol. The microorganisms not only ferment sugars to ethanol, but also generate the biocatalysts – enzymes – that are needed to break down cellulose into fermentable sugars. Mascoma's research is combining naturally occurring metabolic activities into a single microorganism by modifying the fermentative pathways of nature's most efficient processors of cellulose, including the thermophilic anaerobic bacterium, Cl. thermocellum, to produce high yields of ethanol from hardwoods and biomass feedstocks. In addition, the ability to modify the fermentative pathways of a thermophilic anaerobe to achieve high ethanol yield from sugars was previously demonstrated through metabolic engineering of T. saccharolyticum. The practical application of Consolidated Bioprocessing is based on combining new biotechnology and unique but established process engineering. We discuss the four basic steps convert wood to ethanol; (1) feedstock preparation (chipping); (2) simple pretreatment of wood to make it accessible to microbial action; (3) fermentation to ethanol; and (4) product separations for recovery of fuel-grade ethanol and lignin. The design of a commercial facility is being informed by pilot and demonstration scale validation of fermentation parameters and designs that have evolved from work of NREL, DOE and USDA sponsored programs in both the public and private sectors.

Oral Presentation 7-04

Demonstration plant scale production of lignocellulosic ethanol

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The Energy Independence and Security Act (EISA) of 2007 calls for the blending of at least 36 billion gallons of biofuels in 2022. The Act mandates that cellulosic biofuels are to contribute 16 of the 36 billion gallons by 2022 with 100 million gallons by 2010. A number of companies have announced plans to build and operate pilot and demonstration facilities in the United States to validate proprietary cellulosic technologies at scale. Verenium completed construction of a 1.4 MM gal/yr facility in mid 2008 and has been in the process of commissioning the plant. This talk will describe and discuss progress towards completion of the commissioning of Verenium's demonstration plant and commercialization efforts in order to achieve the targets set forth in the EISA of 2007.

Oral Presentation 7-05

Ethanol from wheat straw – A reality in Denmark from November 2009

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In Denmark, DONG Energy subsidiary Inbicon is constructing a plant to demonstrate their proprietary process for conversion of wheat straw to ethanol to be ready for the Copenhagen Climate Summit late 2009. From 30,000 tonnes of wheat straw annually, the plant will produce 1.43 mill. gallon ethanol, 8,250 tonnes of biopellets and 11,100 tonnes of cattle feed. The investment is $56 mill. of which $14 mill. is funded by the Danish government.

In the 1990's, Danish power companies started using biomass for power production. In 2002, a R&D project ("Co-production biofuels") partly funded by the European Commission was initiated to extract more value of the straw. Several technological breakthroughs were achieved and a 1 ton/hr straw pilot plant was inaugurated in 2005. Based on the success of the project, the subsidiary Inbicon was formed to commercialize the technology.

The core technology, a hydrothermal pretreatment and enzymatic hydrolysis, works at high dry matter content, enabling efficient liquefaction with low enzyme doses, a robust fermentation and resulting high ethanol concentration. In addition to ethanol, the process produces a supreme dry biofuel suitable for bio pellets and a C5-molasses, which can be used for animal feed or ethanol production with suitable organisms.

The demonstration plant is the first stage of the Inbicon Biomass Technology Campus. Additional technologies for production of high value products from biomass will be developed, tested and added to the ethanol facility, making the plant an industrial scale biorefinery.

Oral Presentation 7-06

Third generation biofuels from lignocellulosic biomass materials

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In the presentation we will present our integrated concept for production of biofuels and bioproducts using both bio-chemical as well as thermo-chemical reactions. Wheat straw will be our base raw material. The initial step will consist of a pretreatment constructed to make the carbohydrates ready for enzymatic hydrolysis. This pentose fraction can be collected by separation from this stream and used for bioethanol production or as a feedstock for production of new chemicals using microbial catalysts producing chemicals such as succinic acid, acetoin, threonine. In the presentation we will show data from bioethanol production in pilot scale using *Thermoanaerobacter* BGL1. Furthermore, pentoses can be converted using chemicals catalysis into other chemical products, for example, furfural, furfural alcohol, and levulinic acid and it's derivates. The remaining stream contains the solids including the polymers of carbohydrates which can be hydrolyzed by cellulases into mainly glucose and minority portion of other sugars.Lignin-containing residues from biorefineries have previously received little attention except as a fuel for producing the power for fuelling the biorefinery. In the presentation we will show potential alternative ways to thermochemically convert lignin-containing residues into 1) advanced biofuels (hydrocarbons), for example via pyrolysis and/or hydrothermal liquefaction and downstream processing, 2) methane, for example via wet gasification, or 3) chemical products. We will further show our initial economical assessment of the different options and how these different steps will affect the overall economics of the biorefinery.

Oral Presentation 8-01

An up-to-date overview and comparison of sustainability certification schemes for biofuels

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Recently, the use of biofuels is heavily criticised, doubting its GHG impact and potential to reduce emissions. Other objections relate to the possible negative impacts on water, biodiversity and (direct and indirect) land-use changes due to biomass production for biofuels. While the main concerns currently are focussed on especially first generation feedstocks, they are also highly relevant for second generation lignocellulosic feedstocks. With the strong growing increase in biofuels demand, the need to secure the sustainability of biofuels is acknowledged by various stakeholder groups. Developing principles and establishing certification schemes are recognized as possible strategies that help to ensure the sustainable production of biofuels. This paper presents an up-to-date overview of the wide range of efforts undertaken towards the development of sustainability principles, criteria, indicators and biomass certification systems. The stakeholder groups included are governments, international bodies, NGOs and companies. The paper focuses on which key differences are found between the criteria formulated, the methodologies developed and the certification systems envisioned by the various initiatives. Their feasibility, cost effectiveness, and contribution to the removal of trade barriers are evaluated. Special attention is given to (partially) conflicting methodologies, e.g. differing GHG emission calculation methodologies, which can cause major differences in total avoided emissions. The paper analyses these developments and provides recommendations on possible harmonization efforts, with a specific focus on the current developments in the European Union. Within this context, comments and possible solutions to overcome different approaches are obtained from different stakeholder groups as industry and policy makers.

Oral Presentation 8-02

International trade in lignocellulose biomass

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The future use of bioenergy will mostly focus on solid biofuels such as woodpellets and agri- products, as well as liquids for power generation (vegetable oils). Looking at the future developments and intertwining, the raw materials market will also be the source market for the second generation bio liquids such as bio ethanol and biodiesel. In the following graph the interfacing between those markets is highlighted. The distinction between upstream, midstream and downstream is made to clearly split market activities and areas (see fig. 1)

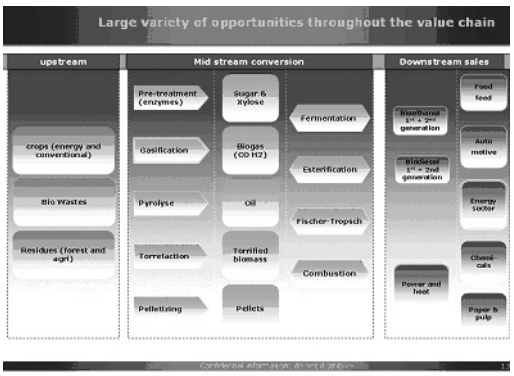

Fig 1.

This presentation will discuss the expected future developments in the international trade of lignocellulosic feedstocks for energy purposes, including the required logistics and the potential impact of the advent of large-scale second generation biofuels production. Moreover, the importance of sustainable sourcing will be highlighting, presenting the Green Gold Label for Solid biomass as an example of a track-and-trace certification system for sustainable biomass, including production, processing, transport and final energy transformation.

On the author:

Peter - Paul (P.J.W.G.) Schouwenberg, Director Biofuels and Development at Duferco Energy International and Task leader of IEA Bioenergy Task40, has long-term experience in the international sourcing of large quantities of solid and liquid biomass for electricity production. In his former position at essent, he was directly involve din the development of the Green Gold Label.

Oral Presentation 8-03

Food and fuel: Investigation into integrating animal feed production with cellulosic ethanol from switchgrass

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As commercial cellulosic ethanol becomes a reality, concerns regarding land use are becoming increasingly prevalent. Critics claim a large cellulosic ethanol industry will result in decreased land available for food production, leading to increased food prices as well as increased carbon emissions due to indirect land use change. However, these concerns assume a "business as usual" approach to farm management. If bioenergy is to become a significant portion of agriculture, then it is likely that the animal feed market will be adapted to reflect these changes. Dedicated energy crops such as switchgrass have the potential to produce more carbohydrates and protein per acre than corn and soy, respectively, thus leading to the possibility that these crops could be integrated into animal feed rations. These feed co-products could be produced on-site at the biorefinery, or more likely at a regional biomass processing center (RBPCs).

We propose several methods for integrating animal feed production with cellulosic ethanol from switchgrass using ammonia fiber expansion (AFEX) pretreatment technology. AFEX-pretreated fiber can significantly increase the digestibility and feed quality of grasses, and thus potentially compete with more traditional energy feeds. Extracting proteins from grasses prior to pretreatment as well as recovering proteins after hydrolysis and fermentation will also be considered. Of particular importance is the quality and digestibility of the proteins, particularly for the essential amino acids. The potential for producing animal feed co-products is considered for both on-site production as well as at RBPCs, and the consequences of each taken into account.

Oral Presentation 8-04

Sourcing Stover: Results from the ISU Integrated Corn Stover Feedstock Supply Systems Project

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Corn stover is widely recognized as the most promising high volume, low cost lignocellulosic feedstock on which to base second generation biofuel production. However, several significant challenges confront this vision. This talk will summarize the results of a four year effort aimed at: (1) developing innovative harvesting and storage technologies to efficiently and economically move corn stover from the field to the factory gate with physical and chemical properties optimal for the conversion processes; (2) identifing genetic varieties of corn with specific properties attractive for biobased industries to enable a breeding program to enhance those properties; and (3) evaluating and optimizing systems of production, harvest and storage for efficiency, and economic and environmental sustainability.

Oral Presentation 8-05

A Life Cycle Impact Assessment Framework for Characterizing Human Health Benefits and Impacts from Emerging Biofuels

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Life Cycle Impact Assessment (LCIA) provides an assessment framework that addresses both benefits and impacts from emerging biofuels on human health from changes in chemical emissions. LCIA addresses the links among emissions, transport, human exposure, and health damage. We have adapted LCIA to compare the impacts of emerging biofuels, such as cellulosic ethanol and butanol, to California reformulated gasoline. This presentation describes the development of characterization factors (CFs), which yield important information on the human health effects and human damage related to the emissions-fate-exposure pathway of chemicals associated with these fuels. CFs track chemical releases to the environment at various fuel life stages including: 1) from drilling and/or harvesting to a refinery; 2) conversion and processing of fuel at a refinery; 3) storage, transport and distribution of the fuel; and 4) fuel combustion. CFs incorporate both an environmental fate factor and the human intake fraction (iF). Systems models that track the exchange of chemicals among air, water, soil, and plant compartments provide fate factors. The iF, which is the ratio of the chemical mass taken in by a population to the mass released, is used to express cumulative inhalation and ingestion exposures for a defined geographic region.
We present the CFs we use to compare emissions at various life cycle stages. Important in this analysis is the consideration of the variation and uncertainty of human exposures related to temporal and spatial scope of a fuel's life cycle, including local (e.g., refinery) and regional (e.g., agricultural systems and storage of fuel).

Oral Presentation 8-06

Process technology and integration options for a sustainable biorefinery

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This presentation will illustrate how advances in process development bring the economically attractive and environmentally friendly production of cellulosic ethanol within reach.

Life Cycle Analysis (LCA), as a holistic approach to evaluate the environmental profile of a biorefinery value chain, has been used side by side with techno-economic evaluations to guide researchers to the most sustainable design alternatives. LCA scenario analyses explain how technology choices, research progress and synergies with co-located facilities affect the cradle to refinery gate footprint of a cellulosic biorefinery.

Oral Presentation ST1-01

One Million Tons of Biomass Per Year-Feedstock Management for Large Scale BTL Plants

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In the next plant generation, synthetic biofuels from Fischer-Tropsch synthesis (BTL) will be produced in large scale facilities of 200,000 – 300,000 tons per year BTL output. Therefore, an amount of around 1 million tons of dry biomass is needed as chipped or pelletized wood or straw for CHOREN's Carbo-V® process. To supply this huge stream of matter, a detailed supply chain management concept has to be established starting from forestry and short rotation coppice plantations to logistics and processing (chipping, drying) of wood up to storage management. CHOREN's Carbo-V® gasification concept is optimized for biomass feedstock with a water content of around 15 % w/w. By these circumstances, the following topics have to be dealt with:

- Sources: all kinds of wood can be used technologically (short rotation coppice, round wood, fresh wood, forest energy wood, waste wood, wood chips) and to a certain extent straw
- Transport and storage of untreated material (rail, road, ship)
- Elimination of contaminants
- Chipping of wood and pelletizing of straw and small wood particles
- Drying of chipped biomass particles
- Biomass storage and storage policy
- On-site biomass transport

We will present a biomass sourcing concept and the results of a supply chain management analysis and engineering study performed to gain insight into designing a specific site to manage a flow of 1 million tons of dry biomass per year from sustainable sources. This first of its kind sourcing and logistic study for large scale biomass gasification in Germany/Europe is proving to be extremely helpful to further understanding of supply concepts.

Oral Presentation ST1-02

POET Update on Project LIBERTY

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As the largest ethanol producer in the United States and in the world, POET is committed to meeting the ambitious targets of the Renewable Fuels Standard through the commercialization of cellulosic ethanol. Project LIBERTY will transform POET Biorefining – Emmetsburg from a strictly grain-to-ethanol plant to include ethanol from cellulose or biomass. Once complete, the facility will produce 125 million gallons of ethanol per year.
Following a successful start-up in the fourth quarter of 2008, POET Research Center in Scotland, S.D. is now producing cellulosic ethanol at a pilot scale, completing a crucial step toward development of commercially viable cellulosic ethanol. The Scotland plant is producing ethanol at a rate of 20,000 gallons per year using corn cobs as feedstock. The $8 million endeavor is a precursor to the $200 million Project LIBERTY that will begin production in 2011.

Oral Presentation ST1-03

Commercialization of Biomass Ethanol at Abengoa Bioenergy

Quang Nguyen, Abengoa Bioenergy, Chesterfield, MO

Abengoa Bioenergy is a world leader in bioethanol production with facilities in operation and under construction in Europe, USA, and Brazil. As part of the diverse portfolio of biofuels, Abengoa is advancing lignocellulosic biomass ethanol commercialization through pilot plant process development (York, NE, USA), commercial demonstration (Salamanca, Spain) and upcoming commercial operation (Hugoton, KS, USA).

This presentation provides an overview of Abengoa Bioenergy's multi-pronged approach on biofuel development and progress on biomass ethanol commercialization.

Oral Presentation ST1-04

Lignocellulosic Ethanol-Breaking Through the Barriers

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Novozymes is, as an independent enzyme technology provider, focusing on enabling the industry in a wider context to commercialize lignocellulosic ethanol. Through an unprecedented research effort with more than 150 scientists, Novozymes is breaking through the barriers for commercialization. Novozymes is developing highly cost effective novel enzyme systems, and has recently launched two new state-of-the-art products. With leading ethanol producers in US, China, Brazil and in Europe, processes are developed and integrated leading to total cost scenarios that could make sustainable 2G ethanol production competitive with gasoline in the near term. Novozymes is working in China with COFCO and Sinopec, two large state owned companies, and together the three parties represent all parts of the value chain. Progress from the joint development project on corn stover to ethanol will be outlined.

Oral Presentation ST1-05

Why Green House Gas Balances are Good for Business

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The British Sugar Group (BSG) is amongst the leading sugar producers in the world. It has 39 factories, in 8 countries, at the last count, processing sugar beet and cane to produce sugar, and a wide range of co-products, including bioethanol. When the UK Government decided to encourage biofuel production it also insisted on carbon and sustainability reporting of all biofuel in the UK. Because of this, BSG designed and integrated their bioethanol plant into existing sugar process to minimise overall fuel consumption. BSG has also worked with its beet growers to optimise agricultural inputs and this has reduced fertiliser consumption significantly over the years. Not only does this reduce green house gas emissions, it also means lower cost. Another area which has improved the cost base, improved profitability and reduced overall GHG emissions has been to diversify into other products from the same inputs to the existing process, like producing tomatoes from the waste heat and CO2 from our power plant and to cogenerate electricity for export to the local grid. It doesn't stop there. We have done a piece of work to benchmark what we do with the worlds best, this has shown that there is great scope to improve in many areas, and also shows, when you look at the world as a whole there is tremendous scope for us all to improve. If we all improve the basics and add on the satellites we will not only improve GHG balances, but also improve profitability.

Oral Presentation ST1-06

Deployment at Scale: A Grand Challenge for Advanced Biofuels

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Perhaps the single most distinguishing feature of the transportation fuel market is its size. To have a meaningful impact in the fuels market, deployment at scale is a key challenge to be addressed. Because even the smallest of changes are difficult to execute at mega-scale, the question of scaling new technologies or manufacturing regimes is arguably best approached by making the most conservative changes to existing infrastructure. One solution to scaling a hydrocarbon-based, advanced biofuel involves retrofitting sugar cane ethanol facilities.

By volume, the largest-scale production of biofuel is ethanol. Sugar cane ethanol facilities in Brazil deploy more than 10 million acres of sugar cane to produce 350 million tons of cane crush which is fermented into 8 billion gallons of fuel. To leverage the infrastructure built around the mills for advanced biofuel production, Amyris employs a "capital light" model, replacing the ethanol-producing yeast with a hydrocarbon-producing yeast. Because a hydrocarbon is produced, rather than a water-miscible alcohol, this advanced biofuel can be adopted into the existing fuel infrastructure. This model presents obvious advantages for rapid scaling and deployment and reveals some of the grand challenges presented to any scale up operation.

Oral Presentation ST1-07

Global Feasibility of Large-scale Biofuel Production

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There is currently great confusion and uncertainty regarding the role biofuels should play in the world's energy future. In response, we have initiated a project to test the hypothesis that the welfare of both humanity and the environment can be better with large-scale production of biofuels than without it. The project is structured in three stages:

1) Hold public meetings at five locations around the world during the second half of 2009 and first half of 2010, to develop a project plan, form a team, and recruit support for stage 2.

2) Answer the question: Is it possible for biofuels to meet a substantial fraction of future world mobility demand without compromising other vital needs: feeding humanity, providing fiber, maintaining and where possible improving soil fertility, air and water quality, biodiversity and wildlife habitat, and achieving large greenhouse gas emission reductions that are not substantially negated by land use changes.

3) Given an affirmative answer to this question, broaden the analysis and team as necessary to address desirable transition paths and policies, ethical and equity issues, impacts of climate change, and local-scale analysis including rural economic development.

Our proposed approach is distinct from prior studies of biomass resource availability and is likely necessary if a low-carbon transportation future is to be realized. Project results will provide critical guidance, both toward the overall feasibility of a biofuel-intensive future, and toward defining the policy and land use trajectories that foster this outcome.

Oral Presentation ST2-01

Overview of Algal Biofuels: From Cell Biology to Biotechnology

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Microalgae are typically aquatic, photosynthetic, oxygenic autotrophs that are smaller and less structurally complex than land plants. Many microalgae have the ability to produce substantial amounts (e.g. 20–50% dry cell weight) of storage neutral lipids/oils mainly in a form of triacylglycerols (TAG). Since TAG can be converted to biofuels (such as surrogates of gasoline, kerosene and diesel), microalgae have been considered a promising alternative, renewable source of feedstock for biofuels. The advantages of algae over oil crop plants for biofuels are that algae have a considerably higher TAG production potential, are a non-food source, can utilize marginal lands and wastestreams (e.g., wastewater and CO2), thereby providing additional environmental benefits. Since the concept of microalgae-based biofuels has been explored to only a limited extent over the past few decades, a scalable, commercially viable production system and process has yet to emerge. In this presentation, an overview of the current status of research on selection of high TAG-producing microalgal strains, the synthesis and regulation of TAG and the effects of environmental and biological factors on cellular TAG accumulation will be provided. An engineered system and process to produce TAG-derived biofuels and the technical limitations associated with existing algal TAG production technologies will be described. Finally, the path forward for microalgae-based biofuels with respect to both challenges and opportunities will be discussed.

Oral Presentation ST2-02

Biofuels from microalgae: Biochemistry and regulation of triacylglycerol accumulation in the model *Chlamydomonas reinhardtii*

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Many microalgae, including Chlamydomonas, accumulate triacylglycerols when cultures encounter certain environmental stresses such as nutrient limitation. However, the regulatory factors and enzymes that govern triacylglycerol biosynthesis in microalgae have not been studied at the molecular level. Chlamydomonas is used as a microalgal model to identify genes and regulatory mechanisms required for triacylglycerol biosynthesis following nutrient deprivation. Multiple global and focused approaches are pursued towards this goal: 1. A gene disruption mutant screen of 32,000 lines yielded 80 putative mutants, some of which are disrupted in genes central to the regulation of triacylglycerol biosynthesis. 2. Global expression analysis identified putative transcription factor genes induced under oil accumulation conditions. New expression vectors were produced for their analysis in Chlamydomonas. 3. Following proteomics analysis of isolated lipid droplets, the expression of a major lipid droplet associated protein was repressed by RNAi in Chlamydomonas resulting in increased oil body size. Betaine lipid biosynthetic enzymes were found associated with lipid droplets and are presumed to be critical to oil biosynthesis. 4. Of five putative genes present in the genome encoding diacylglycerol acyltransferases that catalyze the last reaction of triacylglycerol biosynthesis, two were confirmed to encode enzymes with oil biosynthetic activity following expression in a respective yeast mutant. These newly identified genes will provide novel targets for future engineering approaches towards optimizing microalgae oil production strains.

Funding for this work is provided by the US Air Force Office of Scientific Research and the Michigan Agriculture Experiment Station.

Oral Presentation ST2-03

Strain development of non-model photosynthetic microbes for biofuel production

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Aurora Biofuels has been successful in isolating photosynthetic micro-organisms which meet certain criteria important for the feedstock production process we are designing. These organisms must produce the right amount and types of oil; they must be harvestable; they must be amenable to our specialized pond mixing and oil extraction technologies; and they must grow outdoors stably. However, we have been unbiased in regards to the availability of molecular genetic tools for the organisms that we isolate; and as a result, we are working mostly with non-model organisms. As strain improvement via molecular genetics is essential to our business model, we have had to establish the requisite tools such as transformation technology and whole-genome sequences. We have also stumbled upon some interesting (and very useful) surprises.

Oral Presentation ST2-04

Low-cost photobioreactor technology: Promise, progress, and challenges

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Closed photobioreactors (PBRs) facilitate the cultivation of specific strains of algae and allow optimal growth conditions to be maintained. To date, however, the high capital and operating costs of photobioreactors have prohibited their use for "low-valued" products such as biofuels. Since it's founding in 2006, Solix Biofuels has maintained a sustained effort to develop low-cost, high-productivity photobioreactors. Three generations of PBRs have been developed, each with successively higher productivity, lower capital cost, lower operating cost, and lower energy utilization. Key design details and a performance summary of generation is presented. Extensive process modeling and economic projections have been performed. Increasing efforts are now being devoted to reducing the costs of downstream processing: harvesting, dewatering, oil extraction, and co-product processing. The driving biological factors, design rationale, modeling results, and projected product costs will be discussed. Solix has now begun construction of a large-scale production facility in southwest Colorado, on the Southern Ute Indian Reservation; relevant technology details of this expansion facility will be presented.

Oral Presentation ST2-05

Feedstock-flexible renewable oil production from heterotropohic algae using a proven, scalable system

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Solazyme is a renewable oil production company. The company harnesses the power of microalgae to renewably produce clean and scalable high performance biofuels and renewable industrial chemicals. Solazyme's microbial conversion technology process allows algae to produce oil in standard fermentation facilities quickly, efficiently and at large scale. These oils are tailored not only for biofuel production, but also as replacements for fossil petroleum and plant oils in a range of products running from household cleaning supplies to cosmetics and foods. Solazyme's oils provide compelling solutions to complex issues of fuel scarcity, energy security and environmental impact while fitting into the pre-existing multi-trillion dollar fuel infrastructure. A defined pathway to high production volumes is critical for a new technology to penetrate an industry producing million of barrels a day of products. Solazyme's industrial fermentation has already been demonstrated at the commercial scale.

The company is currently producing thousands of gallons of oil at scale and has refined that oil into the world's first algal based fuels that meet ASTM standards for biodiesel (ASTM D6751), renewable diesel (ASTM D975), and jet fuel (ASTM D1655). Solazyme has road tested these fuels for thousands of miles in unmodified diesel engines.
Solazyme has pioneered the use of cellulosic feedstocks for microbial production of oil. The company has used a combination of genetic engineering and high throughput screening technology to establish proof of concept through high efficiency fermentation on a range of feedstocks, including corn stover, bagasse, sugar cane, switchgrass, and beet pulp.

Oral Presentation 9-01

Assessment of Potential Fermentation Inhibitors in Wet Cake Cellulosic Hydrolysate

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The cellulose-rich fiber byproduct stream of corn dry milling (wet cake), is a potential feedstock for hybrid corn-and-cellulosic ethanol production. To simulate process we prepared a high solids (30% w/w) slurry from wet cake in light stillage from a dry grind ethanol plant using laboratory-scale reactors. The slurry was pretreated under liquid hot water conditions and hydrolyzed with a mixture of cellulases and hemicellulases resulting in a hydrolysate containing 115 g/L glucose, 55 g/L xylose and 46 g/L arabinose. This hydrolysate was subsequently fermented with the Purdue glucose/xylose co-fermenting *Saccharomyces cerevisiae* 424A (LNH-ST). Results of the fermentation showed very strong inhibition effecting utilization of both xylose and glucose.Potential sources include: concentration of secreted yeast metabolic products, osmotic stress or lack of free water from the high total dissolved solids, compounds liberated during the enzymatic deconstruction of the hemicelluloses and concentrated dissolved solids (non-protein) in the enzyme solutions. In order to study the combined effects, artificial hydrolysates were prepared YEP media or corn steep liquor in water with the addition of potential fermentation inhibitors. In comparison fermentations, potential inhibitors were selectively removed from artificial hydrolysates by a bed of adsorbent resins.

Oral Presentation 9-02

Conversion of municipal solid waste into bioenergy

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The amount of municipal solid waste (MSW) is increasing in the developed part of the world. This is apparently an environmental problem but it also holds a large potential for energy production and recycling. However, the major challenge of utilizing MSW is the heterogeneous composition of plastics, biomass and metals.

Here we present a solid-liquid technology for separation of biomass from other waste components followed by possible gasification or fermentation. The philosophy behind this concept is sustainability, no pre-sorting of MSW, full recovery and improved usage of the different waste components. This technology is based on an initial thermal treatment of the entire MSW material which opens up cardboard based packaging and pulp & paper fractions and makes the other organic parts more accessible. Secondly an enzymatic liquefaction is initiated of the biomass fraction which turns this fraction into a pumpable slurry and ease the washing and sorting of non-biomass substances. The third and finishing step is the separation by simple filtration which may include washing for extracting the bound biomass and for cleaning recyclable-non-organics.

Different commercial enzymes have been screened for their effect on liquefaction of municipal waste after thermal treatment of the material.

The overall concept of this process will be shown as well as results obtained so fare from small batch experiments and a pilot plant capable of processing 100 kg/hr continuously. Also required characteristics of the slurry product in order to fulfil demanding properties of different energy systems affecting this project will be discussed.

Oral Presentation 9-03

Evaluation of Target Efficiencies for Solid-Liquid Separation Steps in Biofuels Production

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Development of liquid biofuels has entered a new phase of large scale pilot demonstration. A number of plants that are in operation or under construction face the task of addressing the engineering challenges of creating a viable plant design, scaling up and optimizing various unit operations. It is well-known that separation technologies account for 50-70 % of both capital and operating cost. Additionally, reduction of environmental impact creates technological challenges that increase project cost without adding to the bottom line. Different technologies vary in terms of selection of unit operations; however, solid-liquid separations are likely to be a major contributor to the overall project cost. Despite the differences in pretreatment approaches, similar challenges arise for solid-liquid separation unit operations. A typical process for ethanol production from biomass includes several solid-liquid separation steps, depending on which particular stream is targeted for downstream processing. The nature of biomass derived materials makes it either difficult or uneconomical to accomplish complete separation in a single step. Therefore, setting realistic efficiency targets for solid-liquid separations is an important task that influences overall process recovery and economics. Experimental data will be presented showing typical characteristics for pretreated cane bagasse at various stages of processing into cellulosic ethanol. Results of a generic material balance calculations will be presented to illustrate the influence of separation target efficiencies on overall process recoveries and characteristics of waste streams.

Oral Presentation 9-04

Reactive separations for esterification and purification of individual organic acids from mixed solutions

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Many fermentation systems yield a mixture of products depending on the intended metabolic pathways and conditions within the bioreactor. The recovery and purification of individual species from fermenter effluents can be costly, contributing more than 50% of the overall production cost in some cases. We present here the results of reactive separation studies, building on prior work involving lactate and citrate esters, to simultaneously esterify and recover individual products from aqueous solutions of mixed organic acids. General concepts and limitations of applying simultaneous reaction and separation to mixed acid systems are discussed, and specific results from mixed succinic acid and acetic acid as a prototypical system are presented. Experiments in an elevated pressure, pilot-scale reactive distillation column in our laboratories demonstrate succinic acid conversions greater than 99%, with recovery of diethyl succinate as a pure product stream. Bench studies have focused on characterization of physical properties and phase equilibria of key species in the acetate/succinate system, and a non-ideal kinetic model for simultaneous esterification of the acids has been developed. These physical properties and reaction kinetics have been incorporated into a rigorous simulation of the reactive distillation system using AspenPlus simulation software, facilitating characterization of the pilot-scale results and scale-up to a commercial esterification facility. Reactive separations are thus emerging as versatile, economical, and "green" technologies for the biorefinery, providing opportunity for lower capital costs and greater energy efficiencies than traditional reaction and separation approaches.

Oral Presentation 9-05

Process development of integrated cellulose- and starch-based ethanol production

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The aim of this study is to develop processes for production of ethanol from cellulose-based raw materials, mainly agricultural residues such as wheat straw, barley straw and corn stover (second- generation bioethanol), integrated with starch-based ethanol production (first-generation bioethanol). In this manner higher ethanol yield, lower energy demand and lower production cost can be reached.

The study comprises both experimental studies and techno-economic evaluation of the results using commercial flow sheeting- and cost-estimation programs. Several process configurations for the integration are possible, from integration already at the front end, i.e., after pretreatment of the raw materials, to integration only of the downstream processes, i.e., distillation and evaporation.

Integration of the steam-pretreated wheat straw with liquefied and pre-hydrolyzed starch from wheat (5%+0.5-3% WIS (Water Insoluble Solids) respectively) in SSF (Simultaneous Saccharification and Fermentation) configuration has been investigated, using baker's yeast. Ethanol yields above 80% of the theoretical (from the hexose sugars) and ethanol concentrations around 6 wt-% have been reached. This results in lower total energy demand in comparison with separate production of ethanol in a starch- or in a cellulose-based process.

The study is now continued using genetically modified yeast to also ferment the pentose sugars and further increase the WIS content of the pretreated wheat straw in the SSF step thereby increasing both the yield and the concentration. Furthermore, integration of the steam-pretreated wheat straw with only liquefied starch in order to reduce the availability of glucose in the SSF step is also under investigation.

Oral Presentation 9-06

Fluorescence resonance energy transfer sensors for quantitative monitoring of pentose and disaccharide accumulation in bacteria

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Engineering microorganisms to improve metabolite flux requires detailed knowledge of the concentrations and flux rates of metabolites and metabolic intermediates *in vivo*. Fluorescence resonance energy transfer (FRET) sensors represent a promising technology for measuring metabolite levels and corresponding rate changes in live cells. Sensors for hexose and pentose carbohydrates could help in the development of fermentative microorganisms, for example, for biofuels applications. Arabinose is one of the carbohydrates to be monitored during biofuels production from lignocellulose, while maltose is an important degradation product of starch that is relevant for starch-derived biofuels production. An *Escherichia coli* expression vector compatible with phage λ recombination technology was constructed to facilitate sensor construction and a novel FRET sensor for arabinose was generated. In parallel, a strategy for improving the sensor signal was applied to construct an improved maltose sensor. Both sensors were expressed in the cytosol of *E. coli* and sugar accumulation was monitored using a simple fluorimetric assay of *E. coli* cultures in microtiter plates. The addition of the respective ligand led to concentration-dependent fluorescence resonance energy transfer responses allowing quantitative analysis of the intracellular sugar levels at given extracellular supply levels as well as accumulation rates. The new carbohydrate FRET sensors can be used for *in vivo* monitoring of sugar levels in prokaryotes, demonstrating the potential of such sensors as reporter tools in the development of metabolically engineered microbial strains or for real-time monitoring of intracellular metabolite during fermentation.

Oral Presentation 10-01

Enzymatic synergy examined using an engineered complex of cellulosomal enzymes from *Clostridium thermocellum*

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The bacterium *Clostridium thermocellum* produces a formidable array of enzymes to break down lignocellulosic biomass. Many of these enzymes are organized into structures anchored to the cell surface known as cellulosomes. Organization of cellulosomal enzymes into complexes increases their efficacy both in the cellulosome and in engineered two- and three-enzyme complexes. We have created an assembly based on a chaperonin from the hyperthermophilic organism Sulfolobus shibatae that binds up to eighteen cellulosomal enzymes on the ends of a nine-member double ring. We have characterized the activity of combinations of two, three or four cellulytic enzymes attached to this structure and begun to explore activity on natural biomass substrates using combinations of enzymes including those that degrade hemicellulose. These experiments have yielded new insights into the synergy between cellulosomal enzymes that act on distinct sites on a single substrate and those that act on distinct substrates in within biomass.

Oral Presentation 10-02

The three-dimensional structure of an intact glucoamylase gives insight on how substrate is directed towards the active site

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We present the three-dimensional structure of Hypocrea jecorina glucoamylase at 1.8 Å resolution. The structure model includes both the catalytic domain and the starch binding domain as well as the glycosylated linker segment between these domains. This is the first intact structure of a glucoamylase. Previously, structure models of only catalytic or starch-binding domains have been available and these have been of other fungal and yeast glucoamylases. That the protein is intact in the model allows visualization of the juxtaposition of the starch-binding domain relative to the catalytic domain. One of the proposed starch binding regions on the starch-binding domain are in close proximity of the active site on the catalytic domain. This supports the hypothesis that the starch-binding domain serves to target the glucoamylase at sites where the starch granular matrix is disrupted and where the enzyme might most effectively function. The detailed interactions between the catalytic and the starch-binding domains are confirmed by two independent structure determinations of the enzyme in two different crystal forms. The two structure models exhibit an identical conformation and show the same positioning of the starch-binding domain relative to the CD. This, in turn, suggests that the H. jecorina glucoamylase structure we present not only is independent of crystal lattice contacts but that it also represents the three-dimensional structure found in solution.

Oral Presentation 10-03

Computational Estimates of Free Energy Profiles of Cellodextrin Motion in Cel7A Reaction Tunnel

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The motion of cellodextrin through the tunnel of processive exo-cellobiohydrolases is a fundamental step in the catalytic process by which these enzymes degrade cellulose to cellobiose and other small polysaccharides. We have studied the movement of cellobiose, cellononose, and celloheptose in the reaction tunnel of Cel7A from Hypocrea jecorina (Trichoderma reesii) using molecular modeling and simulation to determine the mechanism of movement and the free energy profile of the movement. Using path sampling methods, we generated reaction paths with associated potential of mean force and have identified the mechanistic contributions to the barriers to motion and explore possible mechanisms for the leaving of cellobiose product and repositioning of cellodextrin chain for subsequent reaction.

Oral Presentation 10-04

A Family of Thermostable Fungal Cellulases Created by Structure-Guided Recombination

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SCHEMA structure-guided recombination of fungal cellulases has yielded a collection of novel, highly thermostable CBH2 chimeras. An appreciable fraction of a sample set of cellulase chimeras were secreted by a heterologous host in catalytically active form. Many of these chimeras have half-lives of thermal inactivation that are greater than the most stable parent cellulase. We predict that the collection of cellulase chimeras contains hundreds of highly stable cellulases. All of the active sequences chosen from the chimeras predicted to be thermostable based on the sample set sequence-stability data retained more activity than the most stable parent upon incubation at elevated temperature. These validated thermostable cellulases have high sequence diversity, differing from their closest natural homologs at up to 63 amino acid positions. Selected thermostable chimeras hydrolyzed phosphoric acid swollen cellulose at temperatures between 7 and 15°C higher than the parent enzymes. These chimeras also hydrolyzed as much or more cellulose than the parent cellulases in long-time cellulose hydrolysis assays and had pH/activity profiles as broad, or broader than, the parent enzymes. Generating this group of diverse, thermostable fungal cellulases is the first step in building an inventory of thermostable cellulases from which optimized enzyme mixtures for biomass conversion can be formulated.

Oral Presentation 10-05

Engineering cellulases on their natural substrates by directed evolution

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Engineering costly cellulases on natural cellulosic substrates is of importance for emerging biomass-based biorefineries. Complicated relationship among heterogeneous cellulose and different action mode cellulase components results in great challenges for cellulase engineering. Most times, cellulase activities on cellulose analog substrates (e.g., soluble substrate or chromogenic substrates) have no relationship with their activities on natural substrates. Directed enzyme evolution is becoming a popular tool, but identification of the desired mutants from a large mutant library remains challenging sometimes.

For beta-glucosidase, we have designed a novel combinatorial selection/screening approach for fast identification of thermostable beta-glucosidase mutants on cellobiose. Several thermostable mutants were identified from a random mutant library of the *Paenibacillus polymyxa* beta-glucosidase. The most thermostable mutant A17S had an 11-fold increase in thermostability at 50°C. In addition, we also attempted to improve the family 48 exoglucanase and the family 5 endoglucanase through directed evolution. The mutant libraries are cell-surface displayed in *E. coli* or secretory across membrane in *Bacillus.* Our preliminary results clearly supported the technical feasibility of cellulase engineering through directed evolution.

Oral Presentation 10-06

Developing Improved Thermostable Cellulases: High-Throughput Cellulolytic Assays and Protein Engineering Strategies

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DNA family shuffling has been employed to improve the thermostability and activity of moderately thermophilic exocellulases from fungal sources. Selection of genes for shuffling was carried out using constrained homology clustering and validated *in silico* using eShuffle. Because cellulases are highly modular, clusters for the catalytic and binding domains were formed separately and recombined to create novel cellulases as the parents for DNA family shuffling.

Conventional cell-based cellulase expression methods are time and labor intensive. Previous efforts to express cellulases in *E. coli* or yeast have often failed to produce active forms. A cell-free protein expression system, on the other hand, can be used as an alternative protein expression tool to address these problems. We have developed a high-throughput cellulase expression and screening platform to generate libraries of four thermophilic archaeal endocellulases, with the aim of improving their properties for industrial application. In addition, carbohydrate binding module (CBM) domains from bacterial cellulases are being added to the archaeal enzymes. The generation of CBM fusions is directed toward improving the catalytic activity of the extremely thermophilic archaeal cellulases toward crystalline substrates.

Finally, successful implementation of directed evolution to improve cellulase activity depends on the screening method used in enzyme selection. The poor correlation between cellulase activity on soluble and insoluble cellulosic substrates requires high-throughput methods for screening cellulase activity on relevant insoluble substrates. Our protein engineering efforts have thus employed high-throughput assays that are compatible with insoluble cellulosic substrates and constraints imposed by directed evolution strategies.

Oral Presentation 11-01

Hydrocarbon Fuels from Plant Biomass

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In this paper we'll discuss the conversion of renewable plant carbohydrates to hydrocarbon fuels, by fermentation of carbohydrates to isobutanol followed by catalytic conversion of the isobutanol to fuel blend stock, gasoline, jet & diesel fuel components. Bioethanol has provided an excellent start to sustainable, domestically produced renewable fuels entering the fuels market. Additionally ethanol has provided an infrastructure of expertise that is extremely valuable to support the development and commercialization of advanced biofuels. One of the remaining challenges is completing the conversion of plant matter into fuels and blend stocks that fit, without special rules, exactly into the existing infrastructure for crude oil derived transportation fuels and vehicles. Ultimately, this means producing sustainable hydrocarbons from biomass. A number of companies are pursuing the production of hydrocarbons from plant biomass utilizing a variety of thermochemical and biochemical approaches. The approaches will be compared and contrasted to the approach proposed in this paper. Data using our approach for the conversion of sugars to isobutanol and conversion of isobutanol to a high value gasoline blend stock, jet fuel and terepthalic acid, will be shown.

Oral Presentation 11-02

Renewable Petroleum™ Products and Technologies: Engineering Microbial Fatty Acid Metabolism for Fuel and Chemical Production

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The challenge to the biofuel industry is to quickly develop products and technologies that enable the rapid and widespread adoption of renewable and sustainable substitutes for existing fossil fuels and chemicals. Success will require products to be "drop in" replacements that leverage existing consumer and distribution infrastructure and for processes to be "feedstock agnostic," scale without competing with food supplies, result in significant reduction in green house gas emissions, and most importantly be cost competitive with existing products. LS9, Inc. has developed a core technology to convert fermentable sugar to a diversity of fuel and chemical products in one-step fermentation processes. Our lead fuel product, UltraClean™ Diesel, is a secreted immiscible product that is recovered by simple centrifugation and is vehicle ready without further chemical processing, such as hydrogenation, cracking, or transesterification. Leveraging the efficient and productive fatty acid biosynthetic pathway in combination with an engineering strategy that places all chemical conversions in a single whole cell catalyst, LS9's Microrefinery™ catalysts enable a specific and efficient route to a high-performing diesel substitute that is competitive with recent oil prices without subsidy. This talk shall discuss the fundamental technology and its application to a diversity of products, but shall focus on the quality, underlying economics, GREET analysis, pilot demonstration, and commercial development plan of UltraClean™ Diesel.

Oral Presentation 11-03

High-Titer Production of Hydroxyvalerates and 4-Valerolactone from Levulinate in *Pseudomonas putida*

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Hydroxyacids and lactones are versatile, chiral compounds that can readily be modified into several useful derivatives. Specifically, these compounds see use in the synthesis of antibiotics, β- and γ-aminoacids and peptides, in fragrances, and as chiral synthetic building blocks. These compounds can also be used directly as nutritional supplements and can be polymerized into biodegradable polyesters with interesting physical properties.

In this work, an economical, high-titer method for the production of 4-hydroxyvalerate (4HV), 3-hydroxyvalerate (3HV), and 4-valerolactone (4VL) from the inexpensive and renewable carbon source levulinic acid was developed. Titers of 4HV and 3HV in shake flask cultures both reached multi-gram-per-liter scale in both minimal and rich media. To achieve these titers, we tested two strains of *P. putida* and examined two enzyme systems for removing CoA acyl carriers from intracellular hydroxyvaleryl-CoA intermediates: the *ptb*/*buk* system and *tesB*. Once a suitable strain and enzyme system was found, the process was optimized at the shake flask scale in minimal and rich media for the high-titer production of both 4HV and 3HV. To produce 4VL from 4HV, a pH-dependent equilibrium between 4HV and 4VL had to be overcome. Because intracellular pH was found to be too high for the appreciable production of 4VL, we employed a membrane-bound, extracytosolic lactonase to perform the lactonization reaction in acidic culture medium and achieved multi-gram-per-liter production of 4VL. To our knowledge, this work represents the first time that these hydroxyvalerates and 4-valerolactone have been produced from a feasible feedstock in shake flasks at the multi-gram-per-liter scale.

Oral Presentation 11-04

Sustainable Chemicals through Biotechnology: 1,4-Butanediol

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The relentless rise and volatility of petroleum prices is stimulating the chemical industry to diversify its raw material base. Genomatica has established an integrated suite of computational and experimental technologies to design, engineer, and optimize novel organisms and bioprocesses for cost-advantaged and sustainable manufacture of chemicals.

In silico metabolic modeling and simulation technologies greatly accelerate the pace of industrial bioprocess development by providing optimum strain designs and engineering strategies, by facilitating data interpretation, and by guiding experimental activities throughout the entire development cycle. Unique pathways to a chemical are identified and verified as superior through genome-scale metabolic models that allow prioritization in terms of parameters such as yield, energy balance and redox balance. Subsequently a strain is designed via the proprietary OptKnock algorithm, which identifies sets of genes that must be deleted in order to tightly couple product formation to growth of the organism. Following introduction of the most attractive biosynthetic pathways and designated deletions, strains are subjected to adaptive evolution methods which use controlled selection pressure to optimize strain performance following genetic manipulations. In addition to achieving superior product yield and productivities, evolved strains are genetically stable and thus ideally suitable for cell recycle or continuous bio-processing with consistent high-level production.

The presentation will highlight successful implementation of this combined computational and experimental approach for engineering a microorganism that produces the industrial chemical 1,4-butanediol (BDO) directly from glucose and sucrose.

Oral Presentation 11-05

Rapid optimization of microorganisms for the cost superior production of chemicals and fuels

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The growing field of bio-refining relies upon the use of microorganisms to convert renewable carbon sources such as sugar into higher value products. Traditional bio-refining processes have taken advantage of the unique abilities of specific micro-organisms to produce desired product, or the genetic engineering of micro-organisms to produce non-natural products. Bio-processes have then been designed around these organisms. These bioprocesses are often very costly in large part due to the complex requirements of the microorganisms themselves, which can necessitate expensive growth conditions as well as separations and processing steps both before and after the micro-organisms' conversion step. OPX has developed several new high-resolution and comprehensive genomics tools that can be used to optimize industrial organisms. We have employed these generalizable methods to very rapidly construct and optimize commercially relevant microorganisms. We are able to optimize micro-organisms that enable both variable and capital cost savings across the entire bioprocess.

In particular, OPX has been able to construct and optimize micro-organisms for the production for several bioprocesses including the biorefining of 3-hydroxypropionic acid. 3-hydroxypropionic acid is a bio-product with several market applications. The most notable being the $7 Billion acrylic acid market, as 3-hydroxypropionic acid is readily converted by conventional methodologies to acrylic acid. Our platform technology has enabled the construction of microbial strains capable of producing commercially relevant titers of 3-hydroxypropionic acid at commercial productivities in inexpensive growth conditions, a strain that will enable a cost competitive bio-processing route to acrylic acid.

Oral Presentation 11-06

Production of terpene based biofuels in S. cerevisiae

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The generation of microorganisms that can produce biofuels similar to petroleum-based transportation fuels would allow the use of existing engines and fuel transportation infrastructure. The corrosivity and high hygroscopicity of ethanol, today's preferred biofuel, are incompatible with existing technologies. "Second generation" biofuels should have better physical properties than ethanol and a higher energy content per unit. Here, a Saccharomyces cerevisiae platform previously engineered to overproduce farnesyl pyrophosphate has been adapted to produce a variety of terpenoid base biofuels. First, a series of terpene synthases were tested for the production of monocyclic, bicyclic, and linear sesquiterpenes in yeast. Next, the production levels of the different sesquiterpenes were optimized and the molecules classified based on their structure and yield as potential biofuel candidates. Finally, the terpene synthases leading to the most promising biofuel candidates were introduced in Escheria coli able to overproduce farnesyl pyrophosphate and tested for biofuel production. This is the first time that such a large array of terpene synthases has been tested for the production of sesquiterpenes in S. cerevisiae. Importantly, some of the successfully produced sesquiterpenes have structures similar to gasoline and jet fuel and may be useful second generation biofuels.

Oral Presentation 12-01

Identification of Desirable Traits in Miscanthus to Enhance Total Sugar Yields in Biological Conversion

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Miscanthus, which has a very high productivity, was selected by DOE as one of the potential biomass crops to support large scale production of fuels. Improving our understanding of features that control enzymatic hydrolysis and how pretreatment alters these features for this prolific crop would be a significant step to identifying better strategies and opportunities to genetically alter the susceptibility of biomass to deconstruction and achieve very high total sugar yields at low costs. In this study, a number of Miscanthus species were screened based on chemical composition and structure to identify the most promising species for more detailed characterization by pretreatment and enzymatic hydrolysis. Then, water-only and dilute acid batch and flowthrough pretreatments were applied to those varieties to define how sugar yields and release profiles vary among species and to develop meaningful cause-and-effect relationships of factors that control deconstruction of hemicellulose, cellulose, lignin, and other subcomponents. Profiles of glucose, xylose, and total sugar release from selected Mischanthus species were determined for various combinations of time, acid concentration, flow rate, and temperature followed by enzymatic hydrolysis of the pretreated solids. The resulting data and models provide a new perspective on how sugar yields vary with species of Miscanthus during pretreatment and biological processes and key features that could govern sugar yields.

Oral Presentation 12-02

Elucidation of Alfalfa Lignin Structures on Gene Down-Regulation

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Independently down-regulation of genes encoding 4-coumarate 3-hydroxylase (C3H) and hydroxycinnamoyl transferase (HCT) has shown to reduce the recalcitrance of alfalfa and thereby improving the yield of simple sugars after pretreatment. One-dimensional (1D) and two-dimensional (2D) nuclear magnetic resonance (NMR) techniques were utilized to identify structural elements of importance to the recalcitrance of genetically engineered alfalfa. After C3H and HCT gene down-regulation, significant structural changes had occurred to the alfalfa lignin. A significant increase of p-hydroxylphenyl unit content was observed in the transgenic alfalfa lignins as well as a concomitant decrease of up to ~70% of the guaiacyl and syringyl units. Quantitative ^{13}C NMR measurement also showed a significant decrease of carboxylic group, methoxyl group and β-O-4 linkage contents in the alfalfa lignins after genetic engineering. ^{13}C-^{1}H HSQC 2D correlation NMR demonstrated an increase of interunit phenylcoumaran and resinol contents for C3H and HCT transgenic alfalfa. In addition, ^{31}P NMR measurement revealed that phenyl hydroxyl group in p-hydroxylphenyl unit was dramatically increased for the transgenic lignins, as well as by over ~50% decrease of guaiacyl hydroxyl group content. The results of these changes in lignin structure and their relationship to recalcitrance will be examined with a perspective to future improvements in plant cell wall design for enhanced sugar production for biofuels.

Oral Presentation 12-03

Small-scale Enzymatic Conversion Screens to Assist in the Development of Improved Energy Crop Varieties

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In the development of improved energy crop varieties, obtaining accurate chemical composition and conversion performance characteristics will be critical. Ceres has previously developed small-scale conversion assays for the assessment of switchgrass biomass. Here we display information on small laboratory-scale, high-throughput assays that can be used to assess the conversion efficiencies of Arabidopsis and sorghum. Both acidic (APT) and basic (BPT) pretreatment methods have been employed. Ceres has generated a collection of thousands of transgenic Arabidopsis lines, each overexpressing a single full length cDNA. A selection of transgenic Arabidopsis lines bearing misexpressed genes relevant to cell wall biosynthesis have been grown to maturity and assessed for conversion efficiency. Additionally, Ceres has available hundreds of genetically diverse sorghum samples, and the biomass collected from a subset of these lines has been assessed for relative digestibility in Ceres' small-scale conversion assays. For both plant species, lines with distinct differences in glucose released per gram dry biomass and/or in percent of theoretical maximum glucose yield have been identified. In the case of the Arabidopsis lines, these conversion assays provide a direct means to identify genes that can influence conversion processing performance, leading to higher rates of conversion and higher final sugar yields. The sorghum assays assist in the identification of sorghum lines with superior conversion characteristics. This information will be invaluable to breeders and genetic engineers in the design of improved energy crop varieties that give higher conversion product yield per ton of biomass input to a conversion process.

Oral Presentation 12-04

Effects of Chemical Pretreatment on Enzymatic Hy drolysis of Lignocellulose Observed by AFM

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Lignocellulsoe has natural resistance to microbial and enzyme destruction often called "recalcitrance". The recalcitrance of lignocellulose is a major barrier to the economical development of biobased fuels and chemicals. Chemical pretreatment is often applied to biomass feedstock to remove lignocellulose recalcitrance for efficient subsequent enzymatic saccharification. The performances of different chemical pretreatments vary significantly. Understanding of the fundamentals of nano-scale phenomena on lignocellulsoe substrate during enzyme actions can provide insight about why some pretreatment is more effective than others. This understanding can lead to developing efficient pretreatment processes and enzyme systems to improve lignocellulose bioconversion. This presentation will provide some observations of the effects of chemical pretreatment on enzymatic hydrolysis of lignocellulose substrate using AFM. Dilute acid, hot water, and SPORL pretreatment were applied to lodgepole pine and eucalyptus wood chips to produce substrates. Enzymatic hydrolysis of the pretreated substrates was conducted at 50°C with enzyme loading of 15 FPU/g substrate od solid. AFM imaging was applied to substrates after enzyme actions for various incubation duration times. The AFM images clearly showed the enzymes attached to the substrate and the destruction of celluloses microfibriles over time after enzyme actions. Furthermore, the effect of CBD on enzyme attachement can be clearly seen from the AFM images. The effects of pretreatment process on the dynamics of nano-scale enzyme destruction of lignocellulose can be cleary seen. When correlating the AFM imaging information with time-dependent quantitative enzymatic cellulose conversion data, the effects of nano-scale enzyme process on macro-scale cellulose conversion can be easily understood.

Oral Presentation 12-05

Understanding Ionic Liquid Pretreatment of Lignocellulosic Biomasses

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Pretreatment of biomass is essential for breaking apart highly ordered and crystalline plant cell walls and loosening the lignin and hemicellulose conjugation to cellulose microfibrils, thereby facilitating enzyme accessibility and adsorption and reducing costs of downstream saccharification processes. Recent reports1, 2 have shown very high yields at very low enzyme loadings. However, pretreatment still remains one of the most costly steps in lignocellulosic biofuel production. Ionic liquids are novel solvents showing great promise for lignin and cellulose solubilization. Instant rejection of dissolved polysaccharides upon addition of anti-solvent shows promise for recyclability in addition to other desired attributes like low volatility, non-flammability and thermal stability. Although ionic liquids have been shown to be very effective in cellulose solubilization3,4 , the disposition of hemicellulose and lignin are not fully understood. The aim of our research is to develop a fundamental understanding of ionic liquid pretreatment by monitoring and analyzing process streams. To that end, we have employed HPAEC, XRD, FTIR, NIR, and SEM to study the impact of ionic liquid pretreatment on switchgrass and corn stover. We will present the results from these measurements in the context of developing and selecting optimized ionic liquid pretreatment conditions for selective depolymerization of either cellulose or lignin, whereby fractionation of different cellulosic and lignin components could be realized.

Oral Presentation 12-06

Single Molecule Tracking of Carbohydrate-Binding Modules Bound to Cellulose Crystals

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To develop more cost-effective approaches to liberate fermentable sugars from recalcitrant biomass, the enzyme cocktail used for saccharification must be improved. We have developed a single-molecule technique based on fluorescence imaging to track the motion of cellulase components with spatial resolution at several nanometers. We used single molecule spectroscopy to study the behavior of carbohydrate-binding modules (CBMs) labeled with quantum dots (QDs) while bound to cellulose crystals. These bio-assembles were subjected to total internal reflection fluorescence (TIRF) microscopy. The concentrations of the CBMs and QDs were optimized to achieve single molecule resolution. This technique revealed a confined nanometer-scale movement of the CBMs bound to cellulose. Although the mechanism of CBM motion is still unknown, the single molecule approach used here offers new opportunities to guide us toward a fundamental understanding of cellulase function, especially the mechanism of the "processivity" of exoglucanse.

Poster 1-07

New Method for Fast Detection of Improved Biodegradability in Genetically Modified Plants

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Plant genetic engineering is considered a potential approach to reduce costs for biofuel production from lignocellulosic material. However, the ability to control cell-wall composition without compromising plant performance is a key objective of bioenergy crop improvement. Plants have been engineered for the production of enzymes within the crop biomass, with an aim to minimize the costs of catalyst production in bioreactors. Future research on the upregulation of cellulose and hemicellulose biosynthesis pathway enzymes for an increase in polysaccharides may also have the potential to improve cellulosic feedstocks. The most successful efforts to date have focused on the modification of lignin quantity and/or quality, in an effort to obviate the need for expensive pretreatment processes. Here we report a method for rapid detection of improved biodegradability in genetically modified plants that vary in lignin content and/or composition. For this purpose, only 50 mg of ground material is needed for liquid hot water pretreatment, and the method allows the pretreatment of up to 9 samples every 10 min per sandbath. Enzyme hydrolysis in the presence of commercial cellulases and β-glucosidase is performed in a final volume of 1 mL for 30 min, at 50 °C, and pH 4.8. The samples are then centrifuged, and the amount of glucose liberated is analyzed via a microplate assay. Using this approach, we have been able to rapidly and reproducibly identify genetically modified plants with improved biodegradability.

Poster 1-08

Progress toward a Renewable, Plant-Based Production System for Methacrylate

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The objective of this DOE-funded research program is to utilize the metabolic pathways that already exist in plants to genetically engineer methacrylate production into a cellulosic ethanol biomass crop, such as switchgrass. Methacrylate could provide a value-added co-product for biomass conversion processes. While methacrylate is a relatively rare metabolite in biological systems, it does appear as an intermediate in primary and secondary metabolic pathways with high carbon flux, namely amino acids and terpenes. To utilize the existing metabolic capacity of plants to produce high yields of chemicals that can be readily converted to methyl methacrylate, we are focusing on the branched chain amino acid degradation pathway. Methacrylate and a number of closely related chemicals are produced as free acids and thioesters of coenzyme A in the catabolic pathway of the branched chain amino acid (BCAA), valine. Under normal conditions, methacrylate and related intermediates do not accumulate to measurable levels because of the catabolic efficiency of the pathway, which is focused on redistributing carbon from the BCAA pool into the TCA cycle. In this presentation, we will discuss the manipulation of BCAA catabolic intermediates in Arabidopsis transgenic lines carrying over-expression constructs of pathway genes and/or T-DNA knock-out lines of them.

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Poster 1-09

Towards a realistic model of plant cell walls via correlative Raman imaging and EM tomography

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Plant biomass is a potential source of large-scale biofuel production as long as the cell wall polysaccharides can be efficiently broken down into sugars that can be fermented into ethanol. Little is known about the precise 3D cell wall molecular organization, although such knowledge would greatly facilitate improved deconstruction schemes as well as rational cell wall engineering. The primary objective of our research is to obtain a realistic model of plant cell walls at molecular resolution, by determining 3D architecture of the cell wall via electron tomography and correlated chemical composition via Raman imaging. 3D architectural and Raman data will be analyzed with the help of sophisticated algorithms, and integrated into a molecular model of the cell wall. As the first step, we have tested different sample preparation methods to obtain the best possible preservation of the cell wall structure and of the Raman signal, including 1) high-pressure freezing and freeze-substitution followed by resin embedding; 2) microwave-assisted TEM processing; and 3) cryo-sectioning of high-pressure frozen samples. We explored differential staining and labeling approaches to increase overall contrast and/or to identify specific cell wall components. Using widefield correlative TEM and optical imaging at high resolution, we have monitored differences and similarities arising from various parts/tissues of the same plant, different ages of the same plant and also different plant species. We discuss our preliminary findings on the different sample preparation methods, 2D widefield TEM and Raman imaging as well as cell wall 3D tomograms.

Poster 1-10

Expression of lignocellulosic-degrading enzymes in transgenic plants

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One of the barriers to more cost-effective conversion of biomass feedstocks to biofuels and chemicals is the high cost of the enzymes used to depolymerize lignocellulose. As a member of the Great Lakes Bioenergy Research Center, this laboratory is pursuing the development of plants as vehicles for the production of lignocellulose-degrading enzymes. The two major objectives of this project are: 1) to take advantage of plant agricultural productivity to both decrease the unit cost and increase the production capacity for these enzymes and 2) to determine if the expression of these enzymes in plants can improve biomass digestibility and effectively lower process costs for ethanol production from biomass. We report initial results for a medium-throughput plant transformation/analysis platform we are developing. As part of the initial phase of this work, we have analyzed the expression of several genes in plants using high-throughput cloning methods and compared these results with previous observations using the same genes. This platform will be used to assess plant expression and delivery methods of several classes of cell wall degrading enzymes provided by other members of the GLBRC.

Poster 1-11

Withdrawn

Poster 1-12

A Rapid Analytical Method for Investigating Genetic Modification of Lignin Pathways in Alfalfa (*Medicago sativa*)

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Genetic modifications of the lignin pathway in alfalfa (*M. sativa*) were studied by two pyrolysis methods and subsequent multivariate data analysis. Two key genes, hydroxycinnamoyl transferase (HCT) and p-coumourate 3-hydroxylase (C3H), in the lignin biosynthesis pathway were downregulated by the expression of anti-sense RNA. Both genes are involved in the initial two steps in the biosynthetic pathways leading to guaiacyl (G) and syringyl (S) lignin but are not involved in production of p-hydroxyphenyl (H) lignin. Pyrolysis-Molecular Beam Mass Spectrometry (py-MBMS) and pyrolysis-Gas Chromatography Mass Spectroscopy (py-GCMS) were used to investigate the impact of changes in total lignin content and ratios of lignin monomers. The resulting mass spectra from the respective mutants were further investigated by multivariate statistical analysis, such as principal component analysis and hierarchical clustering. We found that down regulation of HCT and C3H resulted in reduced S- and G- lignin and an increased the proportion of H lignin. This is consistent with the respective functions these genes in the lignin biosynthesis pathway. We conclude that py-MBMS is a rapid method for estimating lignin content and the ratios of the monolignols. The effect of the genetic modifications on individual components measured in the pyrolysis vapors can easily be seen and the differences quantified with the multivariate analysis.

Poster 1-13

Structural Glycan Composition of Pacific Northwest Grass-Derived Biomass: A Survey

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The structural component compositions of thirty nine non-commercial Pacific Northwest grasses were analyzed in order to develop a database of the grasses that may have application in bioconversion processes. The samples were chosen based on near infrared reflectance data that suggested this group of grasses, collectively, was representative of the broad range of compositions that are likely to be encountered due to genotypic and phenotypic variability. Solvent-extracted samples were prepared by extracting the native grass sequentially with water and then 95% ethanol. Each of the grasses residues was analyzed for glycans, acid-insoluble lignin, acid soluble lignin, and ash. Total glycans ranged from a low of 32% to a high of 50%. Glucan was the major glycan component, typically in the range of 60% of total glycan. Xylan represented about one-third of total glycans while arabinan represented 1.0% to 3.3%. Total glycans tended to increase from the younger stage to the more mature stage. While the amount of glycans varied between species, the ratio of glucan, xylan, and arabinan (12: 7: 1) remained relatively constant. Acid-insoluble lignin ranged from 6.38% to 14.58%, while the acid-soluble lignin ranged from 1.57% to 4.35%. The acid-insoluble lignin of seed mature, flower, boot, and vegetative stages were 12.38%, 11.65%, 9.39%, and 8.10%, respectively. The acid-soluble lignin of seed mature, flower, boot, and vegetative stages were 2.03%, 2.61%, 3.02%, and 3.46%, respectively. Extractives represented 20.29% to 41.55% of the oven-dry grasses.

Poster 1-14

Biofuel feed stock Source – *Jatropha curcas*

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Petroleum dependency is a challenge that can potentially be partly offset by agricultural production of biofuels, while decreasing net, non-renewable carbon dioxide output. Plants have not been domesticated for modern biofuel production, and the quickest, most efficient, and often, the only way to convert plants to biofuel feedstocks is biotechnologically. In the last few years the potential of the drought resistant tropical tree Jatropha curcas L. (Euphorbiaceae) for the production of biofuels and industrial products has been assessed by several groups. Various novel methods for the cultivation and genetic improvement of J. curcas have been presented. A trans-esterification process of the seed oil for its use as a biofuel was evaluated on an industrial scale (1500 t/a). grains to produce bioethanol and biobutanol and oilseeds to produce biodiesel compete directly with needs for world food security. The heavy use of oilseed rape releases quantities of methyl bromide to the atmosphere, which can be prevented by gene suppression. Second generation bioethanolic/biobutanolic biofuels will come from cultivated lignocellulosic crops or straw wastes. These presently require heat and acid to remove lignin, which could be partially replaced by transgenically reducing or modifying lignin content and upregulating cellulose biosynthesis. Non-precipitable silicon emissions from burning could be reduced by transgenically modulating silicon content. There seem to be no health or environmental impact study requirements when the undomesticated biofuel crops are grown, yet there are illogically stringent requirements should they transgenically be rendered less toxic and more efficient as biofuel crops.

Poster 1-15

Characterization of sorghum bmr mutants for biofuel applications

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Genetic improvement of biomass crops can reduce the cost of biomass-to-ethanol conversion. Lignin content and composition affect the conversion of cellulose to monomeric sugars. Sorghum is a promising source of biomass due to its great yield potential and tolerance to stresses. The *brown midrib* (*bmr*) mutants of sorghum are characterized by brown vascular tissue and altered lignin content. We combined genetic and chemical approaches to identify four *bmr* loci represented by the *bmr*2, *bmr*6, *bmr*12 and *bmr*19 allelic groups. We have shown that rapid classification of novel *bmr* lines can be achieved using phloroglucinol-HCl as a histochemical stain. Enzymatic saccharification of stover demonstrated that the mutations in the *bmr*2, *bmr*6 and *bmr*12 groups can increase glucose yields up to 25% compared to wild-type isolines. Characterization of changes in subunit lignin composition in each of the groups by pyrolysis-gas chromatography-mass spectrometry helps predict the genes underlying the mutations. Chemical composition of the *bmr6* group is consistent with reduction of cinnamyl alcohol dehydrogenase (CAD) activity. Analysis of the sorghum genome revealed 14 *CAD-like* genes. Based on their phylogenic relationship and the identification of non-conservative mutations in three allelic *bmr*6 lines, *SbCAD2* was identified as the *Bmr6* gene. In order to expedite the selection of the *bmr* mutant alleles in breeding populations, we have developed molecular markers specific for several *bmr* alleles. We are currently working on cloning additional *bmr* loci from *bmr* lines identified in a TILLING population.

Poster 1-16

Superior Dedicated Herbaceous Energy Crop Varieties

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Ceres used to be a straight-ahead functional genomics company, and the greenhouses were full of transgenic Arabidopsis and rice plants. However, Ceres has now metamorphosed into a comprehensive seed business, complete with traditional and marker-assisted breeding facilities and capabilities, a talented agronomy team that covers relevant areas of the USA, an extensive field-trialing network, seed production acres, and a sales and marketing force. Using the Blade brand name (*www.bladeenergy.com*), we are now marketing seed for superior varieties of both switchgrass and high biomass sorghum for the 2009 planting season. These varieties are targeted specifically for use in biomass conversion processes. This talk will highlight why these varieties are better suited for bioenergy purposes than what has been previously available, and why these factors are important to farmers and biomass processors.

Poster 2-07

Tracking Microbial Community Changes During Decomposition of Switchgrass

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Many compost microbial communities have evolved to decompose lignocellulosic materials similar to those being considered for biofuel production. Furthermore, these communities can tolerate a wide range of environmental conditions, offering great potential for enzymes that will tolerate harsh pretreatment conditions. This study was designed to track changes in microbial and enzyme activity and community composition throughout the course of microbial colonization and decomposition of switchgrass. Switchgrass was inoculated with finished green waste compost from a commercial facility. Temperature was controlled to simulate a composting process with a fast ramp from 30oC to 54oC, 7 days of thermophilic levels at 54oC and a slow decrease back to 30oC over the course of 21 days. Respiration was monitored on-line to track microbial activity and bioreactors were sampled at regular intervals for microbial community and enzyme analysis. Carbon dioxide evolution rates (CER) peaked twice: after one day of composting corresponding to initial consumption of sugars and again after eight days during the thermophilic phase. Comparison of 16S rRNA gene clone libraries from initial and final samples showed a significant increase in microbial diversity on the switchgrass.

Poster 2-08

Current Status of the Department of Energy's Aquatic Species Program Lipid-Focused Algae Collection

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The Department of Energy's Aquatic Species Program (ASP) was funded from 1978 to 1996 in an effort to develop liquid transportation fuels from microalgae. During this time an extensive algae culture collection was amassed from water samples around the United States. Out of more than 3000 strains, 51 were well characterized in terms of growth and lipid production and these are described in the Culture Collection Catalog *(http://www.nrel.gov/docs/legosti/old/3079.pdf*) and addendum *(http://www.nrel.gov/docs/legosti/old/3079a.pdf*). At the close of the ASP, a total of 297 strains of the original 3000, including 37 of the 51 strains listed in the Culture Collection Catalog and addendum, were transferred to the Center for Marine Microbial Ecology and Diversity (CMMED) at the University of Hawaii (UH). The complete list of strains transferred is available in the ASP Closeout Report (*http://www.nrel.gov/docs/legosti/fy98/24190.pdf*). With the resurgence in interest in using microalgae as a feedstock for producing liquid transportation fuels, many inquiries have come up regarding the current status of this important strain collection. Currently, 23 of the 51 strains listed in the Culture Collection Catalog and addendum are still extant and 19 of these strains have been re-established at the National Renewable Energy Laboratory. We present here the current status of these strains including a microscopic characterization of potential lipid vesicles using neutral lipid specific dyes.

Poster 2-09

Withdrawn

Poster 2-10

Biological production of ethanol, xylitol and arabitol by novel, naturally occuring yeast

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Up to date, there is no reported microorganism that is capable of utilizing both, hexose and pentose sugars at the same time, without being genetically modified or co-cultured. A novel, genetically unmodified yeast was identified as being capable of rapid assimilation and catabolism of five and six carbon sugars (arabinose, xylose, galactose, glucose and mannose). This yeast (PTD3) was shown not to be subject to hexose-mediated repression during mixed sugars fermentation. PTD3 produced ethanol of 82% of theoretical during fermentation of glucose, mannose and galactose. It produced considerable amount of xylitol of 96.1% of theoretical when xylose was present in the fermentation media and very high concentration of arabitol during fermentation of arabionse. The high ethanol, xylitol and arabitol yields were obtained without media, aeration, temperature and pH optimization.

This novel yeast has also a high tolerance of inhibitors (furfurals, 5-HMF and acetic acid) during biological production of ethanol and xylitol. PTD3 can effectively ferment five and six carbon sugars present in hydrolysates from different cellulosic biomass (steam pretreated switchgrass, hybrid poplar, and sugar cane bagasse) to ethanol, xylitol and arabitol.

Poster 2-11

Comparative genomics of *Oligotropha carboxidovorans* OM5, a chemolithoautotrophic bacterium

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Oligotropha carboxidovorans OM5 T. (DSM 1227, ATCC 49405) is a chemolithoautotrophic bacterium with the capability to utilize CO (carbon monoxide), CO2 (carbon dioxide) or syngas (gas mixture that contains varying amounts of CO and H2 generated by the gasification of organic wastes). Previous reports showed that the megaplasmid pHCG3 encoding the carbon monoxide dehydrogenase/ acetate synthase (CODH/ACS) enzyme complex is involved in the assimilation of CO or syngas. The present study aimed at sequencing and annotation of the circular chromosome to identify pathways responsible for assimilation of carbon that is fixed by chemolithoautotrophy into fatty acids. The knowledge of the complete genome also enabled comparative genomics studies with relatively close bacterial species. Interestingly, fatty acid methyl ester (FAME) analysis of *O. carboxidovorans* grown in the presence of acetate and syngas showed that the bacterium produces specific fatty acids that are components of biodiesel

Poster 2-12

Withdrawn

Poster 2-13

Pyruvate Induced Metabolic Shift in Cellobiose Fed *Clostridium thermocellum* ATCC27405 Batch Cultures

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Growth, end-product synthesis, and enzyme activities involved in pyruvate catabolism, H2 synthesis, and ethanol production were studied in Clostridium thermocellum ATCC 27405 grown in batch cultures on cellobiose, pyruvate, glycerol, and combinations of these substrates. Cells could not use pyruvate or glycerol as a sole carbon source, but did metabolize pyruvate in the presence of cellobiose resulting in increased acetate, CO2, H2, lactate and biomass production, and a marginal decrease in formate and ethanol production with increasing pyruvate concentrations (40-120 mM). Comparable concentrations of NaCl did not have an impact on generation time or end product ratios in cellobiose grown cells, eliminating the possibility of a sodium pyruvate induced salt effect. Activities of enzymes involved in pyruvate catabolism and ethanol synthesis did not change, while methyl viologen, ferredoxin, and NAD+-dependant hydrogenase activities increased 10-fold and NADP+-dependant hydrogenase increased 5-fold. The presence of glycerol had no effect on growth or end product synthesis in cellobiose grown cells. End product profiles suggest that carbon from additional intracellular pyruvate flows through pyruvate:ferredoxin oxidoreductase leading to hydrogen and ATP synthesis pathways, and not through pyruvate:formate lyase and NAD(P)H reoxidizing pathways yielding ethanol. The presence of lactate under elevated pyruvate concentrations may be a consequence of buildup of glycolysis intermediates, including fructose-1,6-bisphosphate, an allosteric activator of lactate dehydrogenase, resulting in an alternative method of NADH reoxidation. We demonstrate here that the redox state of a substrate may have important implications on final end product profiles.

Poster 2-14

Effect of Gas Sparging on Pyruvate Catabolism during Batch Fermentation of *Clostridium thermocellum* ATCC 27405

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Growth, end-product synthesis, enzyme activities and associated transcription involved in pyruvate catabolism, H2 synthesis, and ethanol production were studied in the cellulolytic anaerobe, Clostridium thermocellum ATCC 27405, during batch fermentation of cellobiose to determine the effect of elevated N2 and H2 sparging on metabolism using a 14 liter fermenter. End product profiles revealed increases in acetate, CO2 and H2 production under high N2 sparging with respect to standard N2 gas flow rates. Alternatively, elevated H2 sparging shifted carbon and electron flow towards the production of formate and ethanol. Transcription levels of key genes involved in pyruvate catabolism as well as hydrogen and ethanol synthesis and associated enzyme activities (pyruvate:ferredoxin oxidoreductase, pyruvate:formate lyase, lactate dehydrogenase, NAD- and NADP- dependent alcohol dehydrogenase, methyl viologen- ferredoxin-, NAD- and NADP-dependent hydrogenase) revealed no significant differences under the conditions tested, with the exception of the hydrogenases. The results presented here suggest that gas sparging can be effectively used to shift carbon and electron flow and the observed shifts at the pyruvate branch-point are principally influenced by the availability of reduced electron carriers (NAD, NADP, ferredoxin) and thermodynamic considerations.

Poster 2-15

Development of a Robust Yeast Biocatalyst for Low pH Lactic Acid and Cellulosic Ethanol Fermentation

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Common characteristics are required for an economically viable biocatalyst for cellulosic ethanol and lactic acid production for commodity applications like Poly Lactic Acid (PLA). These include high yield, fast fermentation, robust growth in simple media, and tolerance to organic acids at low pH. Cargill started with non-conventional yeast naturally possessing some of these characteristics and successfully developed it to efficiently produce a new end product (lactic acid) or to ferment new sugars (pentoses). Replacing yeast's ethanol pathway with lactic acid pathway was straight forward. Development of a strain capable of producing polymer grade lactic acid at commercially interesting titer, yield and productivity required concerted utilization of genome wide tools, targeted modifications, evolution and classical mutagenesis. The developed strain and low pH fermentation process offers considerable cost savings over conventional lactic acid processes.

Cargill has previously demonstrated efficient fermentation of xylose to ethanol in yeast (USPatentApp 10/554887). We are now combining our xylose fermentation technology into the acid tolerant yeast first developed for lactic acid production. Goals have been set for ethanol production from mixed sugars (dextrose, mannose, xylose, and arabinose) in the presence of 10 g/L acetate at 40°C and at a pH less than 5.0. Under these conditions Cargill host can utilize 80 g/l of dextrose and 80 g/l of mannose in less than 36 hours, producing ~ 70 g/l ethanol. A xylose utilization pathway has been engineered into this host and efficient fermentation of xylose to ethanol demonstrated both in defined medium and in hydrolyzate.

Poster 2-16

Fed-batch schemes and yeast adaptation for improving ethanol production at high substrate loading

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Renewable lignocellulosic biomass is expected to be the major future bioenergy feedstock. In this context, the simultaneous saccharification and fermentation process (SSF) has become one of the most successful methods for ethanol production since early 90's. Nowadays, fed-batch SSF process is appearing as a promissing alternative allowing conversion of higher substrate loading.

One of the limitations when using lignocellulosic materials at high substrate loading is the presence of inhibitors, including weak acids, phenolic compounds and furan derivates produced during biomass pretreatment which affect yeast fermentation. Thus, the employment of more tolerant yeast is required for the development of large-scale ethanol production. Since not only glucose is contained in the pretreated material an efficient pentose fermentation is also required for making the process profitable.

In the present study, directed evolution of the xylose fermenting yeast *Saccharomyces cerevisiae* F12 was performed by sequential transfer of cultures to diluted prehydrolysate media with increasing concentration of inhibitors. The evolved strain was tested in prehydrolysates from steam-pretreated wheat straw obtaining in all cases higher ethanol concentration and faster sugar consumption than the parental strain. In the best case, an increase of 34% in the ethanol concentration was observed together with 45% increase in the xylose uptake. Differences were also found in the levels of glycerol, acetate and xylitol which reflected better growth rates and cell viability when using the adapted strain.

Hydrolysates that were found to be unfermentable with the parental strain could be fermented with the evolved strain both in batch and fed-batch SSF.

Poster 2-17

Improved Xylose Consumption in Recombinant *Saccharomyces cerevisiae* Strains

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Increased demand for ethanol has been observed during the last years due to rising petroleum prices and concerns about a greenhouse effect. Currently ethanol is produced from sugar in Brazil and starch in USA. However, to meet the global demand for bioethanol novel feed stocks requiring new technologies have to be explored. Residues from the agricultural and forest products sector as well as dedicated energy crops represent such a feedstock. It is a lignocellulosic raw material composed of cellulose, hemicellulose and lignin. However, cost competitive ethanol production from lignocellulose requires that all carbohydrate components are completely converted to ethanol.

Baker's yeast, *Saccharomyces cerevisiae*, is a GRAS microorganism and the most extensively used fermentation organism in industrial ethanol production due to its high ethanol productivity and tolerance to industrial fermentation conditions. However *S. cerevisiae* can not naturally ferment pentose sugars, which make up a significant fraction of the carbohydrates in agricultural residues and in hardwoods. Therefore metabolic engineering have been extensively explored to introduce pentose utilization pathways in *S. cerevisiae*. In the current study mayor enzymes of different xylose utilizing pathways were introduced in *S. cerevisiae*. The resulting strains were assessed with respect to enzyme kinetics and rate controlling metabolic reactions during growth in xylose medium.

Poster 2-18

Yeast strains for ethanol production from lignocellulosic hydrolysates in situ detoxification

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Yeast strains of Y1, Y4, and Y7 exhibiting high conversion efficiency of sugars and high ability of tolerating/metabolizing inhibitors in the dilute-acid lignocellulosic hydrolysates developed in our lab were presented in this work. Strains Y1 and Y4 consumed glucose completely in dilute-acid lignocellulosic hydrolysate in situ detoxification within 24h and the highest ethanol yield reached to 0.49g and 0.45g ethanol/g glucose, corresponding to the maximum theoretical value of 96% and 88.2%, respectively. Strain Y1 could metabolize xylose to xylitol with the yield of 0.64g/g xylose, whereas, Y4 was not able to utilize xylose as a substrate. Strain Y7 could consume sugars (glucose and xylose) in hydrolysate in situ detoxification within 72h and a high ethanol yield (equivalent to 93.6% of the maximum theoretical value) was also achieved. Y7 is one of the most efficient yeast strains reported so far for ethanol production from non-detoxified dilute-acid lignocellulosic hydrolysates. It may offer huge potential for improving the economics of the bio-ethanol production from lignocellulosic hydrolysates.

Poster 2-19

The Effect of Carbon Source and Oxygen Level on Global Gene Expression Analysis in *Pichia stipitis*

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The haploid yeast *Pichia stipitis* is closely related to yeast endosymbionts of passalid beetles that inhabit and degrade white-rotted hardwood. It has the highest native capacity for xylose fermentation of any known microbe, and is capable of using all of the major sugars found in wood. This ability to utilize xylose and other sugars is significant as biofuels become increasingly important.

We have used NimbleGen expression arrays to study global gene expression patterns in *P. stipitis* CBS6054. Unlike *S. cerevisiae*, which regulates fermentation by sensing the presence of fermentable sugars, *P. stipitis* induces fermentation in response to oxygen limitation. To examine the effect of carbon source and aeration level on this yeast, we cultivated *P. stipitis* on glucose or xylose both aerobically and under oxygen limitation in pH controlled bioreactors. Comparison of gene chip results from these various cultivations will allow for the identification of genes that are induced under oxygen limitation and those that may be affected both by oxygen level and carbon source. We have also examined cellobiose or arabinose as a carbon source, which has revealed highly specific induction patterns in some cases. These studies will provide invaluable insight into growth on these substrates by *P. stipitis* and the genes responsible for fermentation of the extremely abundant sugar, xylose. Identification of these genes will yield targets for the future engineering of improved lignocellulose fermenting yeasts.

Poster 2-20

Withdrawn

Poster 2-21

Metabolic Engineering of *Escherichia coli* for Efficient Conversion of Glycerol into Ethanol

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During production of biodiesel from vegetable oils or animal fats, glycerol is produced as an unavoidable byproduct that makes up about 10% (w/w) of the total product yield. To utilize the potential surplus of this byproduct, we have developed a metabolically engineered *E. coli* that can efficiently convert glycerol into bioethanol. Using elementary mode analysis, we have designed a mutant *E. coli* that can operate only according to efficient pathways for converting glycerol into ethanol under optimal growth conditions. The mutant is also designed to tightly couple cell growth and ethanol production to facilitate metabolic pathway evolution. The operation of the designed pathways in the mutant has been enforced by implementing 9 gene knockout mutations. Characterization of the mutant in controlled bioreactors shows that the mutant is able to convert 40 g/L of glycerol into ethanol in defined medium within 48 hours and achieves at least 90% of the theoretical ethanol yield (0.5 g ethanol/g glycerol). We demonstrate that the performance of the mutant closely matches the theoretical prediction.

Poster 2-22

Withdrawn

Poster 2-23

Identification of *Saccharomyces cerevisiae* genes involved in the resistance to phenolic fermentation inhibitors

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Saccharomyces cerevisiae was exposed to toxic concentrations of three phenolic phenylpropane derivatives: coniferyl aldehyde, ferulic acid and isoeugenol. DNA microarray analysis was employed as one of the tools to generate a set of candidate genes for deletion mutant analysis to determine the potential contribution of the corresponding gene products to the resistance against toxic concentrations of phenolic fermentation inhibitors. Three *S. cerevisiae* deletion mutants with increased sensitivity to coniferyl aldehyde were identified: *yap1D*, *atr1D,* and *flr1D*. The rate of reduction of coniferyl aldehyde to coniferyl alcohol decreased six-fold when the gene coding for the transcriptional activator Yap1p was deleted and to a lesser extent (three-fold) when the Yap1p-controlled genes encoding Atr1p and Flr1p were deleted. Growth, glucose consumption and ethanol formation progressed after a lag phase during which coniferyl aldehyde reduction and coniferyl alcohol formation occurred. The results link *ATR1*, *FLR1* and *YAP1* by their ability to confer resistance to the same compound and show that deletion of any of these three genes impairs the ability of *S. cerevisiae* to withstand coniferyl aldehyde and detoxify it by reduction. Furthermore, the results suggest that overexpression of *ATR1*, *FLR1* and *YAP1* is of interest for the construction of novel yeast strains with improved resistance against inhibitors in lignocellulose hydrolysates.

Poster 2-24

Aspergillus fumigatus JF1: An Ionic Liquid Tolerant Fungus Isolated from Compost

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Pretreatment of lignocellulosic biomass enables more efficient enzymatic hydrolysis of cellulose and xylan to simple sugars for conversion to biofuels. Recently, ionic liquids, organic salts that are liquids at or slightly above room temperature, have emerged as promising alternatives for pretreatment. Ionic liquids have been shown to solubilize intact biomass and facilitate more rapid enzymatic hydrolysis of biomass polysaccharides. However, liquid retained after pretreatment inhibits enzymatic hydrolysis of the polysaccharides. We have initiated experiments to identify ionic-liquid tolerant microbes that express lignocellulose-deconstructing enzymes that may be more tolerant of these liquids. We have focused on using compost as the inoculum for enrichment experiments because of the diversity of lignocellulose-utilizing microbes in compost. Aspergillus fumigatus JF-1 was enriched from cultures with 1% 1-ethyl-3-methylimdazolium acetate (Emim+OAc-), a commonly used ionic liquid, as the sole carbon source and mature compost as the inoculum. A. fumigatus JF-1 grew with up to 5% Emim+ OAc- as sole carbon source, but higher concentrations of the ionic liquid were inhibitory. When A. fumigatus JF-1 was cultured on switchgrass and corn stover, high levels of cellulase and xylanase activity were detected in the culture supernatant. Enzymatic activities were largely retained when the assays were repeated in the presence of 5% Emim+OAc-. We are using biochemical and proteomic methods to identify cellulases and xylanses expressed by A. fumigatus JF-1 in presence to determine if Emim+OAc- has an influence on protein expression. We are also testing other isolates to determine if ionic-liquid tolerance is a general property of saprophytic fungi.

Poster 2-25

Converting C5 and C6 sugars from hydrolyzed pretreated lignocellulosic materials using _Thermoanaerobacter_ BG1L1

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Economic conversion of lignocellulosic biomass materials into bioethanol requires conversion of both C5 and C6 sugars during the fermentation following pretreatment and enzymatic hydrolysis. Conventional yeast *Saccharomyces cerevisiae* lacks the metabolic pathways necessary for conversion of C5 sugars into ethanol. While several genetically engineered strains have been shown to convert C5 sugars, little data exist in the public literature on actual performance of these strains with pretreated lignocellulosic materials.

Thermoanaerobacter BG1 is a thermophilic bacterium isolated from a hotspring on Iceland and having the ability to use both C5 and C6 sugars simultaneously. Strain BG1L1 is a lactate dehydrogenase deficient mutant produced in our laboratory. This strain has been tested with mixed straw as raw material in both laboratory and pilot scale over extended periods of time. Besides this the strain has further been tested in laboratory scale on a large variety of biomass material such as straw, bagasse, willow, soft and hard wood, corn fibers, corn cobs and DDGS.

In the presentation we will show data from experiments with BG1L1 growing on different hydrolysates as well as the performance of this strain with several sugars present. Especially the performance of the strain when grown on xylose and arabinose will be presented in detail showing the major potential for using this bacterium for conversion of corn cobs, corn fibers and DDGS.

Poster 2-26

The model filamentous fungus _Neurospora crassa_, a great system for studying lignincellulose degradation

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Neurospora crassa is a well-known model organism for studying genetics, biochemistry and fungal biology. In nature, *N. crassa* grows on dead plant material, particularly members of the grass family and was described as cellulose degrader over 30 years ago. Unlike other filamentous fungi, *N. crassa* has comprehensive genetics and molecular biology tools and unique functional genomics resources, which include a near full genome deletion strain set and whole genome microarrays. *N. crassa* can grow on plant cell walls or crystalline cellulose (Avicel) as a sole carbon source. We identified the transcriptome associated with growth of *N. crassa* over a 10 day time course on ground Miscanthus stems; over 1000 genes increased in relative expression level. When compared to *N. crassa* transcriptome when grown on crystalline cellulose, an overlap set of 129 genes was identified at an early time point; the functional annotation of these genes showed an enrichment for proteins predicted to be involved in carbohydrate metabolism, but also a significant number of genes/proteins of unknown function. The secretome associated with *N. crassa* grown on Miscanthus or Avicel was determined by MassSpec analysis; at least 85 proteins were identified. A detailed functional analysis of the strains containing mutations in genes encoding these 85 proteins and subsequent bioechemical analyses is an ongoing study. I will also discuss the identification of regulators that affect cellulase gene expression and plans to explore cellulase synergism, using N. crassa as a model celluloytic fungus.

Poster 2-27

***Zymomonas mobilis* systems biology studies to elucidate process relevant inhibitor stress responses and tolerance mechanisms**

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Robust process-tolerant and inhibitor-resistant microbes are recognized as key short-term technological goals for the rapid expansion of cellulosic-ethanol production. *Zymomonas mobilis* is a promising ethanogenic bacterium due to its productivity, high level of ethanol tolerance and its ability to be genetically manipulated. In a series of studies, we have investigated the effects different inhibitors on *Z. mobilis* using genetics and systems biology tools to better understand the physiology of the organism and its stress responses. We observed maximum specific growth rates were not dramatically different between aerobic and anaerobic conditions, yet oxygen did affect the physiology of the cells leading to the buildup of metabolic byproducts that ultimately led to greater differences in transcriptomic profiles in stationary phase. In stationary phase cultures, there was only 1.7% of the amount of ethanol present aerobically as there was anaerobically. Similarly, the effect of ethanol on *Z. mobilis* fermentations was profiled using microarray, proteomics, and metabolomics. We have resequenced the genomes of an acetate tolerant mutant and the wild-type strain using comparative genome sequencing via microarray, next-generation 454-pyrosequencing and Sanger sequencing, which identified a 1.5 kb deletion in the mutant strain and many SNPs in both strains. A locus was identified that conferred acetate tolerance in *Z. mobilis* through systems biology tools, mutagenesis and complementation experiments. Finally, we have updated the *Z. mobilis* ZM4 genome annotation using a new annotation pipeline and 454-pyroresequencing, transcriptomics, and proteomics data to facilitate the future *Z. mobilis* studies. An overview of these studies will be presented.

Poster 2-28

Characterization of Four *Clostridium* species for Ethanol Production

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This research aims to address a piece of the energy crisis by looking for more efficient methods of ethanol production from lignocellulose. Four *Clostridium* species were characterized: *C. phytofermentans*, *C. cellulolyticum*, *C. cellulovorans*, and *C. thermocellum*. These bacteria are capable of breaking down cellulosic substrates and fermenting them into a variety of products, including ethanol. Six substrates were used: cellobiose, crystalline cellulose, fibrous cellulose, xylan and rice straw (both raw and pretreated). Both celluloses were combined with xylan for a total of 8 treatments per bacterium. Each bacterium was grown on each substrate in triplicate. Gas production and products were measured using a pressure transducer and high performance liquid chromatography (HPLC), respectively. Products that were measured are ethanol, formic acid, acetic acid, propionic acid, butyric acid and lactic acid, as well as residual soluble carbohydrates. Pressure was measured daily and products were measured after gas production had ceased. Preliminary data has shown that *C. phytofermentans* and *C. cellulolyticum* can produce ethanol yields up to 0.4 g/g on cellobiose. These two bacteria have shown a preference for xylan over both types of cellulose while *C. cellulovorans* has the highest preference for crystalline cellulose of the four *Clostridium* species.

Poster 2-29

The Endogenous Molecular Basis for Improved Xylose Utilization of *Saccharomyces cerevisiae*

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Although varied recombinant *S.cerevisiae* strains capable of utilizing xylose have been constructed, the cofactor and global metabolic imbalance caused by the heterogonous *XR/XD* genes have been extensively demonstrated. In this study, a mutant strain *S.cerevisiae* 9763v, without any heterogonous genes, displayed significantly improved ability to utilize xylose. The growth rate of the mutant was 0.094 g of DM liter^{-1} h^{-1}, almost 6 times higher than the reported recombinant strain with *XYL1* and *XYL2* from *P. stipitis*. Additionally, the mutant showed a considerable xylose conversion that was 42% higher than the parental strain. Specific activities of XR, XDH and XK in the mutant displayed 2- to 5-fold of those in the original strain. Interestingly, the enzymatic activities in the glucose and xylose co-fermenting cells was up to 10-fold higher than in those grown solely on xylose. The DNA microarray results presented strong evidence that ethanol production in the mutant was mediated by endogenous xylose metabolism pathway with much higher transcriptional level of genes encoding XR, XDH and XK, genes involving in the pentose phosphate pathway and glycolytic metabolic pathway. More importantly, the glucose derepressible XR, XDH and XK of the mutant were also revealed by higher expression level of the relative genes in the glucose and xylose co-fermenting cells compared to the xylose growing cells. The results could be of significant importance in developing native xylose fermenting *S.cerevisiae* strain with innovate enzymatic design and modification of the endogenous XR, XDH and XK, as well as metabolic engineering of xylose metabolism pathway.

Poster 2-30

Improved galactose fermentation by *Saccharomyces cerevisiae* through inverse metabolic engineering

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Biomass from marine plants has several interesting attributes which make it a potential renewable source for production of biofuels. In terms of mass per unit area, yields of marine biomass are higher than yields of terrestrial lignocellulosic biomass. Marine biomass does not contain recalcitrant lignin or crystalline cellulose and is therefore depolymerized more easily than lignocellulosic biomass. Relatively high rates of carbon fixation by marine plants also make them attractive for carbon dioxide sequestration and recycling. One of the abundant carbohydrates in marine biomass is galactose. Although *Saccharomyces cerevisiae* is capable of fermenting galactose into ethanol, ethanol yield and productivity from galactose are significantly lower than those from glucose. An inverse metabolic engineering approach was undertaken to improve ethanol yield and productivity from galactose in *S. cerevisiae*. Specifically, we introduced a genome-wide perturbation library into *S. cerevisiae*, and then screened fast galactose-fermenting transformants. Characterization of genetic perturbations in the isolated transformants revealed novel targets which elicit enhanced galactose utilization in yeast. Interestingly, most of them are not directly related to galactose metabolism. Of the identified genetic perturbations, overexpression of a well-known transcriptional regulator in a truncated form drastically increased ethanol yield and productivity from galactose as well as from a mixture of glucose and galactose. These results suggest that global reconfiguration of sugar metabolism is more effective than overexpression of a single metabolic gene in the galactose assimilation pathway for efficient galactose fermentation in *S. cerevisiae*.

Poster 2-31

Molecular and physiological characterization of heterologous xylose transporters in recombinant xylose-utilizing *Saccharomyces cerevisiae*

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Baker's yeast *Saccharomyces cerevisiae* has been modified by metabolic engineering to ferment the pentose sugar xylose. Xylose is commonly found in forest and agricultural residues and is important for process economics of ethanol production from lignocellulose biomass. Recombinant xylose-utilizing *Saccharomyces cerevisiae* however lack specific transporters for xylose. Consequently this substrate enters the cell through non-specific hexose transporters. Compared to glucose, these transporters have poor affinity for xylose, and it has been hypothesized that this step may control the rate of xylose utilization. Furthermore, the high affinity hexose transporters responsible for xylose uptake, show significant repression by glucose during co-fermentation of glucose and xylose. To improve xylose uptake and reduce repression by glucose, we have expressed the previously identified xylose transporters Gxf1 (*Candida intermedia)*, Sut1 (*Piccia stipitis*) and At5g59250 (*Arabidopsis thaliana*) in recombinant *Saccharomyces cerevisiae*. Resulting strains were characterized in terms of xylose transport kinetics following cultivation in different media. The strains were also compared in aerobic and anaerobic batch cultivation at different substrate concentrations. Results are discussed in relation to different industrial fermentation techniques and the composition of substrate feeds.

Poster 2-32

Regulation of pfl in *B. coagulans*

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The sporogenic lactic acid bacterium, *Bacillus coagulans*, grows and ferments sugars such as glucose and xylose at 50-55 °C and pH 5.0, the optimum temperature and pH for the activity of commercial fungal cellulases. Because of these properties, simultaneous saccharification and fermentation (SSF) of cellulose to products by *B. coagulans* requires less cellulase for optimum volumetric productivity than at a sub-optimal temperature for SSF utilizing yeast. During growth in pH-controlled fermentations of glucose at 50°C and pH 5.0, *B. coagulans* strain 36D1 produced lactate as the fermentation product. Although the genes encoding pyruvate formate-lyase (PFL) are present in the genome of *B. coagulans* strain 36D1, formate, the distinctive product of PFL activity, was not detected in the broth of the pH 5.0 culture either during the growth phase or early stationary phase. However, growth of *B. coagulans* at a medium pH of 7.0 supported PFL activity as evidenced by accumulation of formate during the late-log phase of growth. In agreement with this observation, the *pfl* mRNA was about 3-times higher in cells grown at pH 7.0 compared to cells grown at pH 5.0. The *pfl* mRNA was detected only in anaerobically grown cells and not in cells grown with aeration. These results suggest that the transcription of *pfl* in *Bacillus coagulans* is controlled by the level of oxygen, culture pH and growth stage.

Poster 2-33

Study of cell viability and morphology of *Zymomonas* in the presence of inhibitors

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Fermentation by microorganisms remains to be a critical step for optimization in the conversion of biomass to ethanol to make the process economical. It is well known that pretreatment of feedstocks generates hydrolysates containing toxic compounds (acetate, furfural, HMF, etc), which can severely inhibit the growth and fermentation performance of microorganisms. Although a great deal of research has been conducted to evaluate the ethanologen tolerance to the inhibitors in hydrolysates, this work has rarely provided insights into the mechanisms of toxicity. Using the ethanologen, *Zymomomas mobilis* 8b as a model organism, we present tools for examining hydrolysate toxicity using flow cytometry and fluorescence microscopy analyses of cells stained with dyes such as Syto 9 and propidium iodide grown in the presence of specific inhibitory compounds as well as hydrolysate. Development of these tools can lead to research for improving the cell viability under the stress of hydrolysates which will ultimately enhance the fermentation performance of an organism.

Poster 2-34

Engineering of yeast strains capable of broad substrate utilization in alcohol fermentation - Part I: Identification of founder strains and gene candidates

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Alcohols such as ethanol and butanol produced directly from renewable biomass are considered as an important alternative energy source for sustainable development of human society. Conversion of low-value biomass and industrial organic waste into alcohols, however, has remained a significant challenge due to rarity of natural organisms able to do so and the performance limitation of existing engineered microbes. Working with a collection of over 400 biomass-utilizing or alcohol-producing fungi/yeast strains, we have begun a two-pronged approach to address the need for alternative energy sources: 1) isolation and characterization of natural fungi able to ferment lignocellulose to alcohols and 2) genetic engineering of high alcohol-producing yeast strains capable of using a wide-range of biomass and organic waste. Here we report the progress on selection of stress tolerant founder strains, selection of desired genes or gene clusters from *Trichoderma reesei*, strategy of strain construction, and characterization of resultant yeast strains that are able to digest pretreated woody materials and ferment them into alcohols. A single organism capable of simultaneous saccharification of biomass and fermentation of alcohols (SSF) has the advantage of simplified industrial process and reduced production cost.

Poster 2-35

Rapid fermentation of glucose, xylose and cellobiose by the wood-boring beetle-associated yeast, *Spathaspora passalidarum*

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The efficient fermentation of xylose and cellobiose is one of several significant challenges in the bioconversion of lignocellulosic hydrolysates to fuel ethanol. These sugars comprise the majority of a plant cell wall; however most fermentative yeasts are unable to use them. Recently, Nguyen, et al. (2006) described a novel ascomycetous yeast, *Spathaspora passalidarum*, isolated from the gut of the beetle, *Odontotaenius disjunctus*. This insect is commonly found in decaying logs; therefore organisms located in its gut would be exposed to carbon sources commonly found in lignocellulosic hydrolysates.

Here we describe the growth and fermentation characteristics of this yeast when cultivated on multiple carbon sources, including glucose, xylose, or cellobiose. Initial experiments indicate that it exhibits a distinctly different growth potential from previously characterized species when cultivated in medium with xylose or cellobiose as sole carbon sources, and shows excellent growth potential when grown on all of above sugars. Significant ethanol production was observed under oxygen limitation, and its capacity for growth under severe oxygen limitation exceeded that of other native xylose fermenting yeasts. Initial observations indicate that this yeast may even surpass *Pichia stipitis* in its ability to ferment xylose. Further characterization of *S. passalidarum* will provide great insight into native mechanisms of lignocellulose degradation and fermentation by microbes.

Nguyen N, Suh S, Marshall C, Blackwell M, 2006. Morphological and ecological similarities: Wood-boring beetles associated with novel xylose-fermenting yeasts, *Spathaspora passalidarum* sp. nov. and *Candida jefftriesii* sp. nov. Mycol. Res. 110:1232-1241.

Poster 2-36

Evaluation of engineered xylose-fermenting industrial strains of *Saccharomyces cerevisiae* for improved ethanol production from lignocellulosic feedstocks

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Saccharomyces cerevisiae is currently used to produce ethanol from glucose, but it cannot utilize five-carbon sugars contained in the hemicellulose component of biomass feedstocks. Hemicellulose can make up to 20-30% of biomass and is primarily composed of xylose. Enzymes from native xylose-assimilating organisms have been transferred to *S. cerevisiae* allowing fermentation of xylose. However, efficient conversion of xylose to ethanol is limited, putatively by cellular redox imbalance, low flux of xylose into the pentose phosphate pathway, and lack of efficient xylose transport into the cell. Genetic background has been demonstrated to play a vital role in the fermentation capacity and stress tolerance of laboratory and industrial yeast strains. The goal of this study was to compare xylose fermentation properties of several industrial yeast strains in order to identify a genetic background conferring improved xylose fermentation. Six industrial strains of *S. cerevisiae* from the Agricultural Research Service (ARS) culture collection were engineered to express the *Pichia stipitis* genes encoding xylose reductase and xylitol dehydrogenase, as well as the *S. cerevisiae* xylulokinase gene. Each gene was expressed from a different constitutive, high-level promoter. The three genes were stably integrated at the HO endonuclease site on chromosome IV. The resulting strains were analyzed to determine xylose consumption rates and ethanol productivities. One of the strains showed superior xylose growth and consumption compared to our haploid lab strain and other engineered industrial strains. Xylose fermentation data for the different strains will be presented.

Poster 2-37

The effect of raw glycerol discharged after biodiesel manufacturing in the xanthan gum production synthesized by *Xanthomonas* isolated in Brazil

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The cultural conditions for xanthan gum production by three strains of the *Xanthomonas* sp isolate in Brazil were investigated and optimized by response surface methodology, to maximize xanthan production in batch experiments using a synthetic broth (MPI+II) and glycerol. The individual and interactive effects of three independent variables (substrate concentration (glycerol and sucrose) (0 – 100%), agitation rate (180-250 rpm), time of cultivation (72–120 h)) on xanthan gum and biomass production were studied, using a face-centered composite design of experiments. Overall optimization allowed us to point out an optimal range of the three independent variables. Experimental design methodology were performed and the maximum productivity obtained was 0.331 g/L.h. The viscosity analysis was performed for aqueous solutions 3%, at 25, 45 and 60ºC. The polysaccharides synthesized in media with glycerol and sucrose was largely indistinguishable from xanthan gum produced only glycerol. The rheology (apparent viscosity) presented similar values compared to those of the literature for other biopolymers.

Poster 2-38

Metabolic engineering of a novel thermophilic ethanologen *Geobacillus thermoglucosidasius* M10EXG for enhanced ethanol production

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The thermophilic bacterium *Geobacillus thermoglucosidasius (Gth)* M10EXG is a facultative anaerobe that has an optimal growth temperature of 60 ºC. It can metabolize both C5 (xylose) and C6 (glucose) sugars and is tolerant to 10% ethanol, making it an attractive candidate for industrial bioethanol production from lignocellulosic biomass. However, in order to maximize the production of ethanol from the fermentative pathway, it is essential to understand the fermentative metabolism, operational pathways, and the flux through the different fermentative pathways. We have completed a metabolic analysis of the growth of *Gth* M10EXG using both xylose and glucose under varying concentrations of oxygen. As expected, ethanol production is detected only under anaerobic conditions using either xylose or glucose as the sole carbon source. Furthermore, metabolic flux analysis of the anaerobic and aerobic growth using glucose as the sole carbon source shows that 0.6 mol lactate, 0.9 mol acetate, 0.4 mol ethanol, and 1.0 mol formate are produced per mole of glucose metabolized. With recent genome sequencing and metabolic flux analysis completed, we have targeted both lactate and formate production pathways for modification to increase ethanol production. We present results from the metabolic engineering of the aforementioned pathways and the effect on ethanol production.

Poster 2-39

Draft genome sequence, annotation and metabolic pathway reconstruction of the ethanol-tolerant thermophile *Geobacillus thermoglucosidasius* M10EXG

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We report the draft genome sequence, annotation and metabolic pathway reconstruction of the ethanol-tolerant thermophile *Geobacillus thermoglucosidasius M10EXG* (Gth M10EXG). Gth M10EXG is a facultative anaerobe that can ferment a range of C5 and C6 sugars and shows growth in media containing up to 10% v/v ethanol and is therefore an excellent candidate for simultaneous saccharification and fermentation during bioethanol production from lignocellulosic biomass. In order to understand the mechanisms of ethanol production, ethanol tolerance and to engineer optimum performance in an industrial bioethanol plant we decided to sequence the genome and do metabolic reconstruction of the pathways present in Gth M10EXG. The draft genome has 3.77 million base pairs arranged on 80 contigs and 4420 genes have been identified. A total of 2711 transcription units and 344 pathways have been assigned. We will discuss the unique metabolic pathways identified in Gth M10EXG and compare the genome to closely related species *Geobacillus kaustophilus* and *Geobacillus thermodenitrificans*.

Poster 2-40

Heterologous expression of multiple cellulolytic enzymes in *Zymomonas mobilis*

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Consolidated bioprocessing (CBP) has the potential to reduce ethanol production costs from lignocellulosic biomass by reducing the production cost of saccharolytic enzymes. CBP requires a microorganism that can carry out both the enzymatic depolymerization of plant cell wall polysaccharides and the fermentation of the resulting sugars to ethanol. One microorganism that shows great promise in developing CBP is the facultative anaerobic gram-negative bacterium *Zymomonas mobilis*. *Z. mobilis* can achieve a higher ethanol yield than most yeasts, has been metabolically engineered to use xylose and arabinose, has a naturally high tolerance to inhibitory compounds found in lignocellulosic hydrolysates, and has been successfully used to express heterologous proteins. We describe here the expression of a hemi-cellulolytic enzyme (*xynA* from *Thermomyces lanuginosus*) and a cellulolytic enzyme (*E1* from *Acidothermus cellulolyticus*) in both *Z. mobilis* and *E. coli*. We use multiple promoters, terminators and plasmid backbones. We additionally explore the use of codon optimization in enhancing heterologous expression in *Z. mobilis*. We report here that *Z. mobilis* is capable of expressing both XynA and E1 protein. Additionally, we show that both of these enzymes are catalytically active, providing a preliminary validation of using *Z. mobilis* as a CBP host.

Poster 2-41

Characterization of biohydrogen production in metabolically engineered *Escherichia coli* strains

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Microbes have diverse biosynthetic pathways to produce molecular hydrogen and potentially hold the key to the viable macroscale utilization and production of hydrogen from renewable sources. However, low production yield has been a major limiting factor for large-scale biohydrogen production because of various metabolic bottlenecks. Dark fermentations seem to hold the best promise for biohydrogen production due to low costs and relatively high production yields. Because of the availability of metabolic pathway information and took kits for *Escherichia coli*, this organism was chosen for this study. *E. coli* strains were engineered for greater production of hydrogen by using a combinatorial strategy of over-expressing the components of the hydrogen-evolving complex, interruption of the uptake hydrogenases, and the elimination of competing metabolic pathways.

We present the results comparing several strains for hydrogen production and metabolite formation during batch fermentations at a constant pressure of 760 mm Hg and utilizing a rich synthetic media. The base strain had uptake hydrogenases 1 and 2 deleted along with *hycA*, which is responsible for repressing the hydrogen-evolving complex. Additional strains included the over-expression of *hycEG,* encoding the main subunits of hydrogenase 3, and the interruption of lactate and succinate formation through the elimination of *ldhA* and *frdBC*. Acetate production was also interrupted using paired termini antisense RNA against the *ackA* gene within the acetate kinase-phosphotransacetylase operon. This work provides for the possible application of this knowledge towards developing a commercially efficient hydrogen-producing strain of *E. coli.*

Poster 2-42

Engineering tolerance to spent sulfite liquor (SSL) by genome shuffling *Saccharomyces cerevisiae* leads to increased ethanologenic capacity

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Lignocellulosic substrates derived from waste biomass have become an attractive feedstock for the production of inexpensive, more environmentally-friendly biofuels. For example, spent sulfite liquor (SSL), a carbohydrate-rich effluent produced in sulfite pulping, can be used to add value to the pulp and paper industry by using the sugars it contains to produce ethanol. However, using SSL in such a capacity requires a robust, ethanologenic microorganism that can withstand the substrate toxicity that is due to the presence of inhibitory compounds like furfural, 5-hydroxymethylfurfural (HMF) and acetic acid. *Saccharomyces cerevisiae* is currently used for the production of ethanol from SSL. This industrially well-established yeast, though a robust starting organism for SSL fermentation, will still succumb to toxicity and inhibition, especially in the most inhibitor rich forms of SSL such as hardwood SSL (HWSSL). To establish a *S. cerevisiae* strain that can overcome such a complex and incompletely understood form of inhibitory pressure, a genome shuffling method was developed to create a better SSL fermenter. This method aims to improve polygenic traits by generating a pool of mutants with improved phenotypes, followed by iterative recombination between their genomes. Through five rounds of shuffling and screening, three strains were obtained that are able to not only survive in HWSSL, but grow to a limited extent. Our results show that the tolerance of these strains to SSL translates into an increased capacity to produce ethanol over time using this substrate, due to continued viability of the yeast population.

Poster 2-43

Withdrawn

Poster 2-44

Production of xanthan gum from sisal juice and glycerol

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The xanthan gum is a biopolymer produced by Xanthomonas, has the ability to form viscous gels and solutions in water, gum and the more commercially acceptable due to its wide application in food and other products thickeners, stabilizers or viscosificantes. Glucose and sucrose are cited as sources of carbon preferred, however, the use of agroindustrial waste into bioprocess the achievement of low-cost alternative substrate for fermentation process, which reduces production costs and help in the allocation of waste, minimizing environmental problems. This study aimed to investigate the influence of two media fermentative supplemented with juice sisal residual (Agave sisalana) or glycerol and study the conditions of fermentation in the production of xanthan gum in shaker from a native strain of Xanthomonas campestris, and perform the rheological characterization of polymers obtained. The production of the inoculum occurred in Erlenmeyer flasks with agitation of 150 and 180 rpm at 28ºC for 24 hours, in the YM. The experiments for the production of gum were made by fermentation in Erlenmeyer flasks in shaker at 200 and 250 rpm for 96 hours at. The average yield obtained was 2.41±0.02 gL^{-1} with juice sisal residual to 200 rpm in the medium fermentation 01 and 3.48±0.01 gL^{-1} at 250 rpm in the medium fermentation 02, obtained with glycerol 2.08±0.01 gL^{-1} at 200 rpm in the medium fermentation 01 and 0.85±0.01 gL^{-1} at 250 rpm in the medium fermentation 02. The aqueous solutions of gum showed pseudoplastic behavior characteristic.

Poster 2-45

Engineering E. coli to digest and utilize cellulose and hemicellulose as sole carbon sources

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E. coli is widely used for the production of small molecules via metabolic engineering. However, it is unable to use plant biomass as a feedstock. We are engineering *E. coli* to enable growth on cellulose and hemicellulose in two steps: 1) harnessing secretion systems to export milligram quantities of glycoside hydrolases and other biomass degrading enzymes cloned from cellulolytic organisms; 2) enabling *E. coli* growth on the soluble oligosaccharide products of degradation by incorporating permease and beta-glucosidase genes into the chromosome. In order to enhance growth on crystalline substrates such as cellulose, we have also engineered membrane attachments for the secreted enzymes to allow assembly into multienzyme complexes, which are analogous to cellulosomes found in *Clostridia* species. These modifications can be incorporated into *E. coli* strains able to generate valuable compounds including fuels, which will enable production directly from plant biomass.

Poster 2-46

Yeast genes identified in HMF tolerance screen suggest link to other lignocellulosic derived inhibitors including furfural and vanillin

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Due to environmental and economical concerns, there is a high demand for a renewable fuel source. Bio-ethanol from lignocellulosic biomass is one such renewable fuel. Before this can be realized many problems need to be addressed. One major problem is the existence of multiple inhibitors found in a typical lignocellulosic hydrolysate. The more predominant inhibitors include furfural, hydroxymethylfurfural (HMF), and vanillin. These chemicals inhibit growth and fermentation of the yeast, Saccharomyces cerevisiae. In attempt to understand the genes involved in inhibitor tolerance we performed a screen looking for yeast mutants that exhibited altered growth in the presence of HMF. Genes isolated from this screen included those involved in telomere maintenance, protein and ergosterol metabolism, the pentose phosphate pathway, mitochondria and vacuole function, gene transcription, generalized stress tolerance, and genes of unknown function. Interestingly, there were many similar genes and pathways found in the HMF screen compared to the screen for furfural and vanillin tolerance. This suggests that similar pathways exist in yeast for protection against multiple inhibitors found in a lignocellulosic hydrolysate. To further investigate these similarities we analyzed some of these common genes with a focus on genes involved in the pentose phosphate pathway, ergosterol biosynthesis, telomere maintenance, and oxidative stress protection. Yeast cultures with one of these genes mutated, overexpressed, or exogenous gene product added were examined for their ability to grow in the presence of HMF, furfural, or vanillin. In addition, under these same conditions, yeast cell physiology and the accumulation of reactive oxygen species were quantified.

Poster 2-47

Construction of yeast strains producing high concentration of ethanol from tapioca by using various genetic methods

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For the development of yeast strains producing fuel ethanol efficiently, various genetic methods such as screening and selection, γ-irradiation-mutation, mutation by chemical mutagen, mating, protoplast fusion and recombinant DNA techniques were used. Totally 330 strains of ethanol-producing yeast strains were collected and their fermentation capabilities were examined. Strain KK1 was selected as the best ethanol producer by screening and selection of yeasts from soils of distillery. The pH-regulated continuous culture selection method was more efficient for the selection of good hybrid strains than the conventional selection method. From the rare-mating between haploid *Saccharomyces* sp. and polyploid *S. cerevisiae* and by using continuous culture selection method, hybrid clone KK2 was obtained. This strain was γ-irradiated and finally KK2-7 was selected as the best strain. By repeated γ-irradiation, the best strain KK2-7-2 was selected. From mutagenesis by treatment of NTG, strain NT30-9 was obtained. After protoplast fusion, strain F160 was selected as the best strain. Using rare-mating and recombinant DNA techniques, yeast strain capable of directly fermenting starch has been constructed. All the above genetic methods used were efficient in the developing yeast strains with improved ethanol production.

Poster 2-48

Isolation of cellulose and agarose-degrading *Pseudoalteromonas* sp. NO3 from Sea squirt, *Halocynthia rorentzi*

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Pseudoalteromonas sp. was reported for the main cause of mass mortality of sea squirt, *Halocynthia rorentzi*. Cellulose and agarose-degrading *Pseudoalteromonas* sp. NO3 was isolated from the tissue of abnormal Sea squirt. The highest agarase and cellulase activity was found in the culture supernatant of *Pseudoalteromonas* strain. The optimal pH and temperature of agarase and cellulase were determined by a reducing sugar assay, where the optimal pH of two enzymes was 8 and the optimal temperatures was 35°C. A thin layer chromatography (TLC) was used for the determination of agarose and cellulose hydrolysis reaction after incubating 200μl of substrates (agarose and carboxymethyl cellulose) and 25μl of culture supernatants (2.29mg/ml) at 35°C for 2hr. The presence of low molecular size of digested cellulose and agarose on the TLC plate indicated that *Pseudoalteromonas* strain secretes significant quantities of agarase and cellulase into extracellular environment. We showed high-throughput screening assay that agarase and cellulase activity can be directly measured using liquid cultures grown in a microtiter plate instead of separation or purification steps and is fast and easy to perform more adaptable for screening of a large number of samples.

Poster 2-49

Construction of a reporter gene system for use in the ethanologen *Geobacillus thermoglucosidasius* SB2

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The thermophilic bacterium, *Geobacillus thermoglucosidasius* SB2 is a facultative anaerobe that ferments a range of C5 and C6 sugars making it an attractive candidate for bioethanol production. Fermentation of D-glucose by *G. thermoglucosidasius* SB2 results in mixed acid production (ethanol, L-lactate, acetate and formate). Production of unwanted fermentation products can be prevented by creating strains where genes, encoding key enzymes in the synthesis pathways of these acids, have been knocked out. However, for increased carbon flux to ethanol the upregulation of key genes (e.g. the *pdh* operon) under fermentative conditions is advantageous. Increased expression can be achieved by inserting a promoter active under the desired conditions upstream of the gene to be upregualted.

In order to asses promoter strength, a reporter gene plasmid containing the *phe*B gene from *Geobacillus stearothemophilus* DMZ6285, which encodes a thermophilic catechol 2,3 dioxygenase (C23O), was constructed in the *Escherichia coli – G. thermoglucosidasius* shuttle vector pUCG18. Six sequentially shorter fragments of the *pdh*Aα upstream region, based on *in silico* predicted promoter sequences, were cloned 5′ to the *phe*B gene. C23O enzyme assays and qRTPCR data indicates all promoter fragments were able to initiate transcription of *phe*B under aerobic conditions and at a reduced level in micro-aerobic cultures. However, fragments *int1* and *int2*, which correspond to the promoter sequences proximal to the *pdh*Aα gene, were also found to allow expression under anaerobic conditions. This system could be used to elucidate a promoter sequence that allows constitutively high expression of its downstream gene under all growth conditions.

Poster 2-50

D-Lactic Acid Production from Xylose by a New Bacterium Found in Thailand

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Lignocellulosic biomass is one of the most abundant renewable resources on earth, but conversion of biomass to ethanol has many challenges including pentose fermentation. Pentose is usually a result of dilute-acid pretreatment, and the majority of the pentose generated is xylose. Although, several organisms can convert xylose to ethanol but the conversion is still relatively inefficient. However, xylose can also be converted to lactic acid, which is a building block of polylactic acid (PLA), a biodegradable plastic. Our research team has found a new microbial isolate that can ferment xylose to D-lactic acid with the chiral purity greater than 95%. The bacterium was isolated from manure samples from the farm in Northeastern part of Thailand. Addition of poly D-lactic acid to the existing poly L-lactic acid will improve the thermal properties of the resulting polymer. Nonetheless, lactic acid is not the only product produced by this isolate. In this study, the xylose concentration, temperature and pH are varied in order to find the suitable conditions for D-lactic acid production by this isolated bacterial strain.

Poster 2-51

Metabolic engineering of flocculent *Saccharomyces cerevisiae* with genome-integrated $NADP^+$-dependent xylitol dehydrogenase gene for ethanol production from xylose

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Saccharomyces cerevisiae is commonly used for industrial ethanol production. However, it cannot ferment xylose, the second most common fermentable sugar in the hydrolysates of lignocellulosic biomass. Meanwhile, xylulose, an isomerization product of xylose, can be metabolized by *S. cerevisiae*. *S. cerevisiae* transformed with the *XYL1* and *XYL2* genes encoding XR and XDH from *Pichia stipitis* and the *XKS1* gene encoding xylulokinase (XK) from *S. cerevisiae* acquires the ability to ferment xylose to ethanol. However, this approach is insufficient for industrial bio-processes because of the low rate of fermentation and unfavorable excretion of xylitol. The difference in the coenzyme specificities of XR and XDH creates an intracellular redox imbalance that has been implicated as the main cause of xylitol excretion. To reduce xylitol formation during xylose fermentation, we have been examining laboratory recombinant *S. cerevisiae* strains that express a XDH mutant (ARSdR; D207A/I208R/F209S/N211R), which has a complete reversal of coenzyme specificity toward $NADP^+$. In the present study, the flocculent industrial *S. cerevisiae* strain IR-2, which has high xylulose-fermenting ability, was first selected as a host suitable for genetically engineering xylose fermentation. We then constructed a recombinant strain (MA-R5) through the chromosomal integration of the $NADP^+$-dependent XDH gene, as well as the XR and XK genes. MA-R5 had a markedly increased xylose consumption rate and an increased ethanol yield as compared to the reference strain (MA-R4) that expressed wild-type XDH. Furthermore, MA-R5 effectively co-fermented glucose and xylose and produced ethanol with a high yield from the detoxified hydrolysate of wood chips.

Poster 2-52

The role of membrane phospholipids in xylose utilization

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Cellulosic ethanol fermentation process will be economically viable provided both five and six carbon sugars are fermented simultaneously. At present we have major concerns using genetically modified yeast *Saccharomyces Cerevisiae (424A)* including slow utilization of xylose when compared to glucose and inhibition of xylose utilization pathway due to presence of inhibitors which are produced during pretreatment process. In order to have a fundamental understanding of how the xylose utilization pathway is inhibited, one has to first understand the lipid profile of the yeast in pure sugar media and lignocellulosic hydrolyzate. Some of the experiments we will carry out in order to understand the above mentioned process include: (1) Xylose utilizing rates under different temperatures, and the corresponding differences on the lipidome level, (2) Changes of xylose utilizing rate during the process of cultivation in both pure sugar media and hydrolyzate produced by enzymatic hydrolysis of AFEX treated corn stover and the corresponding changes on lipidome level as a function of time and (3) Compare the difference in xylose utilizing dynamics and difference in lipidomes for genetically modified yeast strain 424A to that of native strain. Lipid profiling will be done using LCMS using normal and reverse phase separation.

Poster 2-53

Effect of lignocellulosic inhibitory compounds on the fermentative capacity of different yeast strains

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It would be very desirable if yeasts were able to directly ferment the hemicellulose derived sugars obtained after the steam pretreatment of biomass without the need to detoxify the resulting sugar stream. Several *Saccharomyces cerevisae* strains, such as spent sulfite liquor (SSL) adapted Tembec strains (T_1 and T_2), an ethanologenic strain Y1528 and a wild strain (BY4742) were selected to assess their ability to ferment the liquor derived from steam pretreated Douglas-fir. These strains were selected on the basis of their unique characteristics: the Tembec strains are very robust and are able to survive in and ferment the SSL containing different inhibitory compounds, whereas, the strain Y1528 exhibits an interesting trait of preferably utilizing galactose over glucose and mannose. Initially, none of the strains was able to ferment the liquor which was likely due to the presence of fermentation inhibitors such as furans and lignin degradation products. However, after the addition of glucose to the liquors, the yeast strains produced comparable amounts of ethanol. High ethanol yields were observed with the Tembec strains as compared to Y1528 and BY4742. The higher sugar concentration possibly supported the growth of the Tembec strains even in the presence of inhibitors. We next studied the effect of different fermentation inhibitors (HMF, Furfural, coniferyl aldehyde, cinnamic acid, 4-hydroxybenzoic acid and vanillic acid) on the fermentative capability of the yeast strains. These experiments showed that the degree of tolerance and fermentative capability of yeast strains towards the inhibitory compounds was confined to the specific inhibitor used.

Poster 2-54

Riboflavin production during fermentation of genetically modified biobutanol-producing *Clostridium acetobutylicum*

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Solvent producing Clostridia are well known for their capacity to use a wide variety of renewable biomass and agricultural waste materials for biobutanol production, which make the process economic and friendly to the environment. To explore the possibility of co-production of a high-value product during biobutanol production, the *C. acetobutylicum* riboflavin operon *ribGBAH* was over-expressed in *C. acetobutylicum* on an *E. coli-Clostridium* shuttle vector J-ribGBAH; riboflavin was successfully produced in both *E. coli* and *C. acetobutylicum* using this vector. The *Clostridium acetobutylicum* purine pathway was engineered by over-expression of the *Clostridium purF* gene, which encodes the rate-limiting enzyme PRPP-aminotransferase. The function of the shuttle vector J-purF was verified by its ability to complement an *E. coli purF* mutation. However, co-production of riboflavin with biobutanol by use of the overexpression plasmid J-ribGBAH-purF was not significantly improved because of the strict regulation of the purine pathway. Rational mutation of the *purF* gene by replacement of a few amino acid codons was made to yield plasmid J-purFC. This construct also was verified by complementation of the *E. coli purF* mutation. In *E. coli*, co-expression of *ribGBAH* and *purFC* improved riboflavin production by more than 30%. Function of the co-expression of *ribGBAH* and *purFC* in *C. acetobutylicum* was also examined.

Poster 2-55

A proteomic analysis of granulation in liquid cultures of *Phanerochaete chrysosporium*

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Filamentous fungi are emerging tools for the development of novel industrial bioprocesses. They are potentially important in the current search for environmentally friendly methods for waste disposal, detoxification, biofuels and chemical sources. Their morphological robustness and biochemical diversity confer them adequate characteristics for the high-stress, high-efficiency environments of an industrial process. However, it is precisely their characteristic filamentous morphology what makes them grow optimally on solid substrates, which are undesirable for most industrial fermentative processes. Liquid media composition regulates the size of mycelial clumps so that they remain small in size to increase mass transfer efficiency of nutrients. Our project focuses on the characterization of candidate proteins associated to the production of small granules by the model fungus Phanerochaete chrysosporium. The analysis method is SDS-PAGE electrophoresis for the detection of differentially expressed proteins on small-size granule induction medium and YMPG. These candidate proteins are to be subsequently identified using mass spectrometry. This characterization will provide important information for the creation of a platform that will enable the utilization of other filamentous fungi in the industrial production of important biochemicals.

Poster 2-56

Phenotype MicroArray Profiling of *Zymomonas mobilis* ZM4

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Zymomonas mobilis ZM4 is a model ethanogenic bacterium due to its productivity, high level of ethanol tolerance and its ability to be genetically manipulated. In this study, we developed a Phenotype MicroArray™ (PM) protocol to profile nearly 2,000 *Z. mobilis* cellular phenotypes. The PM panels included assays for carbon, nitrogen, phosphorus and sulfur source utilization, nutrient stimulation, pH and osmotic stresses, and chemical sensitivities with 240 inhibitory chemicals. The PM analysis gave an overview of the *Z. mobilis* physiological characteristics, such as the limited C-source (fructose and glucose) utilization, which was consistent with literature reports. For nitrogen metabolism, it utilized ammonia and, for single amino acids it preferred aspartate, asparagine, glutamine, and glutamate. Likewise, for peptide utilization, it preferred peptides with aspartate, asparagine, glutamate, glutamine, and glycine, although others were used more slowly. *Z. mobilis* appeared to use a diverse array of P-sources with the exception of pyrophosphate and tripolyphosphate. The assays suggested *Z. mobilis* uses both inorganic and organic compounds as S-sources. No stimulation by nutrients was detected, however, there was evidence of partial inhibition by purines and pyrimidines, NAD, and deferoxamine. *Z. mobilis* was relatively resistant to acid pH, tolerating a pH down to about 4.0. It also tolerated phosphate, sulfate, and nitrate but was rather sensitive to chloride and nitrite. *Z. mobilis* showed resistance to a large number of diverse chemicals that inhibit most bacteria. The information obtained provides fundamental insights into the physiology of *Z. mobilis* that may assist future metabolic engineering endeavors.

Poster 2-57

Construction of a novel integration vector for expression of delta 6 desaturase gene in *Lipomyces kononenkoae*

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Gamma-linolenic acid (GLA) is used as a dietary supplement and for treatment of various medical conditions. GLA is synthesized by a delta 6 fatty acid desaturase using linoleic acid (C18:2 $D^{9,12}$) as a substrate. However, as the limit of its resources, to produce high levels of GLA by genetic engineering technologies is more attractive. Oil yeast *Lipomyces kononenkoae*, with high linoleic acid content, is an ideal expression host of delta 6 fatty acid desaturase gene compared to other expression host such as *Pichia pastoris* and *Saccharomyces cerevisia*e. To construct a novel expression vector which can be used in this oil yeast, four main components including rDNA from *L. kononenkoae*, hybrid promoter from pINA1296, hygromycin resistant gene and green fluorescent protein have been re-organized and inserted in the same vector. Results showed that the novel integrated expression vector has been successfully constructed. Furthermore, the delta 6 fatty acid desaturase gene from *Rhizopus stolonifer* has been heterogenerously expressed in *L. kononenkoae*. This is the first report that a novel expression vector has been constructed and proven effective in *L. kononenkoae*.

Poster 2-58

Effects of S-adenosyl-l-methionine on membrane components and ethanol tolerance in *Saccharomyces cerevisiae*

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Ethanol tolerance is an essential factor of metabolic engineering for bioethanol production because ethanol inhibits the growth, fermentation, and viability of cell. However, the research to improve ethanol tolerance of bioethanol producing microorganism have not been effectively proceeded because the cellular toxicity of ethanol and its mechanism have been barely known. We postulated that ethanol effect on membrane fluidity is major factor of cellular toxicity by ethanol. S-adenosyl-L-methionine(SAM), as a universal methyl donor, is consumed in biosynthetic pathway of ergosterol and phosphatidylcholine which are key components in membrane. We discovered SAM accumulating strain of *Saccharomyces cerevisiae* showed improved ethanol tolerance and its composition of ergosterol and phosphatidylcholine decreased. We concluded that SAM effects on membrane can improve the ethanol tolerance of cell by modification of membrane fluidity.

Poster 2-59

Development of two phase fermentation system for the stable and high butanol production using *Clostridium acetobutylicum* ATCC 824

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Biobutanol has many characteristics that make it a better biofuel than bioethanol, now used in the formulation of gasohol. Despite the remarkable advantages of butanol as a fuel, biobutanol production has several limitations, including low values for final product concentration and the phenomenon of degeneration. In this study, we examined the effect of liquid-liquid extraction for the in-situ removal of butanol from fed-batch reactor fermentation broth using *Clostridium acetobutylicum* ATCC 824. A non-toxic immiscible solvent, oleyl alcohol, extracted the majority of the inhibitory butanol from the aqueous broth, resulting in a high butanol production. In the batch culture, without solvent extraction, butanol production ceased after 36 h at a concentration of 8.5 g/L. Applying oleyl alcohol as the extraction solvent, about 72% of the total butanol produced was extracted and an total butanol concentration of 18.9 g/L was achieved, which was 222 % higher than that with the controlled traditional batch process. During butanol fermentation, in addition, the populations of C. acetobutylicum and the portion of degenerated cells were monitored by using multiplex real-time qPCR with newly designed primers and probe sets, C. aceto set and DGS set. By combining the extraction and the monitoring techniques, it could be possible to produce butanol stably in high concentration without failures.

Poster 2-60

Enhanced production of 1,2-propanediol *Saccharomyces cerevisiae* by metabolic engineering and carbon source optimization

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1,2-Propanediol, also known as propylene glycol, is a major commodity chemical with world market of $ 123.6 millions/yr. We metabolically engineered S. cerevisiae strain to improve 1,2-propanediol production. When we deleted tpi1(triosephosphate isomerase) gene of S. cerevisiae to increase metabolic flux to DHAP(dihydroxyacetone phosphate) and introduced mgs(methylglyoxal synthase) and gldA(glycerol dehydrogenase) using a multicopy plasmid, 1.11 g/l 1,2-propanediol was achieved with 2%(v/v) ethanol as a major carbon source. When glycerol was used as a sole carbon source under anaerobic condition, much higher 1,2-propanediol productivity was achieved with overexpression of mgs and gldA. The productivity was enhanced when fps1 gene encoding glycerol transporter was overexpressed in S. cerevisiae. As a result, we got 3.23 g/l 1,2-propanediol concentration during 144h flask cultivation with 10%(v/v) glycerol as a carbon source.

Poster 2-61

Systems Biology: The New Frontier for Bioenergy

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Environmental biotechnology encompasses a wide range of characterization, monitoring and control or bioenergy technologies that are based on biological processes. Recent breakthroughs in our understanding of biogeochemical processes and genomics are leading to exciting new and cost effective ways to monitor and manipulate the environment and potentially produce bioenergy fuels. Indeed, our ability to sequence an entire microbial genome in just a few hours is leading to similar breakthroughs in characterizing proteomes, metabolomes, phenotypes, and fluxes for organisms, populations, and communities. Understanding and modeling functional microbial community structure and stress responses in subsurface environments has tremendous implications for our fundamental understanding of biogeochemistry and the potential for making biofuel breakthroughs. Monitoring techniques that inventory and monitor terminal electron acceptors and electron donors, enzyme probes that measure functional activity in the environment, functional genomic microarrays, phylogenetic microarrays, metabolomics, proteomics, and quantitative PCR are also being rapidly adapted for studies in environmental biotechnology. Integration of all of these new high throughput techniques using the latest advances in bioinformatics and modeling will enable break-through science in environmental biotechnology. A review of these techniques with examples from field studies and lab simulations will be discussed.

Poster 2-62

Role of efflux pumps in *Escherichia coli* solvent resistance

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For microbial fuel production, the efficiency with which fuel can be exported from the cell is likely to have significant influence on production titer. Build-up of fuel molecules may directly reduce titer, and may also cause significant intracellular stress, leading to feedback inhibition of fuel production. Transport systems, such as efflux pumps and ABC-transport systems in bacteria and yeast, are documented to export a broad range of substrates, including solvents, and provide a valuable engineering route to relieve fuel accumulation-related stress and improve production titer.

We focus on investigating the role of native, as well as heterologously expressed, RND efflux pumps in E. coli. Targeted studies focus on the well-characterized E. coli AcrAB-TolC system, and efflux pumps from solvent resistant bacteria such as Pseudomonas putida S12. Because efflux pumps are likely to be specific to certain fuel molecules and stressors, a wider range of native and heterologous efflux pump systems must be tested against different fuel compound exposure, growth conditions, and in different engineered hosts. To address our broad goal of improving solvent resistance using efflux pumps, a high-throughput approach has been initiated to create a library of expression vectors representing all efflux pumps from E. coli as well from other organisms known to be naturally resistant to solvents.

Poster 2-63

Harnessing Genomic Recombination to Improve Microbial Metabolic Phenotypes

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The microbial production of energy, pharmaceutical, and industrial compounds is a growing alternative to traditional, often costly, production processes. Many naturally occurring metabolic pathways of *Escherichia coli* and *Saccharomyces cerevisiae* have been enhanced for increased production of desired compounds. Optimization of metabolic phenotypes still faces many challenges as pathway improvement often requires both the redirection of intermediates and re-establishment of gene regulation. Furthermore, predicting the complement of genes that function cohesively for an organism to achieve a chosen metabolic phenotype may be exceedingly difficult, particularly if those gene products act at a distance from the pathway enzymes themselves. Genome shuffling (GS), a recently introduced strain improvement strategy, addresses these challenges through the use of genomic recombination to increase the genetic diversity of a population. When coupled with phenotypic screening and genome sequencing, GS holds the potential to discover genetic alterations that improve a phenotype as well as establish connections between gene products that may not otherwise be intuited from our current understanding of gene function or metabolic networks. Here, we present our recent efforts to develop protocols for protoplast fusion and genome shuffling towards isoprenoid production in the industrial organisms *E. coli* and *S. cerevisiae*. Through deep sequencing and comparative genomics, we will assess the new genotypes of strains that arise from this approach. As all isoprenoids share common metabolic precursors, the strains and genomic knowledge generated through this research may be applicable to the biosynthesis of a wide number of valuable industrial, pharmaceutical, and energy-related compounds.

Poster 2-64

Glyceric acid production from raw glycerol by acetic acid bacteria

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For the development of a sustainable industrial society, it will be necessary to shift from our dependence on petroleum to the use of renewable resources, and much attention has been paid to biorefineries, which enable the production of biofuels as well as building-block chemicals from biomass. Glycerol is a by-product from biodiesel production, which has increased dramatically during the last 10 years, resulting a large in excess of glycerol, especially in Europe. Therefore, glycerol is an attractive feedstock for producing useful chemicals.

One promising glycerol derivative, glyceric acid (GA), is obtained by metal-catalytic oxidation of glycerol. GA has the potential to be a building block for several chemical compounds used in the pharmaceutical and cosmetics industry. However, little is known about the production of GA from glycerol via bioprocesses. Hence, we investigated the GA productivity of acetic acid bactera.

Gluconobacter sp. NBRC3259 produced 55 g/l GA as well as 34 g/l dihydroxyacetone (DHA) from 167 g/l glycerol during 4 days of incubation in a jar fermentor with pH control. The GA production from raw glycerol was also evaluated after proper pretreatment of raw glycerol samples. Using raw glycerol sample, 40 g/l GA and 23 g/l DHA were produced from 175 g/l glycerol. This study was supported by Industrial Technology Research Grant Program in 2008 from New Energy and Industrial Technology Development Organization (NEDO) of Japan.

Poster 2-65

Transcriptomic analysis of carbohydrate metabolism during ethanol fermentation by *Pichia stipitis*

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Pichia stipitis can ferment both glucose and xylose into ethanol and this organism has shown great potential for the development of novel co-fermentation processes designed to obtain lignocellulosic ethanol production. This study investigated the large-scale gene expression profiles during ethanol fermentation using different carbohydrate substrates. This was carried out by hybridization of cDNAs made from messenger RNA extracted from yeast samples during the fermentation against a specific and specially designed "*Pichia stipitis* microarray". Analysis of the results showed that various genes involved in oxidative phosphorylation, pyruvate metabolism and the pentose phosphate pathway together with a number of high specificity pentose transporters were up-regulated significantly during xylose fermentation. On the other hand, genes involved in nucleic acid, amino acid and hexose metabolism were greatly up-regulated during glucose fermentation. These results suggest that ethanol production is the dominant pathway when xylose is being metabolized, but that assimilation is important during glucose metabolism. Further analysis of the various different pathways involved in carbohydrate metabolism indicated that improvements in high-specificity pentose transporter activity and the regulation of the expression levels of genes involved in ethanol production and the synthesis of cell biomass are necessary to further enhance the co-fermentation efficiency of *Pichia stipitis*. To our knowledge, this is the first report to explore global gene expression profiles during ethanol fermentation by *Pichia stipitis* that has used a specific "*Pichia stipitis* microarray".

Poster 2-66

Development of Recombinant Yeast for L-arabinose fermentation

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For the development of renewable fuel to solve the world's energy problem, bioethanol represents the major renewable fuel for transportation. It can solve the transportation problem and also is a fuel better for the environment. Currently, most bioethanol is generated from starch or sugar, which is in limited supply. Cellulosic biomass, abundant available and renewable, is a promising alternative resource for bioethanol. The hydrolysates of cellulosic biomass contain large amounts of hexoses and pentoses, including glucose, galactose, mannose, D-xylose and L-arabinose.

A lack of natural microorganisms that can convert all hexoses and pentoses to ethanol has been a major constraint. Most industrial ethanol fermentations now use the natural yeast Saccharomyces cerevisiae to rapidly and efficiently convert hexoses from starch and sugar to ethanol. However, the yeast is unable to ferment the pentose sugars (xylose and arabinose). Our laboratory altered the genetic structure of S. cerevisiae by cloning and overexpressing xylose reductase, xylitol dehydrogenase and xylulokinase, which make it possible to convert glucose and xylose to ethanol.

The focus of this work is to establish an arabinose fermentation pathway in our glucose/xylose co-fermenting yeast S. cerevisiae 424A(LNH-ST). We have cloned and overexpressed genes encoding L-arabinitol 4-dehydrogenase and L-xylulose reductase in 424A(LNH-ST). The newly constructed strain can grow on arabinose and ferment arabinose present in cellulosic biomass to ethanol. Our new strain is also capable of co-fermenting a mixture of all sugars (glucose, mannose, galactose, xylose and arabinose) present in hydrolysates from any types of cellulosic biomass to the ethanol.

Poster 2-67

Effect of Na^+, K^+, NH_4^+ and glycerol on the glucose/xylose co-fermentation by recombinant S. cerevisiae 424A(LNH-ST)

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For large-scale industrial production of cellulosic ethanol to be feasible, it is critical to understand the impacts inhibitors which may negatively affect the fermentation. Potential inhibitors to this process include cations such as sodium, potassium, calcium, and ammonium found in the plant biomass and /or used in pH adjustment prior to and during the fermentation, and the accumulation of fermentation byproducts, such as glycerol, due to recycling of stillage. For this reason, bench-scale fermentations were conducted using Purdue recombinant glucose/xylose co-fermenting *S. cerevisiae* 424A (LNH-ST) to test the inhibitory effect of four cations (sodium, potassium, ammonium), and glycerol potentially found in the cellulosic ethanol process. The tested concentration of the cations range from 0.1 M to 0.5 M and the glycerol concentration range from 10 to 30 g/L in fermentation of glucose and xylose in YEP media. From these fermentations, it was found that glycerol and potassium were less inhibitory than ammonium and sodium. Additionally, the impact of all inhibitors was stronger toward xylose utilization than glucose utilization.

Poster 2-68

Metabolic analysis of the effect of acetic acid on the co-fermentation of glucose and xylose by *S. cerevisiae* 424A(LNH-ST)

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Lignocellulosic biomass, primarily composed of cellulose, hemicellulose, and lignin, is a promising renewable feedstock for the microbial production of chemicals and fuels, especially ethanol via fermentation. The major fermentable sugars released from the processing of the lignocellulose are glucose and xylose. However, the primary processing steps required for this conversion also produce a range of compounds that can inhibit the subsequent microbial fermentation. One such inhibitory compound is acetic acid, liberated from hemicelluloses during the pretreatment of the biomass. We previously reported that acetic acid inhibited cell growth, substrate consumption, and ethanol productivity, while it improved metabolic ethanol yield. To explore the effect of acetic acid on a cellular level, a comprehensive analysis of key intracellular metabolites involved in glycolysis and the pentose phosphate pathway was conducted. The Global Isotope-labeled Internal Standard (GILISA) MS quantization method was used for the identification and quantification of the intracellular metabolites of glucose/xylose fermenting *Saccharomyces cerevisiae* 424A(LNH-ST). Metabolic flux analyses were performed and compared between control co-fermentations and co-fermentations with acetic acid (7.5, 10, and 15 g/L) at a controlled pH of 5.5.

Poster 2-69

Effect of soy-based glycerol concentration on 3-hydroxypropionaldehyde production by enteric species

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During soy biodiesel production, a coproduct stream containing glycerol, fatty acid and methylesters of fatty acids results. Raw glycerol is the major coproduct formed during the processing of crude soybean oil to biodiesel. Raw glycerol is considered a low value product but can serve as a substrate for the microbial fermentation of the specialty chemical 3-hydroxypropionaldehyde. This specialty chemical is a precursor for plastic production and has other uses as a food preservative and a tissue fixative. In this investigation, the ability of selected enteric bacteria to convert different concentrations of soy-based raw glycerol to 3-hydroxypropionaldehyde was analyzed. Using a complex medium containing diluted raw glycerol, the enteric bacteria were grown for 24 hours at 28°C. After the cells were collected by centrifugation, they were resuspended in a neutral phosphate buffer containing diluted raw glycerol and semicarbazide hydrochloride. Following the cell suspension being shaken for 24 hours at 28°C, the cells were collected by centrifugation and the resultant supernatant of each suspension was colorimetrically assayed for 3-hydroxypropionaldehyde. The enteric bacterium *Citrobacter freundii* ATCC 8090 produced more than double the concentration of 3-hydroxypropionaldehyde on 10% raw glycerol than on 5% raw glycerol. In contrast, *Enterobacter aerogenes* ATCC 13048 produced more than double the concentration of 3-hydroxypropionaldehyde on 5% raw glycerol than on 10% raw glycerol. Overall, it was shown that the concentration of raw glycerol used to grow enteric species can affect the level of the specialty chemical 3-hydroxypropionaldehyde produced.

Poster 2-70

Design of transcription factor-based in vivo biosensors for improved butanol production in *E. coli*

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The *Pseuodomonads* are well recognized for their ability to rapidly evolve σ^{54}-transcriptional activators to detect synthetic compounds in their environment, many of which are of high interest for replacement using microbial production processes. Here we present a facile strategy for the *in vivo* detection and quantification of structurally-diverse, industrially-important metabolites on the single cell level. We constructed an *in vivo* biosensor responding to C2-C8 linear alcohols from a rationally-designed library of chimeric *Pseudomonad* σ^{54}-transcriptional activators. We focused on a putative alcohol-responsive transcription factor from *Pseudomonas butanovora*, BmoR, and XylR, a well characterized toluene-responsive transcription factor from *Pseudomonas putida*. When transformed into *Escherichia coli* the biosensor yielded a linear response to exogenously added n-butanol up to 0.5% v/v; above which butanol-induced growth inhibition was observed. In butanol production strains of *E. coli* the biosensor demonstrated accurate quantification of butanol titers as compared to gas chromatography-mass spectrometry measurements. We then employed the biosensor for directed evolution of *Escherichia coli* for improved n-butanol production, targeting modifications to the *E. coli* genome and a heterologous n-butanol pathway from *Clostridium acetobutylicum*. This work demonstrates a versatile strategy for rapid design of high-throughput screens and selections targeting for intracellular metabolites. Further, we gained increased insight into possible mechanisms through which the nature is able to evolve the ability to detect and respond to synthetic compounds in the environment.

Poster 2-71

Mixed sugars fermenting *Saccharomyces cerevisiae* strains with optimized pathway combination

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Renewable biofuels, such as bioethanol, can be produced by bioconversion of lignocellulosic biomass from sustainable forestry or agriculture.

Lignocellulosic material is mainly composed of cellulose, hemicellulose and lignin, where hemicellulose is a heteropolymer of hexoses and pentoses (xylose and arabinose). Baker's yeast *Saccharomyces cerevisiae* can rapidly convert hexose sugars to ethanol with high yield and high productivity. In addition, it displays a good tolerance to several fermentation-inhibiting compounds present in lignocellulosic derived media. However, *S. cerevisiae* is not able to utilize pentose sugars, which may constitute a significant portion of the lignocellulosic feedstock.

Since optimal process economy demands exhaustive substrate utilization, the feasibility of the forthcoming switch from oil- to biomass-derived raw materials for the fabrication of fuels and chemicals is strongly dependent on the development of a fermenting micro-organism with a broad range of substrates. Construction of a substrate-broadened *S. cerevisiae* strain entails the introduction of heterologous genes encoding D-xylose and L-arabinose metabolizing enzymes, respectively.

Essentially, two different pathways are available in nature for the catabolism of C5 aldoses: isomerization based pathways and reduction/oxidation based pathways and several metabolic engineering studies showed that their introduction in yeast allows xylose and arabinose utilization to different extent.

For the present study, mixed sugars fermenting *S. cerevisiae* strains were constructed by combining optimized xylose and arabinose utilisation pathways. Comparative evaluation of the different strains capacity of aerobic growth on pentose sugars is presented, as well as substrate consumption rates and products distribution in anaerobic mixed sugars fermentation.

Poster 2-72

Withdrawn

Poster 2-73

Sugarcane Platform for Chemicals and Next-Gen Biofuels

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Though not a new idea, use of bioethanol as a platform for production of chemicals or other fuels is re-emerging as a significant strategy among chemical and energy industry players, often allied with the owners of the carbohydrate feedstocks/bioethanol production. Several projects are underway and others are being studied to convert Brazilian sugarcane ethanol to ethylene for polymers production. Others are proposed for India and elsewhere in Asia. These will use one or another version of long-commercialized, simple catalytic dehydration technology. Others are considering routes to condense ethanol to n-butanol and higher alcohols, or for direct fermentation of cane sugars to other hydrocarbon monomers and fuels, leveraging bioethanol know-how and facilities. This paper will identify and analyze the technologies and economics of these broad cases and the players comprising these developments.

Poster 2-74

Harnessing the Microbial Fermentation of Glycerol for the Production of Fuels and Chemicals

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Glycerol has become an inexpensive and abundant carbon source due to its generation as inevitable by-product of biofuels production. Given the high degree of reduction of carbon in glycerol, fuels and reduced chemicals could be produced from glycerol at yields higher than those obtained from common sugars. Fully realizing this potential, however, requires the metabolism of glycerol in the absence of external electron acceptors (i.e., fermentative metabolism). Unfortunately, only a small group of microorganisms, most of which are not amenable to industrial applications, was known to be capable of fermentative utilization of glycerol prior to our work. In these organisms, the ability to synthesize 1,3-propanediol (1,3-PDO) has long been considered the metabolic property that enables to ferment glycerol. For example, Escherichia coli and Saccharomyces cerevisiae, workhorses of modern biotechnology, do not have the capacity to synthesize 1,3-PDO and therefore have been deemed unable to conduct glycerol fermentation.

Following our recent discovery that the previous view was incorrect and that although E. coli cannot synthesize 1,3-PDO it can indeed ferment glycerol in the absence of external electron acceptors, we have engineered this organism for the conversion of glycerol to fuels and chemicals. Several biocatalysts have been developed for the production of ethanol, hydrogen, formate, succinate, lactate, and 3- and 4-carbon diols from glycerol-rich streams generated during biofuels production (e.g. crude glycerol, thin stillage). This paper will include the discussion of our latest work related to the harnessing of microbial glycerol fermentation for the production of fuels and chemicals.

Poster 2-75

Microbial Conversion of Bio-Oils to Fuels and Chemicals: A New Biorefinery Paradigm

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The use of oils and fats as renewable resources for the production of chemicals and fuels is a promising avenue to establish biorefineries. The highly reduced nature of carbon atoms in these feedstocks, as compared to sugars, would ensure the production of chemicals and fuels at higher yields. However, metabolism of free fatty acids (FAs), the main constituents of fats and oils, requires the presence of an external electron acceptor, which in turn would preclude the synthesis of metabolic products. We propose a new hybrid paradigm to balance cell growth and synthesis of reduced products by using micro-respiratory conditions that lead to a respiro-fermentative metabolic mode.

Ethanol and succinate were chosen as model products and E. coli as model organism to illustrate the feasibility of the above approach. The maximum theoretical yields for the synthesis of ethanol and succinate from FAs are 1.33 g/g and 1.85 g/g, respectively, compared to 0.51 g/g (ethanol) and 1.12 g/g (succinate) for their production from sugars (i.e. 2.6- and 1.7-fold increase by using FAs). To increase the production of ethanol, a mutant of the enzyme acetaldehyde/alcohol dehydrogenase (r-AdhE) was created that is functional in the presence of oxygen. Overexpression of r-AdhE in wild-type E. coli MG1655 grown under micro-respiratory conditions led to a yield of 0.60 g ethanol/g FAs, which already surpasses the maximum theoretical from sugars. Similar improvements in the synthesis of succinate were achieved by engineering the TCA cycle and the glyoxylate shunt to prevent succinate degradation and enhance its synthesis.

Poster 2-76

Development and scale-up of xylose-rich hydrolysate fermentation process for lignocellulosic ethanol production

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Pichia stipitis is widely used in pentose fermentation because of high ethanol productivity. However, the yeast is sensitive to some inhibitors, such as acetic acid and furfural in xylose-rich hydrolysate. Thus, the capability of hydrolysate fermentation by *Pichia stipitis* was often neglected in the lignocellulosic ethanol production process. The study was aimed to investigate the scale-up of rice straw hydrolysate fermentation process. Adaptive evolution of *Pichia stipitis* was first performed to enhance the tolerance of yeast to inhibitors in rice straw hydrolysate. Microarray analysis has been demonstrated the expression levels of alcohol dehydrogenase (ADH), pyruvate decarboxylase and several sugar transports from adapted yeast were up-regulated in comparison of parental yeast. The optimization of fermentation conditions and detoxification procedure were then carried out to enhance the ethanol conversion during the fermentation of hydrolysate. The maximal ethanol yield of total sugars was achieved to 0.44g/g at 5L scale fermentation when rice straw hydrolysate was prepared by a twin-screw extrusion pretreatment with dilute acid hydrolysis. Recently, 100L fermentation experiments were further conducted to optimize scale-up of rice straw hydrolysate fermentation for ethanol production. An ethanol yield 0.43 g/g was obtained by controls of dissolved oxygen in hydrolysate. Thus, the fermentation of xylose-rich hydrolysate by adapted *Pichia stipitis* was shown potential for further scale-up experiment.

Poster 2-77

Evaluation of the Metabolic Burden of Recombinant Cellulase Expression by *Saccharomyces cerevisiae* in Batch Culture

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In an age of dwindling fossil fuel reserves, the abundance, variety and sustainability of lignocellulosic substrates presents an interesting proposition as a feedstock for second generation biofuels. Whereas simultaneous saccharification and fermentation could be carried out through addition of enzymes in the fermentation broth, processing costs can be substantially decreased through expression of these enzymes in a recombinant host with strong fermentative characteristics. Previously, two recombinant strains of *Saccharomyces cerevisiae* Y294 were constructed expressing either an endoglucanase and β-glucosidase from Trichoderma reesi and *Saccharomycopsis fibuligera* (S. cerevisiae CEL 5), respectively, or these enzymes in combination with fungal cellobiohydrolases (S. cerevisiae Y118P). Low biomass and ethanol yields during growth on phosphoric acid swollen cellulose prompted fundamental studies to quantify the metabolic burden incurred through heterologous protein expression by comparison of the kinetic and stoichiometric parameters with S. cerevisiae Y 294 harbouring an empty plasmid as control. In bioreactor-grown batch cultures in a mineral medium with glucose as carbon source, a two-fold decrease in the maximum specific growth rate of strain CEL 5 could be correlated to a four-fold and two-fold greater expression of β-glucosidase and endoglucanase, respectively, when compared with strain Y118P. Whereas the maximum specific rates of glucose uptake and biomass yields for these strains were similar, the greater biomass yield on glucose and glucose uptake rate of the control strain pointed towards a significant energetic demand for heterologous protein expression by the recombinant strains.

Poster 3-07

In-Situ Examination of Biomass Dissolution and Cellulose Regeneration Enabling Cellular level Insight of Ionic Liquid Pretreatment Process

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Cellulosic biomass is a low-cost feedstock with the potential of costing $40/ton competitive with oil at about $13/barrel on an equivalent energy content basis. These materials must be pretreated prior to biological steps of adding enzymes for scaccharification of cellulose or hemicelluloses as yields are too low otherwise to be economically competitive. Promising new approaches, like ionic liquid solvents, are still in their infancy and need further exploration before implementation at biorefinery levels. Quantifying and imaging the mechanism and efficiency of the ionic liquid pretreatment process is thus a critical first step in developing a pretreatment process that minimize the formation of by-products that are inhibitory to fermentation of cellulosic sugars to ethanol. We evaluated a targeted combination of an ionic liquid (1-n-Ethyl-3-methylimidazolium Acetate) with a leading candidate for national energy crop production (switchgrass) to evaluate the efficacy of ionic liquid in breaking down biomass into fundamental biological building blocks. In this study, auto-fluorescent lignin mapping and its de-convolution was used to visualize cellulose in pristine switchgrass stems in order to gain unprecedented cellular level understanding and insight of biomass dissolution during ionic liquid pretreatment. Ionic liquid efficiently solubilized both cellulose and lignin. Cell wall swelling, perhaps due to breakage of inter and intra-molecular hydrogen bonding between cellulose fibrils and lignin, followed by complete dissolution of biomass was observed without using chemicals routinely used in staining, embedding and processing of biomass. In comparison to untreated biomass, ionic liquid pretreated biomass provides enhanced area enabling efficient saccharification with high sugar yields.

Poster 3-08

Efficiencies of Designed Xylanase Combinations in Releasing Sugars from Hemicellulose Extract on Mixed Northeast Hardwood

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One near term option to developing a forest products biorefinery is to carefully pre-extract hemicellulose from incoming wood chips before the main pulping step. This extract can then be fermented to bioethanol or other fermentation products. The release of monomer sugars from a xylan-rich extract, creating a fermentable substrate is a prerequisite for utilization of pulping wood hemicellulose for production of ethanol or other value-added products. Pre-extraction of hemicellulose from northern hardwoods by hot water produces a pH=3.5 extract containing hemicellulose. This project examined the individual and combined effectiveness of six selected, commercial, multicomponent enzyme preparations in catalyzing arabinose and xylose release from hot water hardwood extract: enzyme complex I (from *Aspergillus aculeatus*), enzyme complex II (from *Humicola insolens*), xylanase I (from *Aspergillus oryzae*), xylanase II (from *Aspergillus oryzae*), xylanase III (from *Trichoderma viride*), and xylanase IV (from *Thermomyces lanuginosus*). In this study, different hydrolysis conditions, i.e. enzyme dosage, pH, and temperature, were evaluated for the extent of monomeric sugar yield before fermentation by individual and combined enzyme treatments.

Poster 3-09

Pretreatment of sweetgum (*Liquidambar styraciflua* L.) with dilute sulfuric acid

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The hardwood species Sweetgum (*Liquidambar styraciflua* L.) grows as understory in pine forests, and is widely distributed in the southeastern United States. Sweetgum trees must be harvested prior to logging of the pine forest, and represent a residue biomass that can be utilized in the production of cellulosic biofuels. In the biochemical conversion platform, the conversion of biomass into liquid fuels is centered on the pretreatment of the biomass, followed by enzymatic hydrolysis of cellulose and hemicellulose, resulting in a C5 and C6 sugar streams that can be anaerobically fermented to ethanol by *Saccharomyces cerevisiae*, *Zymomonas mobilis*, *Clostridium* species or other ethanolgenic microorganisms. Dilute acid pretreatment and saccharification conditions were investigated for sweetgum conversion to monomeric sugars. Sweetgum feedstock was received from UA Monticello and stored at 4°C at UA Fayetteville. The ground feedstock (3.175 mm, 5% solids w/w) was presoaked at room temperature in 0.22%, 0.49% or 0.98% w/w sulfuric acid for at least four hours. Pretreatments were carried out in a 2L Parr reactor heated in a sandbath, and consisted of combinations of 0.22%, 0.49% and 0.98% w/w sulfuric acid at temperatures ranging from 140-200°C for times varying between 5 and 80 minutes. The pretreated slurry was analyzed for monomeric sugar and acid content as described in the National Renewable Energy Laboratory TP-510-42623 protocol.

Poster 3-10

Chemical features of solid residues obtained from supercritical water treatment of lignocellulosics

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In this research poplar wood meals were treated with supercritical water for 60s in the temperature range between 325°C and 425°C at the fixed pressure at 220±10atm in order to develop effective saccharification process of lignocellulosics and to analyze chemical feature of solid residues produced from the process. The solid residues obtained from degradation products by filtration were consisted of chemically modified lignin and fibrous materials. Glucose and xylose were identified as main sugar components of fibrous materials, and the highest ratio of glucose/xylose in fibrous materials was achieved at the highest reaction temperature. At the reaction temperature of 425°C the more than 80% of solid residues was composed of lignin. The molecular weight of lignin in solid residues was ranged between 4,000 and 1,500 by GPC analysis, which is only 1/4 to 1/9 folds compared to that of milled wood lignin (MWL) isolated from poplar wood determined to ca. 13,500. This result suggested that lignin was severely fragmented to almost oligo-state molecular weight level. Assuming that average molecular weight of monomeric units of lignin (coniferyl and sinapyl alcohol) could be 200, the lignin is solid residues could be constructed only with 12 to 15 monomeric units. In addition, DFRC analysis suggested that β-O-4 linkage was not detected in the lignin in solid residues, indicating that two major reactions, cleavage of β-O-4 linkage as well as propane side chains, could occur under the condition of high temperature and pressure. Those structural features of lignins were clearly confirmed by ^{1}H and ^{13}C NMR spectroscopic analysis.

Poster 3-11

Value prior to pulping: Extraction of hemicellulose from hardwood

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Extracting hemicellulose prior to pulping is a proposed method of creating new feedstocks in an integrated forest products bio-refinery. Hemicellulose can be extracted without causing significant yield loss to the more valuable pulp product if alkaline chemicals are used to maintain the final extract liquor at near-neutral pH conditions. Acetyl groups liberated from the hemicellulose polymer during extraction are neutralized by the added alkali, and represent a valuable co-product. The aqueous extract contains xylo-oligosaccharides which can be hydrolyzed and utilized by pentose fermenting organisms to produce ethanol or other value-added products.

Mixed southern hardwood chips were extracted with either carbonate or green liquor, a pulping intermediate containing NaOH, Na_2S and Na_2CO_3, at 0, 2, 4 and 6% TTA (total titratable alkali) for each of the two chemicals. The extractions were also performed for each chemical charge condition at low, medium and high H-factors (a kinetic model expressing cooking time and temperature as a single variable) to determine the effect of extraction severity on pulp yield and composition of the extracted liquor. The severity of hemicellulose extraction in terms of time and alkaline charge determines the concentration of acetic acid and monosaccharides available for downstream processing, the pulp yield attainable for extracted fiber, and the accumulation of degradation products such as organic acids and furans. Complete compositional data and the corresponding pulp yield effects will be presented for each of the conditions tested.

Poster 3-12

Bioethanol Production from Steam Exploded Forage Sweet Sorghum at High Solid Content

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The aim of this work was to determine the best operation conditions for steam explosion pretreatment of forage sorghum biomass and to test ethanol production by Simultaneous Saccharification and Fermentation (SSF) process at high pretreated solids loading.

Steam explosion pretreatment of forage sorghum was performed in a 2 L batch steam-explosion pilot unit at various experimental conditions. The effect of pretreatment temperature (180-230ºC) and time (2-10 min) on overall glucose yield was studied using a response surface method according to a rotable central composite experimental design 2^2. Glucose overall yield was calculated taking into account sugar recovery in the liquid fraction and sugar release from pretreated substrate by enzymatic hydrolysis laboratory tests using commercial cellulases. Optima pretreatment condition of 220ºC and 7 min resulted in a overall glucose yield of 93% of the glucose content in raw material.

The pretreated water insoluble solid fraction (WIS) from pretreatment run at optima temperature and time conditions was tested for bioconversion to ethanol by SSF in laboratory fermentors. A presaccharification step of 8 hours prior to SSF was tested in experiments at initial WIS concentrations of 10, 15 and 20% (w/v) using commercial cellulase and β-glucosidase (15-20 FPU and 15-20 IU/g cellulose in WIS, respectively). Fermenting yeast *Saccharomyces cerevisiae* at low inoculum (0.4-0.5 g/L) loading was used in SSF. Main results of this research will be reported.

Poster 3-13

Optimizing the Saccharification of Sugar Cane Bagasse Using Phosphoric Acid and Fungal Cellulases

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At temperatures above 140ºC (10 min), a low level of phosphoric acid (1% w/w on dry bagasse basis) was shown to hydrolyze most of the hemicellulose in sugar cane bagasse into monomers with minimal side reactions while also serving as an effective initial treatment for the enzymatic hydrolysis of cellulose. Up to 40% of the resulting water insoluble solids (WIS) was digested to glucose by low concentrations of Biocellulase W (10 ml/kg WIS; 0.5 filter paper unit/g WIS) supplemented with β-glucosidase. Much higher levels of cellulase (100-fold) were required for complete hydrolysis. After neutralization and nutrient addition, phosphoric acid syrups containing hemicellulose sugars were fermentable by ethanologenic E. coli LY160 without prior purification. Fermentation of these hemicellulose syrups was preceded by a lag which increased with treatment temperature. With further improvements in organisms and optimization of steam treatment conditions, it may be possible to co-ferment sugars derived from hemicellulose and cellulose and eliminate early process steps for liquid-solid separation, sugar purification, and separate fermentations. The incremental increase in fermentable sugars with added cellulase (maximum of 30 kg glucose/L for Biocellulase W) was proposed as an approach to estimate the value of commercial cellulases for biofuel production. Using a phosphoric acid steam treatment at 160°C or above, predicted ethanol production based on solublized sugars per metric ton (dry weight; assuming 90% overall efficiency of ethanol production and recovery) ranged from 66 gal (0.5 filter paper units/g WIS) to 110 gal (50 filter paper units/g WIS).

Poster 3-14

Optimization of Dilute Acid Hydrolysis of Rapeseed Straw as a Pretreatment for Conversion to Ethanol

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Increasing use of the world's oil reserves has prompted much research into finding alternative sources of fuels and valued chemicals. The most likely renewable resource is lignocellulosic biomass--i.e., plant materials such as agriculture and forestry wastes. Of agricultural wastes, rapeseed straw is discarded in the process of harvesting for biodiesel production. The government of South Korea has introduced blending mandates specifically for diesel and this policy results in more hectares planted with rapeseed and it is therefore expected to increase supply of straw for ethanol production. The limiting factor in using lignocellulosic biomass is hydrolyzing the raw material into fermentable sugars. In this work, the dilute-acid hydrolysis of rapeseed straw was optimized. 69.0% of hemicellulose and 53.6% of lignin dissolved from rapeseed straw into the hydrolyzate using 0.77% (w/v) H_2SO_4 for 18 min at 164°C. The composition of pretreated rapeseed straw analyzed is 59.13% (w/w) glucan, 10.3 % (w/w) xylan, presented as XMG and 26.38% (w/w) lignin with extractives. It could be found that 6.4% (w/w) of the xylose and 1.1% (w/w) of the glucose were dissolved as other decomposed materials. From the mass balance analysis, acid hydrolysis gave a higher recovery of hemicellulose in the hydrolyzate, preserving cellulose on pretreated rapeseed straw under the optimized pretreatment condition. In this study, we also investigated the ethanol yield of the pretreated rapeseed straw by the Simultaneous Saccharification and Fermentation (SSF) process and characterized effects of the optimized pretreatment condition using SEM and AFM imaging technologies.

Poster 3-15

The effects of varying pretreatment chemicals on the enzymatic hydrolysis of organosolv pretreated mountain pine beetle killed Lodgepole pine

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Mountain pine beetle killed lodgepole pine (*Pinus contorta*) chips were pretreated by the ethanol organosolv process and the cellulose rich insoluble component was subsequently enzymatically hydrolyzed. The effect of varying pretreatment chemicals on the resulting substrate's ease of enzymatic hydrolysis and physicochemical characteristics was investigated. It was apparent that the different pretreatment chemicals resulted in variations in the chemical composition of the solid and liquid fraction as well as in the extent of cellulolytic hydrolysis. Pretreatment under acidic conditions resulted in substrates that were readily hydrolyzed despite the observed increase in delignification selectivity obtained under alkaline conditions. Furthermore, acidic pretreatments also resulted in a lower cellulose degree of polymerization, shorter fiber lengths and increased substrate porosity. These results strongly suggest that a substrate's susceptibility to enzymatic hydrolysis is influenced by its physical properties (especially enzyme accessible surface area) rather than its chemical composition.

Poster 3-16

The comparative analysis of lignin from steam and organosolv pretreated substrates

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During enzymatic hydrolysis, the lignin component of pretreated lignocellulosic substrates is known to decrease hydrolysis yields and influence the adsorption of cellulolytic enzymes. Although hydrophobic, electrostatic, and hydrogen bonding interactions between lignin and cellulases have been proposed as potential mechanisms for the effects of lignin on hydrolysis, there have been limited fundamental studies in this regard. In this study, steam and organosolv pretreated substrates from softwood (Lodgepole pine), hardwood (Poplar), and an agricultural residue (corn stover) were prepared with subsequent isolation of lignin via two methods. Lignin preparations isolated using protease treatment were compared to those obtained using dioxane extraction with respect to their chemical and physical properties and their effects on enzymatic hydrolysis. It was apparent that the lignin isolated using dioxane contained fewer carbohydrates and residual protein than those isolated using the protease method. The isolated lignin from lodgepole pine softwood seemed to be more inhibitory to enzymatic hydrolysis compared to lignin preparations isolated from corn stover and poplar. Further experiments were also conducted to determine the lignin-related factors that decrease the hydrolysis yields and influence protein content.

Poster 3-17

Application of Chemspeed AUTOPLANT® to Ammonia Fiber EXpansion (AFEX) pretreatment

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It has been shown that various biomass species requires different AFEX pretreatment conditions in order to get higher sugar yields. Also, within a given biomass species, pretreatment conditions may vary depending upon the cultivar, and when and where the feedstock was harvested. Therefore, the AFEX pretreatment itself must be as consistent and repeatable as possible. We have applied the AUTOPLANT®, manufactured by Chemspeed Technologies, Inc, Monmouth Junction, N.J. to the AFEX pretreatment of Corn Stover. The AUTOPLANT® is an automated platform which will hold six ChemSpeed's MiniPlant modules (12 reactor vessels total). Each MiniPlant consists of two individually controlled 100 mL stainless steel reactors with stirrers. It has a robotic arm to deliver a precise amount of water to the biomass in the reactor. We believe that the AFEX pretreatment can be done under controlled conditions (including temperature, pressure, ammonia/water delivery and residence time) using the AUTOPLANT®. The first of its kind, the AUTOPLANT® will quickly identify the optimal AFEX conditions for a given batch of biomass based on maximum sugar conversion using enzymatic hydrolysis. In this work, we will provide results of AUTOPLANT® pretreated corn stover which can comparable to, if not superior to the known results of previous AFEX pretreatment work using corn stover as a substrate.

Poster 3-18

Optimization of rice straw pretreatment by hydrothermal process

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Hydrothermal treatment is an environment-friendly pretreatment process and its effects on lignocellulose biomass are hemicellulose hydrolysis, lignin degradation, cellulose alteration and recovery of polysaccharides. The goal of this study was to define the optimal conditions for hydrothermal treatment of rice straw to provide maximum total sugar yields. In this report, dried rice straw was fractionated by hydrothermal pretreatment, which was carried out at 10% solid loading in the range of 170-220°C for 0-120min. Results show that the solublization of raw material components such as hemicellulose and lignin leads to increase cellulose content in the solid fraction with values between 51%-65%. In comparison to untreated substrate (42.8% dwb), this is an important advantage for the following step of enzymatic hydrolysis. The enzymatic hydrolysis yield increased with pretreatment time and temperature, but xylose degradation in in low xylose recovery became significant. The highest enzymatic hydrolysis yield (91.6%) was obtained at 180°C for 30 min. Furthermore, the optimized conditions were obtained at 200°C for 2 min giving maximum total recovery of 63.6%.

Poster 3-19

Investigation of nitrogen-containing compounds produced during AFEX pretreatment of lignocellulosic biomass

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During pretreatment of corn stover several degradation compounds are produced that may inhibit downstream biological processing. The nature of these degradation products can vary depending on the thermochemical severity of pretreatment. Ammonia Fiber Expansion (AFEX) pretreatment produces a set of nitrogen-containing compounds including phenolic amides, sugar amides, and amines. These compounds are specific to AFEX over other pretreatment methods. Degradation products normally derived directly from plant biomass, such as ferulic acid, p-coumaric acid, and acetic acid, have amide analogs detected in AFEX-pretreated samples. It is important to determine the types and quantities of these nitrogenous compounds in pretreated biomass. The loss of ammonia through undesirable reactions to various inhibitory compounds is a potential hurdle to making cellulosic ethanol economically feasible using AFEX. On the other hand, the process may offer access to valuable co-products derived from these reactions. Described here are the GC-MS and LC-MS methods developed for identification and quantification of some important nitrogenous compounds. AFEX pretreated biomass was also characterized by NMR to study the incorporation of nitrogen to lignin, phenolics and reducing sugar aldehydic groups. Some of these methods were subsequently used to examine differences in degradation product concentration for untreated, AFEX-pretreated, and acid-pretreated corn stover. The effect of AFEX pretreatment severity on the formation of various nitrogenous compounds is also investigated.

Poster 3-20

The optimum of enzymatic hydrolysis from pretreated rice straw by aqueous ammonia

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The bio-ethanol has recently received much attention as an alternative energy. Generally the bio-ethanol is produced by fermenting the glucose obtained from biomass. The typical raw materials for bio-ethanol are biomass such as a sugar cane, wheat, corns, potato, barley, and a sweet potato. The bio-ethanol is renewable because raw materials are obtained from plants. Compare to the gasoline discharging pollutants like carbon monoxide, the bio-ethanol is entirely not exhaust hazardous substance. Bioprocesses for converting lignocellulose to useful materials such as liquid fuels and chemicals have been receiving increasing attention. Pretreatment is an essential element in the bioconversion of lignocellulosic substrates. Pretreatment of lignocellulosic biomass using the percolation process was usually determined to be 150°C. Soaking process was carried out mild conditions at atmospheric pressure of 60°C. Compared two pretreatment processes, we found enzymatic hydrolysis condition of pretreated biomass. In case of a rice straw, compared with previous lignocellulosic biomass, we knew that hydrolysis time was short. Also, the optimum conditions for the maximum glucose conversion rate were found to be a temperature, pH, hydrolysis time. Thus by using the control composite design, it is possible to determine the accurate values of the hydrolysis parameters where maximum production of sugar occurs. This study estimates that a rice straw has the possibility of SSF (Simultaneous Saccharification and Fermentation).

Poster 3-21

Acid Saccharification to Ethanol Production from Red Algae (*Gelidium amansii*)

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Lignocellulose (2nd generation) is difficult to hydrolyze due to the presence of lignin and the technology developed for cellulose fermentation to ethanol is not yet economically viable. Recent advances in the extremely new field of biotechnology for the ethanol production are making it possible to use of macro algal biomass, e.q., red algae, because of their several superior aspects as 3rd generation biomass; no lignin, high contents of carbohydrates as well as very fast-growing rate with the fixation of large amount of CO_2, know as a green house gas. This article, as the basic study of saccharification to ethanol production from red algae, the properties and the optimum conditions of saccharification process were estibilshed. The important independent variable for saccharification process were selected as acid(H_2SO_4) concentration, reaction temperature, reaction time, and S/L ratio by batch reaction.

Poster 3-22

Organosolv pretreatment of *Liriodendron tulipifera* with acid and alkali catalysts

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To confirm the pretreatment behavior on catalyst in organosolv pretreatment, pretreatments of *Liriodendron tulipifera* were carried out. Grounded materials and Meicelase were used. All pretreatments were carried out in minibomb (500ml) and the ratio of mixture was 1:20 (10g/200ml). 50% of EtOH was used as solvent and 1% of H_2SO_4 and NaOH were applied as catalysts. The mixtures were pretreated at 180 and 200°C for 30 and 60 min. Pretreated mixtures were washed with distilled water and divided into the solid and liquid fraction.

First, chemical composition of pretreated material was very similar in case of both catalysts. Hemicellulose was decreased largely but lignin was decreased a little, compared to control. Crystallinity was very different on catalyst. Although, crystallinity of the alkali was almost similar to the control, crystallinity of the acid was increased largely by the degradation of amorphous cellulose. It might indicate that crystallinity is not an important pretreatment factor. In the case of the digestibility, both of acid (8.09→74.21%) and alkali (8.09→63.66%) improved the digestibility, however, pretreatment behavior on catalyst was very different. Alkali improved the digestibility at all conditions, but acid improved the digestibility at specific conditions with 200 °C and 60 min in this study. The SEM image on catalyst was also different. In the case of acid, the increase of pore was observed by the preceding of hemicellulose degradation, and it might improve the accessibility of cellulase. However, fiber exposure by the lignin degradation in alkali catalyst pretreatment might improve the digestibility.

Poster 3-23

Pretreatment of biomass by proton beam irradiation

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Bioethanol is one of anticipated fuels which alternate primary fossil fuel and generally produced from sugar and this sugar is from sugarcane, sugar beet, corn etc. But in these days inedible biomass such as lingo-cellulose from wood are concerned because of increase of crop price. In case of lingo-cellulose, it has not only beta (1→4) linkage of each monomer but also hydrogen bond between those polymers, then the structure is very hard and rigid to degrade into monomers. Algal biomasses also have non-degradable structure and need somewhat pretreatment. Either acid or base treatment has been used for degradation of biomass and gamma ray irradiation pretreatment was also reported.

The objective of this study is to investigate the effect of proton beam pretreatment on biomasses. Concerned biomasses are red algae and cellulose from paper and the effect of each irradiation exposure time on those biomasses was observed. In this study, finally defined is a notable enhancement of the productivity of sugar through the irradiation pretreatment in comparison to non-pretreated biomass and we may expect that the irradiation pretreatment contributes to increase the bioethanol yield.

Poster 3-24

Two-step hot-compressed water treatment of woody biomass to enhance enzymatic digestibility of cellulose and hemicellulose

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Hot-compressed water (HCW) treatment without chemicals such as acids, bases or organic solvents is an environmentally-benign pretreatment method to enhance the enzymatic digestibility of cellulose and hemicellulose components in the lignocellulosic biomass. An enhanced enzymatic digestibility of the HCW-treated cellulose residue is related to the removal rate of hemicellulose and lignin in the feedstock. Meanwhile, the sugars from the hemicellulose fraction are largely decomposed to other compounds such as furfurals in a severe HCW condition that enhances the enzymatic digestibility of cellulose residue. To improve total sugar yields from both components, we investigated the effects of a two-step HCW treatment on the enzymatic hydrolysis of eucalyptus (hardwood) and Douglas-fir (softwood). The optimum conditions of the first HCW treatment for hemicellulose solubilization of eucalyptus and Douglas-fir were at 210˚C for 5 min and at 220˚C for 5 min, respectively. Each of the resultant residues was washed and then treated by the second HCW treatment at 240-260˚C for 5 min to increase enzymatic digestibility of cellulose. The eucalyptus treated with two-step HCW (210˚C/240˚C) was converted to an approximately 490 g of total sugar/kg-wood with a cellulase loading of 10 FPU/g-residue. The Douglas-fir treated with two-step HCW (220˚C/260˚C) was converted to an approximately 440 g of total sugar/kg-wood, though the high cellulase loading of 40 FPU/g-residue was required for enzymatic hydrolysis of the residue. The total sugar yields from these woody biomass treated by two-step HCW were 1.3-1.5 times higher than those by the optimized one-step HCW.

Poster 3-25

Enzymatic hydrolysis and ethanol production from AFEX pretreated bagasse at high solid loading using recombinant ethanologens

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Several million tons of sugarcane baggase and cane leaf matter are produced worldwide. These have a tremendous potential as sustainable lignocellulosic feedstocks for cellulosic ethanol biorefinery. The ethanol yield depends on the efficiency of conversion of glucans and xylans to fermentable sugars using pretreatment and enzymatic hydrolysis without generating byproducts that are toxic to fermentative microorganisms. Ammonia fiber expansion (AFEX) is a promising pretreatment method operates at milder conditions and does not degrade polysaccharides. The ammonia used in the AFEX process can be recovered and reused. In this work, the sugarcane bagasse and cane leaf matter were pretreated independently by AFEX under different conditions and hydrolyzed by a mixture of cellulase, xylanase and b-glucosidase enzymes. The best pretreatment conditions were identified based on higher glucan/xylan conversion during enzymatic hydrolysis. Using this optimized conditions larger batch of pretreated bagasse and cane leaf matter were hydrolyzed at high solid loading (up to 24%). Further, the hydrolyzate of both bagasse and cane leaf matter were fermented to ethanol using different recombinant ethanologenic strains of yeast and bacteria. For the ethanol fermentation process, the hydrolyzate was used as the sole carbon source with out any external nutrient or mineral supplementation. The kinetics of sugar consumption and ethanol production for different fermentation process are discussed.

Poster 3-26

Towards consolidated enzymatic biomass conversion

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The conversion of biomass into fermentable sugars is a critical aspect of realizing biofuels as an alternative to petroleum-based fuels. Lignocellulosic biomass is of particular national interest because it is abundant and is not a food crop. While the cellulose and hemicellulose components of lignocellulosic biomass can be enzymatically hydrolyzed to release sugars, the encrusting lignin component cannot. Expensive and energy-intensive non-biological pretreatments are currently required to degrade lignin enough to allow enzymes access to the cellulose/hemicellulose. This hurdle has limited the use of lignocellulosic biomass on an industrial scale. In nature, some organisms have been identified that can degrade lignin. For example, the white rot fungus *Phanerochaete chrysosporium* utilizes a number of secreted peroxidases, in combination with small molecule cofactors and mediators, to catalyze the degradation reaction. Our goal is to develop a process for degrading lignocellulosic biomass, via the following objectives: (1) Define the key enzymes, mediators, and cofactors in the fungal degradation of biomass, (2) Characterize the detailed interaction of those key molecules with biomass, and (3) Combine cellulose, hemicellulose, and lignin degrading enzymes with mediators and cofactors in a consolidated biomass conversion process. A multidisciplinary approach, which includes various imaging techniques, enzymatic analyses of lignin degradation, theoretical modeling of biomass degradation, and identification of metabolites and expressed proteins by mass spectrometry, is being used to tackle this difficult problem. In this presentation, we discuss our latest data and progress.

Poster 3-27

Hemicellulose hydrolysis in South America lignocellulosic biomass by steam explosion

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Hemicellulose from lignocellulosic materials constitute a large potential source of fermentable sugars to be used for fuel production. Sources from the Bolivian Altiplano include e.g. Paja Brava (*Stipa ichu*), quinoa straw (*Chenopodium quinoa* Willd.) and Curupaú (*Anademanthera columbrin*). Those sources all have a high carbohydrate content and are already used for various purposes. The aim of the present study was to determine conditions for a good recovery of hemicellulose, primarily pentose, sugars from these materials. The three feedstocks were subjected to steam explosion using different catalyst agents. The Paja Brava and quinoa straw were SO_2-impregnated (2.0 to 2.5% w/w) and steam pretreated at temperatures between 190 and 200°C, and holding times of 300 to 600 s. Curupaú hardwood, on the other hand, was hydrolyzed with dilute sulfuric acid (H_2SO_4 = 0.5 – 1.5 % (w/w) at a temperature of 180 to 210 °C, and a holding time between 300 and 600 s.

Acid hydrolysis gave a good recovery of pentose sugars with only minor amounts of degradation products in terms of furan compounds. Only minor proportion of the lignin was solubilized. At the conditions in the study, SO_2 was the most effective catalyst tested for extraction of xylose. The quinoa and Paja Brava gave the highest xylose yields, 62% and 65%, respectively. A reasonable xylose yield (58%) was also found from the CurupaÃº. Acid catalyzed steam explosion thus appears to be a suitable pretreatment process for extraction of hemicellulose sugars from these feedstocks to be used in fermentation processes.

Poster 3-28

Alkaline Pretreatment of Recycle Newsprint and Its Mill Sludge

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Pretreatment of two different softwood-based lignocellulosic wastes (recycle newsprint and recycle newsprint mill sludge) was investigated. Pretreatment was done by aqueous ammonia and by sodium hydroxide with or without supplementation of anthraquinone. In all cases there was a substantial degree of delignification ranging from 20 to 50%. After pretreatment, 90-95% of the cellulose and 50-80% of hemicellulose were retained in the solid. Under this scheme, both cellulose and hemicellulose are enzymatically hydrolyzed to respective sugars. This is a significant economic benefit since it eliminates the need of detoxification of hemicellulose sugars this is required in non-alkaline pretreatments. For treated recycle newsprint mill sludge, the overall enzymatic digestibility was in the vicinity of 70% with 15 FPU/g-glucan loading of Spezyme CP. With newsprint feedstocks, the digestibilities were lower than that of treated sludge. In order to achieve higher enzymatic digestibility of cellulose and hemicellulose, external xylanase was supplemented. Xylanase supplementation increased not only the digestibility of hemicellulose but also the digestibility of cellulose. With xylanase addition, the overall sugar yield has increased by 10% to 20%. Both treated feedsotcks were tested as substrates for production of ethanol through fermentation using the cellulase and two different microorganisms -Recombinant *E. coli* KO-11 (SSCF) and *Saccharomyces cerevisiae, NRE- D_5A* (SSF). The fermentation process proceeded in a normal pattern without any major difficulty. The ethanol yields based on glucan were in the range of 70-80% of the theoretical maximum.

Poster 3-29

Starch hydrolysis evaluation for the production of fermentable sugars

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Development of a fermentation process using economic carbon sources is extremely important for commercial scale biofuel production. Profitability of the fermentation process depends mainly on the pretreatment given to the raw materials. In this work, the best acidic and enzymatic hydrolysis conditions for yucca and malanga starch were studied through a statistical study in order to obtain the highest yield of fermentable sugars. An analysis of variance study (ANOVA) and a 2^3 factorial design were performed to study three important factors in acidic hydrolysis: temperature, H_2SO_4 concentration and time. We observed with a 95% of confidence, that temperature is a factor with an important effect over hydrolysis and that the best hydrolysis conditions for the yucca and malanga starch were: 5% H_2SO_4, 5 h and 95°C; obtaining yields of 0.47 gr glucose/gr of yucca flour and 0.52 gr glucose/gr of malanga flour. A 2^3 central design and a response surface analysis were performed in order to establish the best conditions for the enzymatic hydrolysis of yucca and malanga flour starch with α-amylase and amiloglucosidase in synaeresis. Three of the factors considered important for the reaction were studied: temperature, pH and hydrolysis time. Response surface analyses showed that the response was minimal in the central points for both tubers and that, with a 95% confidence, the best conditions for the enzymatic hydrolysis were: 73.6°C, pH 4.08 and 3 h, with a yield of 0.27 g glucose/g of yucca flour and 0.16 gr glucose/gr of malanga flour.

Poster 3-30

Ammonia recovery in AFEX biomass pretreatment systems

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Ammonia Fiber Expansion (AFEX) is an effective and promising pretreatment process for the conversion of cellulosic biomass to ethanol and other useful products. In the AFEX process, ammonia is allowed to contact moist biomass and then absorb into biomass fibers at a moderately elevated temperature and pressure. After contacting for an adequate time period, pressure is released, and ammonia is partially removed as flashed vapor. It is envisioned that in commercial AFEX plants, the ammonia should be recovered and re-used to reduce material costs. Energy and capital costs for ammonia recovery should also be minimized, as they will contribute to overall operating and capital costs of commercial AFEX plants. In this study, we investigate the energy requirements and vaporization rates for recovery of ammonia remaining in the pretreated biomass after the initial flash. Vaporization rates of ammonia from selected biomass feedstocks are measured and used in a proposed model of an ammonia recovery operation for a commercial AFEX plant. The calculated heat and mass transfer coefficients for ammonia in moist biomass will be presented. Different approaches for minimizing ammonia recovery costs will also be discussed.

Poster 3-31

Polysaccharide decomposition in hemp woody core induced by electron beam irradiation

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The electron beam irradiation was applied as a pretreatment for the enzymatic saccharification of industrial hemp biomass woody core with doses of 0 (control), 150, 300 and 450 kGy. The higher irradiation dose resulted in the more extraction with 0.5% sodium hydroxide solution extraction at room temperature. The electron beam irradiation induced chain scission in both polysaccharides and lignin. The higher solubility of treated samples came from xylan degradation induced by electron beam irradiation based on carbohydrate compositional analysis by ^{1}H-NMR spectroscopic method. The changes in micro structure of hemp resulted in the better response to enzymatic hydrolysis with commercial cellulases (Celluclast 1.5L and Novozym 342). The higher improvement in enzymatic hydrolysis by the irradiation was found in the hydrolysis of the xylan than that of the cellulose in polysaccharides of hemp.

Poster 3-32

Chemical composition of the sugarcane bagasse

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The sugarcane bagasse is, as any lignocellulosic material, mostly constituted by cellulose, hemicellulose and lignin. These three components amount to more than 90% of the dry weight of the fiber. The ash content is, in general, low. In turn, the non-fiber compounds, commonly referred as extractives, may represent a significant portion of the dry weight in the raw material. In the present study, samples of raw bagasse were extracted with water, ethanol and water followed by ethanol. Then, the extracted bagasses had their chemical compositions determined by a series of analyses based on the methods proposed by Browning (1967), with modifications. As can be seen in Table 1, the water extraction removed 5.8% of extraneous materials from the bagasse fibers. The ethanol extraction led to a similar content of extractives (5.6%). On the other hand, the sequential extraction with both solvents reduced the dry weight of the raw material in 9.4%, thus showing that these two solvents dissolved structurally different compounds. This assumption was further confirmed by the relative absorption spectra of the extracts (Figure 1).

Table 1: Chemical composition of the extracted samples of sugarcane bagasse

	Extractant		
	Water	Ethanol	Water + Ethanol
Cellulose (%)	42.50	45.25	46.82
Hemicellulose (%)	24.88	27.29	26.84
Lignin (%)	20.90	18.93	19.76
Ash (%)	1.64	1.64	1.64
Extractives (%)	5.83	5.64	9.38
Sum (%)	95.75	98.75	104.44

Figure 1: Relative absorption spectra of the aqueous and alcoholic extracts

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Poster 3-33

The effect of wet oxidation pretreatment method on rape straw fibres for bioethanol production

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Rape plant is extensively used in biodiesel and food oil production industry. The leftovers of the plant, the straw is typically burned or spread back to the land. Rape straw is rich in sugars, and if properly pretreated with chemicals and enzymes, can be well fermented into ethanol by yeasts. In this study, rape straw fibres are pretreated by oxidants, such as oxygen gas and hydrogen peroxide at increased temperature. Focus is given in the oxygen pressure, the concentration of hydrogen peroxide, the effect of washing of the fibres after the treatment, the effect of recycling of the water phase, and pre-soaking of fibres.

The output of the wet oxidation pretreatment is a fibrous cake enriched in cellulose, and a water phase consisting of oligomeric derivatives of hemicellulose. Lignin, the third main component of biomass, is partly left in the fibres and partly oxidised or hydrolysed to phenolic compounds in the water phase. The fibrous cake can be used for ethanol production through simultaneous saccharification and fermentation (SSF), using cellulolytic enzymes and a C6-fermenting organisms e.g. *S. cerevisiae*. The pretreatment should be effective enough to induce break down of the coherence of the cellulose fibrils through removal of lignin. On the other hand, the pretreatment should be mild enough to avoid formation of degradation products of sugar and lignin, which are inhibitory for SSF. Enzyme digestibility of cellulose enriched filter cakes, and degree of inhibition under SSF, are used as tools for assessing the pretreatment strategies.

Poster 3-34

Withdrawn

Poster 3-35

Compositional changes in sugarcane bagasse on low temperature, long-term ammonia treatment

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An investigation into a simple atmospheric pressure pretreatment process using dilute ammonia solution, over a 40 day period enhanced the enzymatic cellulosic digestibility by ten folds. Sugarcane bagasse was stored in 0, 0.03, or 0.3% ammonium hydroxide for 40 days, at 30°C under atmospheric pressure in a closed vessel. Samples were withdrawn at 10 day intervals, and the changes in amounts of structural carbohydrate and lignin in the solids and organic acids and total phenolic compounds in the liquid stream were determined. Total microbial counts were also monitored. This process removed up to 40% of the lignin, with little loss of retained glucose or xylose.

Poster 3-36

Bioconversion of cellulose wastes using cellulolytic biofilms

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Celulose wastes as solid pollutants are considered in view of their lack of toxicity on the one hand and their recalcitrant nature on the other. The microbial degradation of cellulosics is frequently discussed, and the contrast between its success in handling natural cellulosic wastes versus its failure to cope with man – made refuse is described. The research carried out in the past decade has demonstrated that cellulolytic microorganisms are mostly provided with cell surface multienzyme conglomerates, which are capable of solubilizing solid cellulosic substrates. These complexes include cellulose binding and enzymatic components and serve as a substrate-targeting carrier, efficiently delivering hydrolytic component to the cellulose, if this solid substrate is physically contacted by cell surface. Therefore, a progress in establishing more efficient arrangement for technological solubilization of solid cellulosic wastes could be achieved using cellulolytic biofilms, formed by direct colonization of these wastes by cell populations of cellulolytic strains of adequate properties. In this context, the aim of presented experimental work was to characterize taxonomically not related strains to select these ones that could be engaged in above biofilm-based technology development. The capacity of respective strains to utilize different cellulosic substrates as well as to manifest cellular adherence mechanisms under the effect of physiological factors modulating the composition of cellulase system, is described. Moreover, a method enabling to imaging solid cellulose colonization (biofilm formation) was developed and found amenable to image analysis.

Poster 3-37

Biological Conversion of Municipal Solid Waste to Ethanol

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Municipal solid waste (MSW) is an attractive cellulosic resource for sustainable production of transportation fuels and chemicals because of its abundance, the need to find uses for this problematic waste, and its low and perhaps negative cost. However, significant heterogeneity and possible toxic contaminants are barriers to biological conversion to ethanol and other products. In this study, we obtained six fractions of sorted MSW from a waste processing facility in Fontana, California: 1) final alternative daily cover (ADC Final), 2) ADC green, 3) woody waste, 4) grass waste, 5) cardboard, and 6) mixed paper. Application of dilute sulfuric acid pretreatment followed by enzymatic hydrolysis gave the highest sugar yields in the cardboard and ADC final fractions for enzyme loadings of 100 mg enzyme protein/g sugars in the solids prior to pretreatment. However, treatment with our protein detoxification technology before adding enzymes improved sugar yields at low enzyme loading of 10 mg enzyme protein/g sugars in raw materials. For example, pretreatment with 1% dilute sulfuric acid for 40 min followed by BSA supplemented enzymatic hydrolysis at an enzyme loading of 10mg enzyme protein/g glucan recovered 72% of the potential glucan and 76% of potential xylan in solution from ADC final. These results will be incorporated into an economic model to estimate the economic feasibility of converting MSW to ethanol and identify opportunities for improving the economics.

Poster 3-38

Activity and Function of Ionic Liquids for Lignocellulose Dissolution and Hydrolysis

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Ionic liquids (ILs) are organic molten salts and show promise as solvents for lignocellulosic materials. The solubilities of lignocellulose components as a function of anion and cation properties are not well understood. Our objective is to obtain the thermodynamic phase behavior for lignocellulose in various ILs and ILs with organic cosolvents. This will help identify ionic liquid-cosolvent combinations that are efficient for biomass dissolution and separation of its components. The values of infinite dilution activity coefficients for various organic co-solvents in ionic liquids, the solubilities of sugars such as xylose, arabinose and glucose in ionic liquid-cosolvent combinations are predicted by a priori thermodynamic models (COSMO-RS, conductor-like screening models – real solvents). In addition, from COSMO-RS calculations, we provide QSPR (quantitative structure property predictions) of several ionic liquids, cosolvents and saccharides. Toward depolymerization of both the cellulosic and lignin components of biomass , ionic liquids have been screened for maintenance of enzyme stability and activity. GFP fluorescence has been employed as a reporter of enzyme stability. While imidazolium-based ILs showed a loss of conformation after 30 minutes, IL/cosolvent solutions were able to retain protein conformation.

Poster 3-39

Saccharification of ionic liquid pretreated biomass with different enzyme mixes

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The ionic liquid pretreatment of lignocellulosic biomass produces amorphous or partially crystalline cellulose and enhances the saccharification rates of biomass sugars. The high crystallinity of cellulose poses an impediment to hydrolysis enzymes for the cellulosic portion of biomass. Different pretreatment conditions have been explored to produce amorphous cellulose to increase accessible surfaces to the enzymes used in saccharification. Variable enzyme mixes have been used to study the saccharification kinetics in an attempt to explore a simplified enzyme component mixes and to reduce the overall cost of the process. Cellulose and biomass pretreated with ionic liquid have been characterized to assess the crystallinity and elucidate the acessibility of cellulase enzymes to biomass. Saccharification results are compared for pretreated biomass of variable crystallinity.

Poster 3-40

Biotransformation of coffee pulp/husk by ligniculous fungi

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Agro-industrial residues have been used as efficient substrates in several bioprocesses, the utilization of these residues as substrates for subsequent bioprocess contribute to solve pollution problems, and also is a way to improve the management of sources, which will be conducted to integrated processes, where the "wastes" could be used for example to generate energy, allowing a total sustainability of the process. In Puerto Rico, coffee is one of the most important crops, where commonly are processed by wet method. As consequence of this, is necessary establish new treatments to reduce the pollution from this source, because coffee Pulp/Husk contain a toxic (caffeine) that limited its use for animals. In the present study will be evaluated twelve strains of ligniculuos filamentous fungi (*Gloeophyllum trabeum; Pleurotus ostreatus; Phanerochaete chrysosporium; Alternaria alternate; Chaetomium globosu; Microsphaeropsis sp.;Phialocephala dimorphospora; Lecythophora hoffmannii; Ceriporiopsis subvermispora; Xylaria polymorpha; Daldinia concentrica; Postia placenta)* which will be maintained in PDA (Potato Dextrose Agar) used for the degradation of caffeine and lignin from coffee pulp/husk.

A Solid State Fermentation will be carried out. Experiments will be conducted with a pH (4.5-5.5) and moisture (60-70%) and on the addition of nutrient solution. Content of lignin, caffeine and lipids will be monitored. Caffeine and Lignin will be measure by HPLC and lipids will be measure by Soxhlet method. Also, this study will be conducted to obtain some by-products generated from fermentation. As a result the authors expect obtain a free caffeine biomass to use as animal feed and, oil as by-product.

Poster 3-41

Bioethanol Production from Plasma Assisted Pretreatment of Wheat Straw Fermenting C5 and C6 sugars

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With the aim to develop progressive pretreatment technologies, the effect of O_3 generated in an atmospheric pressure low temperature air plasma, for degradation of lignin was investigated. It was shown that this process can be described via an "instant analysis" correlating the amount of O_3 consumed, with the amount of lignin degraded and the sugars developed in the sample. Lignin was degraded up to 95% but cellulose was found to remain unaffected for more than 7 hours pretreatment. This analysis allows to produce specifically either pretreated biomass with a very low amount of lignin (1%) or pretreated biomass that consists of a compromise between degraded lignin, fermentation inhibitors and the maximal amount of fermentable sugars for yeast. We investigated the potential of *Pichia stipidis* (ATCC 58785 and ATCC 58376) as a natural strain to ferment C5 and C6 sugars to bioethanol.It was found that the maximal amount of fermentable sugars was produced after 1 hour pretreatment and estimated by enzymatic hydrolysis (~25g $100g^{-1}$ cellulose, 12 g $100g^{-1}$ xylose) with respect to the dry mass. The O_3 consumption was 0.2 g O_3 per g wheat straw (1 mm, 50% DM). The optimal air flow rates were 0.6 L min^{-1} with the mini reactor (ø 7 cm) or 12 L min^{-1} with the multi-layer reactor (ø 30 cm) with an O_3 concentration of 1 %.

Poster 3-42

Effect of Solvent Extraction on Pretreatment Process of *Pinus rigida*

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To confirm the effect of solvent extraction on pretreatment process, pretreatment of *Pinus rigida* was studied with several solvents.

Materials were ground and sieved through 40-mesh screen for the study, and Meicelase, which is commercial cellulase derived from *Trichoderma viride*, was used for enzymatic hydrolysis. Holocellulose/α-Cellulose content (TAPPI), lignin content, structural carbohydrates (NREL procedures), carbon types (^{13}C NMR), crystallinity (powder XRD), extracts analysis (GC-MS) and pore-size distribution (BET) were analyzed. Materials were extracted by petroleum ether, ethyl acetate and 70% methanol solution (v/v). Pretreatment of the extracted materials were carried out in the minibomb, and 10g of materials with 200 ml of 2% dilute sulfuric acid solution (w/v) were pretreated at 200¢ªC for 90 min. Then, residual materials were used for enzymatic hydrolysis. Enzymatic hydrolysis was carried out with 1g of pretreated material in 100 mL of 50 mM acetate buffer (pH 5.0) containing 40 mg of the enzyme powder, and the samples were incubated at 45 ¢ªC of 250 rpm in a rotary shaker for 48 hr.

Enzymatic hydrolysis yield of materials pretreated by dilute acid only was 20.50, while that of the solvent extracted materials was up to 37.52%. It might indicate that solvent that used in extraction process removed some extractives which acted as an inhibitor of enzyme. Thus removal process of these extractives before pretreatment could enhance enzymatic hydrolysis yield of soft woods, especially *Pinus rigida*.

Further analysis will be conducting to investigate the effect of solvent extraction process and to find out this obstructive extractives.

Poster 3-43

Development of a continuous AFEX process

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It has been shown that pretreating biomass with concentrated ammonia in the AFEX (Ammonia Fiber Expansion) process increases yields of fermentable sugars. Up to now, the AFEX process has been conducted as a batch process. However, conducting AFEX as a batch process limits the ability to commercially apply the process. Recently MBI has developed an innovative continuous AFEX (CAFEX) reactor capable of providing five critical functions required for the completion of the AFEX treatment. These functions are: 1) pressurizing the moistened biomass and ammonia, 2) mixing and generating a homogeneous mixture of liquid ammonia and moistened biomass, 3) heating the moistened biomass and ammonia, 4) providing adequate residence time, 5) releasing the pressure quickly. A prototype designed to process 300lb/hr of dry biomass has been built and constructed. The CAFEX prototype accomplishes several of the aforementioned functions simultaneously and also is capable of meeting AFEX process conditions. Since there are no moving parts the MBI CAFEX reactor has no dynamic seals that pose a risk of ammonia leakage into the work environment. Currently we are using distillers dried grains and solubles (DDGs) as a model feedstock to test the validity and functionality of our CAFEX reactor. The efficiency of the process is evaluated via enzyme hydrolysis. Hydrolysis of the DDGs treated in the continuous AFEX process are benchmarked with hydrolysis of theDDGs treated in the batch AFEX reactor under comparable conditions. The detail of the process and the results will be presented in this paper.

Poster 3-44

Identification and Quantitation of Water Extractives in Sorghum

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Sorghum has gained interest as a biomass feed stock with its high crop yields and drought tolerance. Its value as a feedstock for bioprocessing is inherently dependent upon detailed knowledge of its chemical composition. Currently accepted analytical procedures for compositional analysis of biomass enable near-quantitative mass closure on a dry-weight basis. However, total water- and/or ethanol-soluble materials are quantified gravimetrically and identified only as 'extractives'. Reported compositional analyses of water-soluble materials in corn stover and switchgrass have shown that fermentable sugars (primarily glucose, fructose, and sucrose) represent as much as ~26% and ~27% of the dry weight of extractives respectively (~4% and ~7% of the total dry weight of each feed stock respectively). Preliminary data indicates that water-soluble sugars may represent an even larger fraction of extractives in sorghum extracts. The analytical techniques developed to determine the composition of water-soluble materials in corn stover and switchgrass are being applied to assess the composition of water-soluble materials in sorghum samples. Sorghum results will be compared with previous analyses of corn stover and switchgrass extracts and presented in the context of their potential impact on biomass processing, feedstock storage, and future analyses of feedstock composition.

Poster 3-45

Conversion of switchgrass to sugars and ethanol using dilute ammonium hydroxide pretreatment

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There has been growing interest in producing switchgrass as an energy crop because as a perennial it can be grown on erosion-prone land, has low energy/water requirements, and is a native species of the with much genetic variability. However, further conversion research is needed to realize its potential as a feedstock for ethanol production. In this study, switchgrass was converted to ethanol by *Saccharomyces cerevisiae* following dilute ammonium hydroxide pretreatment. Switchgrass was treated at various ammonium hydroxide loadings and reaction temperatures and times. Following pretreatment, the ammonium was removed by evaporation and the samples evaluated for release of glucose when digested with GC220 cellulase (30/g glucan), Novo188 b-glucosidase (40 U/g glucan), and Multifect Pectinase (50 ul/g). When ammonium hydroxide loadings were varied between 4–10%, pretreatment with 8% w/v was determined to be sufficient to maximize glucose yields (78% of max). Samples were subsequently treated for up to 20 min at 160, 170, and 180°C. The highest glucose yields were observed when treated at 180°C for 10 and 20 min (78 and 81% of max). Switchgrass treated at optimal conditions was evaluated for fermentability using *S. cerevisiase* D5A in a simultaneous saccharification and fermentation culture. No lag phase was observed and the fermentation efficiency, based upon glucose conversion to ethanol, was 70% after 48 h. Further work is in progress to evaluate the effect of harvest maturity on yield and to characterize the effect of enzyme loadings on final ethanol yields

Poster 3-46

Profiling of Oligosaccharides and Related Degradation Products Generated by AFEX Pretreatment of Lignocellulosic Biomass using LC/TOF-MS

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Pretreatment of biomass helps enhance accessibility of recalcitrant cell wall sugar polymers to enzymatic processing. Characterization of chemical transformations of soluble oligosaccharides and other degradation products has remained elusive, but this information is critical for optimization of pretreatment conditions for maximizing enzymatic digestibility.

This study presents methods for quantitative profiling of oligosaccharides and related degradation products following Ammonia Fiber Explosion (AFEX) pretreatment of corn stover using different thermochemical severity conditions. Solid phase extraction conditions were optimized for enrichment of oligosaccharides using porous graphitized carbon, and LC/TOF MS using electrospray ionization with multiplexed collision induced dissociation (CID) was used to analyze the degradation products. The study revealed presence of oligosaccharides up to degree of polymerization (DP) 22, with and without various chemical modifications including acetylation. Multivariate statistical analyses highlighted variations in oligosaccharide content among untreated and different severity AFEX-treated corn stover. Chemical characterization of AFEX byproducts reveals the extent of oligosaccharide modification using different pretreatment conditions, and provides valuable information for process optimization.

Poster 3-47

Hydrothermal pretreatment of switchgrass: Effect of temperature and potassium carbonate on enzymatic reactivity

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Switchgrass is emerging as a potential lignocellulosic biomass for ethanol production. Unfortunately, switchgrass has very low enzymatic reactivity; hence, pretreatment is required to enhance the reactivity for an efficient conversion to fermentable sugars. Hydrothermal pretreatment of biomass has attracted much attention as a non-toxic, environmentally benign and inexpensive media for chemical reactions. Increase in ionization constant of water with temperature causes hydrolysis of hemicelluloses, degradation of lignin and cleavage of acetyl group during hydrothermal treatment. The process mainly removes hemicelluloses and resulting structural change after the pretreatment improves the accessibility and hydrolysis of cellulose.

Hydrothermal pretreatment of switchgrass was carried out in a flow-through reactor in at 150-180 °C and 35-136 bar. The process removed nearly 50 wt% of lignin and 55-80 wt% of hemicelluloses. The hydrolysate (liquid fraction) is acidic (pH ~3), which can be reduced by an small (0.45-0.9 wt%) addition K_2CO_3 which in turn retains the majority of hemicelluloses in the solid fraction. Enzymatic reactivity of after pretreatment was tested with cellulase enzyme at 15 FPU/g glucan. In addition , the changes in crystallinity and surface morphology were examined by X-ray diffraction and electron microcopy, respectively. The pretreated substrates at 150 °C (with or without K_2CO_3) were further studied for ethanol yield by fermentation using E. Coli KO11. The effect of temperature, pressure, and use of K_2CO_3 in hydrothermal pretreatment of switchgrass is discussed with respect to its enzymatic digestibility.

Poster 3-48

Novel approach to prediction of hydrolysate fermentability based on chemometric modeling of spectroscopic data

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Production of ethanol from biomass has been a subject of extensive research. To date, a variety of different pre-treatment technologies have been identified that render lignocellulosic biomass accessible for ethanol production. The hydrolysate that is formed during this pre-treatment process contains degradation products which may decrease the fermentability of the hydrolysate. Fermentability can be measured in several ways. Typically, batch fermentation studies are carried out, followed by comparison of results to a control which contains only the fermentation medium and microbe. These methods are time consuming and labor intensive which makes them unattractive to researchers. We are currently exploring a novel way to predict fermentability of hydrolysates based on chemometric modeling of spectroscopic data. Twenty pretreatment conditions, resulting in hydrolysates of variable composition, were selected for model development and validation. For each hydrolysate, spectroscopic information and relative fermentability were determined in independent experiments. These data were then evaluated chemometrically, using commercially-available software, in an attempt to identify the degree of correlation and predictive capabilities of models that varied with respect to the type of spectroscopic information and/or chemometric approach utilized.

Poster 3-49

Rice Straw Oxidation Using Hypochlorite-Hydrogen Peroxide for Bioconversion to Ethanol

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Rice straw is a lignocellulosic biomass that is a renewable organic substance and alternative source of energy. Presently, rice straw was pretreated in a novel manner using a hypochlorite-hydrogen peroxide (Ox-B) solution. The optimum pretreatment condition was analyzed by response surface methodology and the pretreated rice straw was hydrolyzed using exo-glucanase, endo-glucanase, hemicellulase, and β-glucosidase of Accellerase (endo-glucanase equivalent activity of 1,250 CMC U/g pretreated rice straw for 24 h). The optimum condition was 60 min pretreatment using Ox-B solution containing 20 ml NaClO and 100 ml hydrogen peroxide for 1 g rice straw in 220 ml total reaction volume, and 47.3 mg glucose and 72.5 mg xylose were obtained from 1 g rice straw. The structural change of rice straw after pretreatment and enzyme hydrolysis was examined by scanning electron microscopy. Following enzyme hydrolysis, *Saccharomyces cerevisiae* and *Pichia stipitis* were inoculated for ethanol production. Ox-B solution treatment was an essential step for efficient hemicellulose hydrolysis. With the initial 5% sugar concentration, the final ethanol concentration was about 1.67%, which is 87.3% of the stoichiometric and fermentation efficiency yield.

Poster 3-50

Investigating Residence Time Distribution and Effects on Performance in Continuous Biomass Pretreatment Reactor Designs

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Dilute sulfuric acid pretreatment of corn stover has been demonstrated in the National Renewable Energy Laboratory's bench scale batch pretreatment reactor and 1/4 tonne/day continuous horizontal pretreatment reactor. Operation of the continuous reactor has resulted in lower xylose yield and higher degradation product formation compared with the batch reactor at same conditions. Independent testing of the continuous reactor has revealed a broad residence time distribution, which explains reduced pretreatment performance as unconverted xylan and xylose degradation products result from insufficient and excessive reaction time, respectively. To improve residence time control by reducing back mixing and removing stagnant areas, the reactor's original interrupted-flight auger and smooth tube shell have been replaced with a continuous-flight auger and tube containing anti-rotation bars. The measured residence time distribution and corresponding pretreatment performance of both continuous reactor designs will be discussed and recommendations will be provided.

Poster 3-51

Inhibition Effects of Dilute-Acid Prehydrolyzates on Enzymatic Hydrolysis and SSCF of Solka Floc

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The effects of dilute acid pretreatment liquor on enzymatic hydrolysis of lignocellulosic biomass and microbial conversion to ethanol production were investigated. The treatment of biomass with acid removes the hemicellulose portion along with degradation and extraneous components that are potentially toxic to biological reactions. For the cellulosic ethanol process to be economically feasible, cellulose as well as hemicellulose must be utilized. Therefore, the hydrolysates of the pretreatment containing hemicellulose sugars need to be co-processed with the pretreated solid during the bioconversion process. In this work, the prehydrolyzates from dilute-acid treatment of corn stover were added to a SSCF reactor converting Solka Floc to ethanol in order to verify its inhibition effects. The enzymatic reaction was inhibited significantly reducing the reaction rate by 65% with 1:1 mix. The toxic effect on the microorganism was even higher showing negligible ethanol production in the SSCF. This inhibition effect was further studied to identify the individual inhibition effect and the type of inhibition on the enzyme. Inhibition by individual component of HMF, furfural and xylose at concentrations normally found in the prehydrolyzate was found to be much lower than that of the liquor from the acid pretreatment. The inhibition is therefore caused primarily by the organic acids, acid soluble lignin, and its degradation products. The inhibition data of these components on the enzymatic reaction and the toxic effects on the microbial growth and cellular reactions are analyzed, and the individual and conjugate effects are discussed.

Poster 3-52

Efficacy of lime and ammonium hydroxide for conditioning dilute acid pretreated corn stover hydrolysates

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Dilute acid pretreatment of lignocellulosic biomass releases hemicellulosic sugars and improves the enzymatic digestibility of the treated solids, but the hydrolysate is often toxic to microorganisms. Several processes have been proposed to reduce hydrolysate toxicity including treating the liquor with lime or ammonium hydroxide. We investigated the impact of both of these chemicals on the performance of two different process configurations for producing ethanol from corn stover. In one process configuration, the hydrolysate liquor was removed from pretreated slurry and treated with lime or ammonium hydroxide. The liquor was recombined with the solids, the solids were enzymatically hydrolyzed to glucose with cellulase and the resulting sugar solution was fermented to ethanol using a glucose-xylose fermenting *Z. mobilis*. In the second process configuration, the whole slurry was treated with lime or ammonium hydroxide, and then the procedure used for the first process configuration was followed. Both process configurations were tested at a 15% and 20% (w/w) total solids loading at the enzymatic hydrolysis step. Neither lime nor ammonium hydroxide had a large impact on performance regardless of the process configuration or solids loading. For the first process configuration at a 20% total solids loading, cellulose to glucose yields of 74.3% and 75.6% and ethanol yields of 79.3% and 79.6% were achieved using lime or ammonium hydroxide, respectively. However, compared to lime, ammonium hydroxide eliminates sugar losses seen during the overliming process, the need to dispose of gypsum, and the potential for deposition of gypsum in downstream process equipment.

Poster 3-53

Residual calcium in neutralized hydrolysates can destroy HPLC columns

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HPLC (high pressure liquid chromatography) columns containing weak anion exchange resins in the lead form (eg. BioRad Aminex HPX-87P) are frequently used to quantify the carbohydrates (sugars) that are found in lignocellulosic hydrolysates (sugarcane bagasse, sorghum, etc.). These columns are expensive and are easily damaged by both extreme pH (5>x>9) and ash. The newer NREL (National Renewable Energy Laboratory) procedure specifies the use of calcium carbonate ($CaCO_3$) rather than barium hydroxide ($Ba(OH)_2$) to neutralize the hydrolysate prior to analysis via HPLC. Since this change was made, we noted a column-mortality rate exceeding that observed when using $Ba(OH)_2$. The columns were irrevocably damaged, and could not be regenerated. The cause of this was observed to be excessive Ca^{2+} remaining in the hydrolysate after neutralization. The phenomenon is described here, along with a quick colorimetric assay which can be performed to determine if the samples are likely to cause column damage. It was found to be simpler and less expensive, overall, to return to the use of $Ba(OH)_2$.

Poster 3-54

Evaluation of lignocellulose dissolution in ionic liquids as a pretreatment strategy for ethanol and lignin production

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Ionic Liquids (ILs) are liquid salts with extraordinary dissolution behaviour and physical properties. For example, 1-n-butyl-3-methylimidazolium chloride (bmimCl) is able to completely dissolve lignocellulosics. The components of lignocellulosics can be recovered and partially fractionated by the addition of antisolvents. It is known that the cellulosic fractions recovered from bmimCl are more amenable to enzymatic hydrolysis than cellulosic fractions recovered from other pretreatment processes. The utility of this dissolution strategy as a biomass pretreatment for enzymatic saccharification and bioethanol production is evaluated by determining the kinetics of dissolution of sugarcane bagasse in imidazolium salts over a range of temperatures and biomass loadings; assessing the choice of antisolvent; quantifying the recovery of the IL and biomass fractions including lignin; and comparing the ease of enzymatic saccharification of the cellulosic products from the ILs with that of dilute acid pretreatment. All cellulosic products recovered from IL dissolution have enhanced saccharification kinetics. However, the biomass loading and choice of antisolvent have a significant impact on the IL recovery and consequently on the economic feasibility of IL-based pretreatment processes.

Poster 3-55

Mechanism of delignification in the ionic liquid tetradecyl(trihexyl)phosphonium chloride

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While dissolution and derivitization of cellulose in Ionic liquids (ILs) has been demonstrated at laboratory scale, the dissolution of lignocellulose is difficult due to the presence of inter-dispersed lignin in biomass matrix.Consequently dissolution of biomass in imidazolium ILs require longer reaction times and higher temperatures than for dissolution of pure cellulose.Some ILs that do not dissolve cellulose (e.g. tetradecyl(trihexyl)phosphonium chloride) are able to dissolve lignin rapidly at low temperatures.These ILs have potential as delignification solvents in biomass pretreatments.Tetradecyl(trihexyl)phosphonium chloride can effectively delignify sugarcane bagasse.It is found that the mechanism of delignification in the phosphonium IL is similar to an acid-organosolv pulping.This delignification reaction is studied over a range of temperatures and compared to acid-organosolv delignification.Phosphonium IL delignification is observed with very short reaction times and at atmospheric pressures.At high temperatures lignin recondensation reduces the effective delignification.However delignification in phosphonium ILs can be optimised to reduce the effect of unwanted condensation reactions.The results indicate that the ILs in the tetra alkyl phosphonium series are effective delignification or pulping solvents.

Poster 3-56

Ethanol production from sweet sorghum by a dilute ammonia solution

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The Audubon Sugar Institute (ASI) is working diligently to enhance the productivity and profitability of the state of Louisiana by developing integrated technologies to convert energy crops such as sugarcane, sweet sorghum, energy cane and Miscanthus into value added products like alternative fuels, specialty chemicals, biomaterials and animal feeds. An ethanol process, utilizing dilute ammonia-treated sweet sorghum bagasse as feedstock material, has successfully been developed at ASI. This process resulted in at least 80% cellulose digestibility and 90% theoretical ethanol yield at 10% (dry weight) solids loading. Organic acids, glycerol, HMF and furfural concentrations were determined.

Poster 3-57

Rapid Analysis Methods to Predict Component Concentrations (Liquor and Solid) in a Pretreated Slurry Stream

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Accelerated research efforts to develop viable and economical solutions for the production of ethanol from lignocellulosic material has resulted in a need for fast, accurate, and inexpensive methods for compositional analyses of biomass used as process feedstock. One of the challenges faced by analytical chemists is the development of analytical methods for very rapidly measuring the composition of intermediate process streams generated from the dilute-acid pretreatment of biomass. This work documents the development of two rapid analytical techniques based on Near-Infrared (NIR) spectroscopy for the compositional analysis of pretreated corn stover slurries and associated liquors. The liquor method employs transmission NIR spectroscopy to measure the composition of dissolved constituents in pretreatment liquors. The slurry method employs transflectance NIR spectroscopy to measure the composition of the insoluble solids fraction in pretreatment slurries. The results of this effort have demonstrated that it is possible to obtain compositional data for dilute acid pretreated slurries and associated liquors without the need for time and labor intensive sample preparation steps (washing, drying, and milling) required by wet chemical and other NIR spectroscopic techniques. Compositional data for these process streams can now be made available to researchers in minutes rather than days, and could be used by industry to optimize process performance.

Poster 3-58

Production of bioethanol in pilot-plant scale using dilute-acid hydrolysis of spruce

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The interest for large-scale production of bioethanol is increasing, but full-scale plants are not yet a reality. However, pilot and demo plants are under construction at several locations in Europe and the US. The complexity of the plants can be very different, but the goal is the same: to gather data for techno-economic calculations to estimate capital and operational costs for full-scale plants.

In the municipality of Sveg in Sweden, a pilot-plant is in operation. It is the first step in an intended biorefinery, based on an already existing pellet-production facility. In the biorefinery concept utilization of the raw material is paramount. Therefore, not only bioethanol will be produced, but also solid fuel (pellets), waste heat and carbon dioxide. The latter two will be used in greenhouse cultivation. The purpose is to optimize batch-wise dilute-acid hydrolysis and fermentation of various raw materials, e.g., pine, spruce and hemp. The capacity of the plant is 1/20 of a full-scale plant and an increase to production scale will be done by increasing the number of the already full-sized reactors.

Extensive investigations have been performed in laboratory scale, regarding hydrolysis and fermentation conditions. The results will be validated in the pilot-plant and, if necessary, the pilot reactor will be modified to find optimum large-scale conditions. In the biorefinery concept, the economics of the process is depending on a mixture of products; therefore, it is vital to gather data for all products to be used for techno-economic calculations. Results from the studies will be presented.

Poster 3-59

Understanding the impact of corn stover compositional variability on pretreatment performance

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Corn stover is a potential large-volume lignocellulosic biomass feedstock that can be converted to fuels and chemicals. While most research efforts are focused on improving conversion yields, little is known about how the large compositional variability inherent in corn stover affects conversion yields. This study focused on assessing the impact of stover compositional variability on xylose conversion yields during dilute-acid pretreatment and on the enzymatic cellulose digestibility of the resulting treated solids. We pretreated seven compositionally-diverse corn stovers obtained from various locations throughout the United States. Each corn stover lot was pretreated at three different conditions in triplicate in a pilot-scale continuous reactor. At a medium pretreatment severity, monomeric xylose yields ranged from 30% to 70% for the different stover lots, and corresponding enzymatic cellulose digestibilities ranged from 68% to 95%. Similar results were seen at the other pretreatment severities. We found that xylose yields and enzymatic digestibility decreased with increasing acid neutralization capacity or soil content of the stover. Xylose yields also increased to a lesser extent with increasing xylan content of the stover. No other significant correlations between the stover's component concentrations and conversion yields were found. Apparently, the compounds that neutralize acid are predominately associated with external contaminants on the stover (i.e., soil). The same conversion yields could likely be obtained with different stover lots by appropriately adjusting the acid loading during pretreatment to compensate for the neutralizing effect of the external compounds.

Poster 3-60

Comparison of Kinetics of Xylose and Lignin Removal During Hot Water and Dilute-Acid Pretreatment of Corn Stover using a Continuous Flow-Through Reactor

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A flow through reactor (FTR) was used to determine the kinetics of xylose and lignin removal during hot water and dilute-acid pretreatment for bioethanol production. The removal rates of xylan and lignin during hot water (HW) and dilute-acid flow-through (DA) experiments with corn stover were studied between 170°C and 230°C for HW and 150°C and 210°C for DA. During all FTR pretreatments, insoluble dark precipitates were observed in the effluent and were characterized as lignin-carbohydrate complexes (LCC). Oligomeric and monomeric xylan was measured in the effluent during all of the FTR experiments. At temperatures beyond 200 °C significant xylan degradation to unknown products was observed. Total xylan removed was proportional to lignin and acetate release over the reaction time. Increases in pretreatment temperatures from 200°C to 230 °C did not significantly enhance the kinetics of xylan, lignin or acetate removal. Melting and mobilization of lignin also likely contribute to the process of xylan release. The results show that a flow through reactor is suitable for kinetics studies because the products are removed from the reaction zone, therefore less sugar degradation and lignin condensation reactions occur as compared to a batch reactor system.

Poster 3-61

Effect of Moisture Content on the Ensilage of Sugar Beet Pulp and Tomato Pomace

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Seasonally produced biomass such as sugar beet pulp (SBP) and tomato pomace (TP) must be stored properly to meet the demand of year-round biofuel production. Ensilage was studied to preserve SBP and TP from degradation of energy components. The moisture content (MC) of the feedstock is a critical factor affecting the performance of ensilage as well as the cost of feedstock storage and transportation. Two lactic acid bacteria (LAB) strains, including *Lactobacillus brevis* B-1836 and *Lactobacillus fermentum* NRRL B-4524 were used for SBP ensilage. The raw SBP as received (80% MC) and air-dried (10% MC) were run as controls. The other tested MCs of SBP were 80%, 55%, and 30%. A TP-isolate denoted UTP 4 and a TP-extract containing naturally occurring microorganisms were used to ensile TP. Five different MC levels, including a raw control (60% as received), air-dried control (10% MC), 60%, 45%, and 30% were investigated. Except for the controls, all other tested MCs were achieved by air drying followed by rehydration. For both SBP and TP, the ensilage of the raw control was competitive with ensilage with LAB inoculations tested at the as-received MC. The 10% MC treatment did not exhibit any change during ensilage; indicating drying the biomass can effectively stabilize SBP and TP during storage. The ensilage was significantly improved as the MC decreased from 80% to 30% for SBP and from 60% to 30% for TP. Our results suggest that partial drying followed by ensiling may be a good approach for biomass stabilization.

Poster 3-62

Leaching of Food Industry Residues to Improve Feedstock Quality and Resource Recovery

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Food processing wastes and residues such as fermented grape pomace (FEGP) from wineries and sugar beet pulp (SBP) from sugar producers are potential feedstocks for biofuel and other bioenergy conversion using both thermochemical and biochemical approaches. However, high concentrations of alkali metals and chlorine in these feedstocks promote ash slagging and fouling as well as corrosion in high temperature conversion systems. Residual ethanol and other organic compounds can be inhibitory to further microbial fermentation. Earlier research has shown leaching to be beneficial in reducing concentrations of deleterious constituents in biomass. Leaching was studied for its application in extracting inorganic and organic constituents from FEGP and SBP. Samples of each feedstock were leached in water at ambient temperature for 30 or 120 minutes at dry solid-to-liquid ratios of 1/20 and 1/50 kg/L. Leachates were analyzed for organic acids, water soluble carbohydrates, ethanol, potassium, sodium, and chloride. Leaching removed 82% of sodium, 86% of potassium, and 76% of chlorine from SBP. Leaching reduced total ash concentration in FEGP from 8.2% to 2.9% of dry matter, and from 12.5% to 5.4% in SBP. Glycerol (7-11 mg/dry g), ethanol (131-158 mg/dry g), and acetic acid (24-31 mg/dry g) were also extracted from the FEGP. Propionic acid (89-100 mg/dry g) and glucose (27-33 mg/dry g) were extracted from the SBP. These results suggest that leaching is a beneficial pretreatment step for improving the quality of food processing residues for thermochemical and biochemical conversion. Some compounds in leachate may possibly be recovered for higher value uses.

Poster 3-63

Sugar Beet Pulp Storage via Ensilage: Effects on Sugar Yield upon Enzymatic Hydrolysis

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Sugar beet pulp (SBP) contains more than 40% carbohydrate and less than 2% lignin; therefore, it could be a promising feedstock for bioethanol and other biofuel production without severe pretreatment. In this research, the ensilage process was studied for storage and pretreatment of SBP for the purpose of sustainable ethanol production. Two lactic acid bacteria (LAB) strains, including *Lactobacillus brevis* B-1836 (LAB #120) and *Lactobacillus fermentum* NRRL B-4524 (LAB #137) were used to ensile SBP in 1L reactors at 26°C for 90 days. The effects of LAB loading level and SBP packing density were investigated using response surface methodology. Ensilage without LAB inoculation was conducted as a control. The ensiled SBP was hydrolyzed with commercial cellulases to study the effect of ensilage on the enzymatic digestibility of SBP. A particularly interesting finding was that the ensilage process had a pretreatment effect on the SBP. Enzymatic digestibility of SBP was increased by 4-35% for inoculated treatments and 20-30% for the controls, compared to untreated SBP. The improvement in enzymatic digestibility of SBP could be due to pectin and hemicellulose removal and/or cell wall structural changes caused by microbial activity during the ensiling process. Therefore, the ensilage process could be a suitable biological pretreatment method for SBP. Inoculation with LAB strain #137 tended to have the most positive effect on enzymatic digestibility of SBP with the lowest losses of hemicellulose and cellulose. Performance of this strain depended on packing density and level of inoculation.

Poster 3-64

Optimization of Enzyme Cocktail for Alkaline Pretreated Switchgrass

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Switchgrass is one of the most promising energy crops for production of cellulosic ethanol. The major concern in producing economically feasible cellulosic ethanol is delignification of biomass and optimization of enzyme mix. In this study, pretreatment of Dacotah switchgrass using aqueous ammonia was investigated by applying two different modes of operation: ammonia recycle percolation (ARP), and soaking in aqueous ammonia (SAA). Two alkaline reagents, NaOH and aqueous ammonia, were used for pretreatment of switchgrass. Dacotah switchgrass was found to be recalcitrant even at high temperatures (130^{0}C for SAA and 170^{0}C for ARP), for further improvement of delignification and retention of hemicelluloses in biomass H_2O_2 and anthraquinone were used along with alkaline reagents. Five different pretreatment schemes were used: aqueous ammonia alone, aqueous ammonia and H_2O_2, aqueous ammonia and anthraquinone, NaOH alone, and NaOH and anthraquinone. The pretreated samples were subjected to enzymatic digestibility test by using commercial enzymes, like Spezyme CP, Accelerase, Multifect Xylanase and β-Glucosidase using different combinations. The results were analyzed to assess the overall effectiveness of the pretreatment and to determine the optimum enzyme mix.

Poster 3-65

Pretreatment of Seaweeds for Production of Chemical Intermediates

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Biotechnology for macro- and microalgae began to develop in the middle of the last century. Recently, there are used to several applications such as nutritional additives of food and animal feed, cosmetic additives, and bioenergy sources, etc. Seaweeds mainly divided into three kinds such as brown, red and green seaweed. They have a high content of carbohydrates, easily degradable and making them a potential substrate for the production of liquid fuel. In the past, most of the work of bioconversion using seaweed had been related to methane production. Recently, the production of liquid fuels such as bioethanol, biobutanol and biodiesel from seaweed and microalgae has lately attracted considerable attention. A lot of research is performed about different pretreatment methods to enhance the digestibility of cellulosic materials. Nevertheless, the pretreatment method of seaweed has received very little attention. In this study, we investigate the pretreatment method of seaweed, and evaluate the properties of seaweed extract for using potential substrate for the synthesis of chemical intermediate and bioenergy.

Poster 3-66

A comparison of lime and sodium hydroxide pretreatment for delignification and enzymatic hydrolysis of rice straw

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Fresh-harvested and air-dried rice straw was pretreated either by hydrated lime ($Ca(OH)_2$) or by sodium hydroxide (NaOH) in sealed 250-ml containers. A full factorial experiment including parallel wash-only treatments was designed for both pretreatments to investigate the alkaline loading and pretreatment time for delignification. Alkaline loadings for lime pretreatments were 0, 5% and 10% of biomass, and 0, 2% and 4% of biomass for NaOH pretreatments. Reaction time was set at 1, 2 or 3 hours for both pretreatments. Water-to-dry biomass loading ratio was 10 g/g and 5 g/g for lime pretreatment and sodium hydroxide pretreatment, respectively. Reaction temperature was held constant at 95°C for the lime pretreatment and 55°C for the sodium hydroxide pretreatment. The range of delignification was 13.1 to 27.0% for lime pretreatments, and was 8.6 to 23.1% for NaOH pretreatments. Both alkaline loading and reaction time have a significant positive effect on delignification. Additionally, higher temperature also aids the effect of delignification. Delignification with water alone ranged from 9.9% to 14.5% for pretreatment at 95°C, but there was little effect observed at 55°C. The post pretreatment wash step is not necessary for subsequent enzymatic hydrolysis under the design pretreatment conditions. Cellulase and β-glucosidase were added at a dosage 15 FPU/g glucose and 15 CBU/g glucose for enzymatic hydrolysis of pretreated biomass. Hightest glucose yield was 176.27 mg/g 105°C dried biomass (48.53% hydrolysis yield) in lime pretreated and unwashed biomass, and was 142.26 mg/g 105°C dried biomass (39.17% hydrolysis yield) in NaOH pretreated and unwashed biomass.

Poster 3-67

Biodrying as a pretreatment of horticultural wastes

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In this work, biodrying process was studied as a technology to reduce mass of horticultural wastes in order to improve handle and transport of organic wastes. During biodrying water is evaporated and some organic matter is degraded by microorganisms, thus organic waste is stabilized. Six static piles were prepared inside a greenhouse, where three contained whole wastes, three with shredded wastes and a test pile outside. Ventilation duct was put in two piles for aeration improving.

Air temperature and relative humidity were monitored inside and outside the greenhouse. Mass loss, humidity, organic mater and total nitrogen in the wastes were measured. No difference in mass loss was observed between piles with and without ventilation duct, and between the shredded and whole wastes, but the loss of mass and weight were lower in the pile outside the greenhouse. Piles inside the greenhouse showed decreases of 80% and 75% in weight and volume, respectively. The data obtained in this work suggest that stabilization and volume reduction as result of biodrying improve the handle and transport of horticultural wastes minimizing the pollutant impact

Poster 3-68

Simplex optimization and mathematical modeling of wheat straw dilute acid hydrolysis

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Wheat straw is an interesting biorefinery raw material, due to its abundance, chemical composition, and cost. Among the different pretreatments suitable for its processing, dilute acid hydrolysis still presents some benefits due to its simplicity. Nevertheless, it requires a careful optimization to avoid excessive by-products formation and catalyst spending. An attractive and simple optimization approach is the Sequential Simplex Method, an iterative procedure that enables to rapidly screen a large area of operational conditions and effectively encircle the optimal.

In this work, dilute acid hydrolysis of wheat straw was optimized to selectively hydrolyze the hemicellulose fraction and obtain a pentose-rich fermentable hydrolyzate. The influence of time (up to 180 min), and sulfuric acid concentration (up to 4%, w/w) were studied. The hydrolyzates obtained in the optimized conditions mainly contain free sugars (total content higher than 46 g/L). The main potential microbial inhibitors found were acetic acid, furfural, and HMF, in concentrations lower than 4.8, 1.7 and 0.3 g/L, respectively.

Empirical models describing the influence of the studied variables on sugars and by-products formation were validated for the entire domain. Sulfuric acid concentration was found to be the most influential variable, although both variables are statistically significant for xylose recovery. Interaction effects play a significant (negative) role. Data was also modeled based on the combined severity parameter (CS) and the results of these two approaches are compared and discussed. These hydrolyzates were easily utilized by *Debaryomyces hansenii*, a natural pentose assimilating yeast.

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Poster 3-69

Biofuels Production with Cattails from Constructed Wetlands

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The feasibility of conversion of the cattails in the constructed wetlands of the North Carolina A&T Farm into ethanol and hydrogen was investigated. Using the cattails to produce renewable energy will add value to the land as well as reduce emissions of greenhouse gases by replacing petroleum products. Pretreatment of the dried cattails with hot water, dilute sulfuric acid or sodium hydroxide was followed by solid-liquid separation and enzymatic hydrolysis and fermentation of using *Saccharomyces cerevisiae* (ATCC 24858), *Yamadazyma stipitis* (ATCC 58784) and *Enterobacter aerogenes* HU101. Trials gave an average conversion efficiency of 43.4% for the pretreated solids alone which, in conjunction with the crop yield for the cattails, would give up to 4,012 liters ethanol per hectare, a favorable comparison with corn stover's 1,665 L/ha at a 60% conversion rate. Given the high potential – 9,680 L/ha at 60% conversion efficiency for solid and liquid streams – and the social and environmental benefits gained by adding value to the waste management system and reducing carbon emissions otherwise made by gasoline, it is recommended that further studies be made using cattails as a feedstock for biofuels.

Poster 3-70

Integration of particles of different sizes in a hydrothermal process for the pre-treatment of agro-industrial residues such as wheat straw

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The recent year's extensive research focusing on processes for the integral use of lignocellulosic materials has brought the possibility of production of several commodity chemicals; particular attention has been paid to fuel-ethanol production. Wheat straw, an abundant by-product from worldwide wheat production, has been studied in this work, aming at evaluating the influence of the particle size distribution, through the integration of different proportions of particles of different sizes, in a hydrothermal process, based on the utilization of water as sole fractionation agent, as pre-treatment of agro-industrial residues. The milled material was initially separated into fractions (a. > 1.0mm, b. 1.0-0.5mm, c. 0.5-0.3mm and d. < 0.3mm) and then mixed in different proportions. Considering the values of water absorption capability in each sample, hydrothermal processes were evaluated in different conditions of time and temperature. Thus, water was added to a wheat straw sample (solid/liquid ratio, 1:10w/v) and placed in a closed and pressurized vessel, taking into account the moisture content. After each treatment, the liquid phase (hemicelluloses fraction) was separated from the solids. The hemicelluloses were precipitated with three volumes of 95% ethanol and dried for yield determination. The operation at 200ºC/30 min (containing 10% of a., 40 % of b., 40% of c. and 10% of d.) showed to be the most efficient. This process has been developed aiming at a more efficient utilization of agro-industrial wastes on autohydrolysis processes for hemicelluloses extraction during the initial stage of straw integral use.

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Poster 3-71

2-D NMR Investigation of the Effect of Ionic Liquid Pretreatment on the Chemical Composition and Structure of Switchgrass

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One of the biggest challenges in the production of biofuels is the development of an efficient process to increase the rate of hydrolysis of lignocellulosic biomass into its component sugars. The use of ionic liquids as 'green' biomass solvents for pretreatment processing has recently gained attention due to their ability to drastically reduce the crystallinity of cellulose and dissolve biomass. Through ionic liquid pretreatment, the rate of enzymatic hydrolysis of the biomass is markedly increased compared to other pretreatment techniques. In order to understand the mechanism of ionic liquid treatment on biomass, high resolution 2D NMR technique was employed. Here we present the first chemical characterization of switchgrass, a leading candidate for biofuel production, using 2D NMR and examine the changes in its composition that result from ionic liquid pretreatment.

Poster 3-72

Comparison of the Enzymatic Hydrolysis of Ionic-Liquid Pretreated Energy Crops

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The development of techniques that enable the efficient conversion of biomass into fermentable sugars is essential if lignocellulosic biofuels to be energetically and economically feasible. The crystaline and fibrous nature of cellulose is one of the main factors affecting the rate of enzymatic hydrolysis of biomass into its component sugars by cellulases. In order to increase the efficiency of this process, biomass is treated to open up the cellulose fibers. Current pretreatment technologies typically require high temperature, pressure or extremes in pH, that can result in the formation of undesirable byproducts and also necessitate high capital costs. A novel pretreatment technology that utilizes ionic liquids, salts that have a melting point of below 100C, has recently drawn a great deal of attention due to their ability to drastically increase the rate of enzymatic hydrolysis of crystalline cellulose. Some of these liquids have the ability to dissolve cellulose at relatively low temperatures and upon reconstitution, yield amorphous cellulose. Complete saccharification of microcrystaline cellulose treated with the ionic liquid 1-ethyl-3-methyl imidazolium chloride was achieved in 2 hours using a commercial cellulase derived from the fungus Trichoderma reesei. To examine the widespread applicability of this pretreatment technology, the rate of hydrolysis and resulting sugar yields of 5 different herbaceous and woody biomasses, pretreated with the ionic liquid EMIM Acetate, was examined.

Poster 3-73

A catalyzed wet-explosion of wheat straw

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The individual and interactive effect of different pretreatment conditions on the release of arabinose, glucose, xylose, and acetic acid from wheat straw during pretreatment and on dehydration of pentoses to 2-furaldehyde, and hexoses to 5-hydroxymethyl-2-furaldehyde (HMF) during pretreatment of wheat straw, were evaluated in a three-factor central composite design template. The template comprised 17 different combinations of temperature (-1, 0, 1), residence time (-1, 0, 1), and H_2SO_4 concentration (-1, 0, 1) with three center points. In all pretreatments, a fixed amount of air was used as oxidizing agent.

H2SO4-catalyzed wet-explosion of wheat straw was optimized in such a way, that the right combination of temperature, residence time, and sulfuric acid concentration in a wet-explosion process resulted in optimal recovery of the heteroxylan-derived sugars (arabinose, and xylose), ideally in a monomeric form, and that the formations of inhibitors (primarily 2- furaldehyde and HMF) were minimized. In addition, enzymatic hydrolysis with commercial available cellulase preparations was used to select the optimal pretreatment condition for wheat straw.

Poster 3-74

Ethanol production from cashew apple bagasse: Improvement of enzymatic hydrolysis by microwave-assisted alkali pretreatment

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Energy consumption has increased steadily as world population has grown and more countries have become industrialized. The fossil fuels have been the major resources to meet the increased energy demand. Moreover, its inevitable depletion and the increased concerns of greenhouse gas emissions have resulted in a worldwide interest in exploring renewable energy such as fuel ethanol. Lignocellulosic materials appear as a feedstock and low-cost biomass that can be used as alternative raw materials for ethanol production. Cashew apple bagasse (CAB) is the result of the industrial process of cashew apple, a pseudofruit native of the Northeast region in Brazil, for juice production. Therefore, CAB appears as an alternative raw material for ethanol production. In this study, the objective was to evaluate the microwave-assisted alkali pretreatment and to investigate the effects of process parameters on enzymatic hydrolysis of CAB. Furthermore, the hydrolysate was fermented to ethanol using *Saccharomyces cerevisiae*. The microwave oven pretreatment was carried out with CAB presoaked in NaOH solutions with different concentrations (0.0, 0.2, 0.4 and 0.6 mol.L^{-1}) for 15 and 30 minutes and power of 600W and 1000W. The enzymatic hydrolysis was carried out at 45°C using 2 and 16% (w/v CAB), 150 rpm and pH 5.0. Fermentation assays were carried at 30°C, 150 rpm, pH 5.0, and an initial cell concentration of 10.0 g.L^{-1}. Results showed that microwave-assisted alkali irradiation is an efficient pretreatment method to enhance CAB digestibility.

Poster 3-75

Measurement of total lignin mass balance closure after dilute sulfuric acid pretreatment from herbaceous feedstocks

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Determining the lignin mass balance closure in biomass materials after dilute sulfuric acid pretreatment is an important element to optimize pretreatment conditions for bioethanol production. Previous research at NREL has found the lignin mass balance closure determined in pretreated corn stover appears to be greater than the original lignin in the unpretreated material. We investigated the factors affecting lignin measurement in dilute acid pretreated feedstock using combinations of nitrogen analysis, ^{13}C cross polarization/magic-angle spinning (CP/MAS) solid-state nuclear magnetic resonance (NMR) spectroscopy, removal of lignin using acid chlorite bleaching, and pretreatment with ^{13}C-labeled sugars. It was found that remaining protein in acid insoluble residue is 0.5-1.0 wt% of original corn stover and only minimal contamination due to carbohydrate was observed. However, degradation products were present in acid insoluble residue suggesting that low molecular weight compounds can be condensed on to the biomass substrate after pretreatment. The acid-soluble lignin released during the pretreatment was determined by UV-Vis spectrometry to be an unrealistic 22-30% of unpretreated feedstock lignin. However, no substantial intensity assigned to lignin peaks could be detected in the pretreatment hydrolysate by quantitative ^{13}C liquid-state NMR. This indicates interference from low molecular weight compounds or sugar degradation products may be affecting the UV-Vis method. We are attempting to improve the determination of soluble lignin content after pretreatment using isolated lignin from native corn stover.

Poster 3-76

Optimization of the SPORL Pretreatment of Corn Stover for Ethanol Production

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SPORL (Sulfite Pretreatment to Overcome Recalcitrance of Lignocellulose) is a robust pretreatment technology of lignocellulosic feedstock for cellulosic ethanol production. The pretreatment consists of a short chemical treatment of feedstock with acidic sulfite solution followed by a mechanical size reduction (defiberization). Our preliminary results indicated that the SPORL is a novel and feasible pretreatment technology and meets the criteria for a commercially viable pretreatment process: (1) economical conversion of both the cellulose and hemicellulose to fermentable sugars, (2) low energy consumption in substrate size reduction and surface development, (3) high-value utilization of lignin, (4) mature technology for recovery of pretreatment chemical (sulfite), and (5) low risk in commercialization by using existing infrastructure and mature capital equipment in paper industry.

In this study, the SPORL process was optimized for corn stover pretreatment for cellulose ethanol production. The pretreatment parameters (chemical charge, temperature, catalyst, reaction time etc.) were optimized through a designed experiment to maximize sugar recovery and enzymatic saccharification. Mass balance of the major components (cellulose, hemicellulose and lignin) of corn stover during the pretreatment was conducted. Enzymatic hydrolysability of the pretreated corn stover and fermentability of hemicellulose-derived sugars in the pretreatment liquor were evaluated. The SPORL was also compared with the dilute acid pretreatment. The results indicated that the SPORL produced more digestible substrate and formed fewer inhibitors to fermentation than the diluted acid pretreatment.

Poster 3-77

Non-natural reactions to convert cellulosic biomass to fuels and chemicals

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Natural conversion of cellulosic biomass to energy by free-living or gut-symbiotic microbes is slow, partly because biodegradation of dead plant tissues must be compatible with living organisms. Industrial conversion of cellulosic biomass can use a wider range of reaction conditions and intermediates to proceed at a faster rate; however, these reactions are often incomplete or produce undesirable side-products such as inhibitors. We propose the use of non-natural enzyme-catalyzed reactions that are both selective and more efficient. Specifically, we propose use of enzymes with the potential in multi-reaction conditions to release the required sugars from a complex multi-component material like cellulosic biomass. Here, we present work on an engineered perhydrolase with improved substrate recognition and that catalyzes formation of peracetic acid more efficiently than its natural counterpart by 100-fold. Peracetic acid is a strong oxidant that effectively alters lignin allowing subsequent release of sugars (saccharification) by cellulases and xylanases. We have generated up to 70 mM peracetic acid in situ, resulting in up to 45% lignin reduction and up to 97% sugar release in saccharification efficiency. At this time, we are optimizing reaction conditions to improve potential involvement of endogenous acetate groups and toward matching saccharification reaction conditions. Our goal is to allow, for the first time, a consolidated approach that includes both the pretreatment and saccharification steps.

Poster 3-78

Xylan hydrolysis during hot-water pretreatment of corn stover

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The objective of this work was to study the kinetics of hemicelluloses extraction and to evaluate factors affecting the rate of xylooligomer and xylose formation during hot-water pretreatment of corn stover. The pretreatments were conducted in a batch reactor at 160-210°C with reaction times of up to 90 min. Yields of xylooligomers, xylose, and sugar degradation products, i.e., furfural, were determined. Under these conditions, sugar yields of up to 92% (as total xylose) were obtained. At the maximum of total xylose recovery, about 85% of the xylose was recovered in the oligomeric form. The release of acetic acid in the hydrolyzate followed a similar kinetic trend to that observed for total xylose release. The time of maximum total xylose recovery corresponded to the same reaction time at which the maximum acetic acid was recovered for all reaction temperatures. This indicates the influence of acetic acid in determining the rate of xylan hydrolysis.

Poster 3-79

Method Development for Assessing Feedstock Reactivity Using an Automated Solvent Extractor

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The development of standard methods for the assessment of cellulosic feedstocks for biofuel production is important to allow accurate comparisons of feedstocks across research groups. In this study we discuss current efforts to develop a standard pretreatment methodology for assessing the susceptibility of feedstocks to pretreatment for hydrolyzing structural sugars. We investigated the applicability of an Automated Solvent Extractor (Dionex Corp., model ASE350) for conducting bench scale batch pretreatment experiments. The ASE350 was selected for this research because it is a commercially available system, is fully automated, has the ability to run 24 samples sequentially, and has the possibility to carry out solid-liquid separation, a time consuming step in biomass analysis. Dilute sulfuric acid pretreatment was carried out on corn stover as a method to asses the significant operating factors of the ASE350, as well as explore the region of greatest feedstock response to pretreatment. A factorial designed experiment was used to determine the effect of time, temperature, acid concentration, flush volume, rinse volume, and other equipment operating parameters on xylose yield from pretreatment, which is one indicator of pretreatment efficacy under dilute acid conditions. We report on the relative effects of ASE350 operating parameters on xylose yield in pretreatment, as well as the reproducibility of the equipment and processes. Results showed that temperature and acid concentration had the greatest effect on xylose yield, while flush volume and purge time did not impact yields.

Poster 3-80

Method Development to Determine the Acid Concentration of Acid Impregnated Biomass

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Dilute-acid pretreatment will most likely be the initial process in cellulosic biofuels production. Effective acid impregnation of biomass is required to achieve high xylose yields and minimize degradation products during pretreatment, as well as prepare the biomass surface for enzymatic hydrolysis. Biomass neutralization capacity, structure, acid loading, and acid solution saturation affect impregnation efficacy. To compare various impregnation processes, a rapid, accurate, and reproducible method of determining the "in situ" acid concentration in biomass is needed. Previous methods are time consuming and ineffective for low acid concentrations, particularly below 0.6%, because of biogenic acid production. Homogenization, incubation, and refrigeration were the three different methods analyzed to extract acid from impregnated biomass. Homogenization was tested using two different devices, a blender and a homogenizer. We analyzed 16 biomass samples representing 8 different impregnation conditions. The varied factors among each sample condition included the impregnation reactor (spray or soak), acid concentration (0.2 wt% or 1.08 wt%), and particle size (¼ " or ¾"). We found that biogenic acid production at low acid impregnation concentrations (<0.6% H_2SO_4) is exhibited during incubation. A single-factor, 95% confidence ANOVA test showed that homogenization and refrigeration are both suitable methods to determine biomass acid concentration. However, because refrigeration took 48 hours and homogenization took 15 minutes, the latter was found to be the most suitable method of ascertaining biomass acid concentration.

Poster 3-81

Microbial pretreatment on corn stover by the white rot fungus *Ceriporiopsis subvermispora* for enzymatic digestibility

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Microbial pretreatment on corn stover by an effective lignin-degrading fungus *Ceriporiopsis subvermispora* was studied. The substrate characteristics such as moisture content and particle size were studied for obtaining maximum sugar yield. The main and interactive effects of moisture content, particle size and cultivation time on enzymatic digestibility of corn stover was obtained. The fungal-treated corn stover was mainly analyzed for lignin degradation and cellulose conversion.

Poster 3-82

Withdrawn

Poster 3-83

Weak Acid Hydrolysis of Sugarcane Bagasse for Ethanol Production

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Sugarcane processing generates a large volume of bagasse. Disposal of bagasse is critical for both agricultural profitability and environmental protection. In this study, we demonstrate that cane processed bagasse could be used to produce fuel grade ethanol without saccharification. A chemical pre-treatment process using alkaline peroxide and acid hydrolysis was applied to remove lignin, which acts as physical barrier to cellulolytic enzymes. Yeast Saccharomyces cerevisiae ATCC strain 765 was used in the experiment. The pre-treatment process effectively removed lignin. Ethanol production in the culture sample was monitored. The results indicate that acid hydrolysis produced the most ethanol from the residue. More ethanol was produced from bagasse treated with 0.8M H2SO4 for 18 days compared to alkaline pretreated residue at 2% H2O2 (pH 11.5) for 48 hours and fermented for 21 days. This preliminary study showed that ethanol production from post-harvest sugarcane residue such as bagasse is possible without the addition of cellulase enzyme. The ethanol yield in our study is eight times lower than the theoretical yield as per National Renewable Energy Laboratory(NREL) calculation. In this study, we achieved a significant removal of lignin from the bagasse, which resulted in higher production of ethanol.

Further research is needed to optimize the conditions for maximum production of ethanol from bagasse.

Poster 3-84

LHW pretreatment and enzymatic hydrolysis of rapeseed straw

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Rapeseed cultivation is deserving a growing interest because rapeseed oil can be used for biodiesel production, adding interesting features (e.g. freezing point) for biodiesel to comply with EN 14214 quality norm. After seed harvesting, rape straw is left in the fields and usually eliminated by burning. Rapeseed straw is a lignocellulosic material containing on average 33% cellulose and 22% hemicelluloses (dry basis). This work examines the possibilities of using rapeseed straw as feedstock for fuel ethanol production. The raw material was submitted to Liquid Hot Water pre-treatment. The pretreated solid was further submitted to enzymatic hydrolysis, while the liquid fraction issued from pre-treatment was analyzed for sugar composition. A 2^2 factorial design was used to evaluate the influence of temperature (170-210ºC) and time of pre-treatment (10-50 min) on the measured responses. Following first trials results, a composite central design was performed and response surface methodology was applied to determine the optimal operation conditions for either maximum glucose enzymatic yield or maximum xylose recovery in the liquid fraction. The maximum glucose recovery in the pretreated solid (68.3% of that in the raw material) resulted at 213.5 ºC and 42.4 min. If the glucose released by enzymatic hydrolysis together with the xylose recovery in the liquid is chosen as optimum condition, the best result was found at 191 ºC and 34 min, corresponding to 62.5% glucose yield, while sugars in the liquid fraction were 5.5 and 42.3% of the glucose and xylose present in the raw material, respectively.

Poster 3-85

Monitoring Process Streams towards Understanding Ionic Liquid Pretreatment of Switchgrass and Cornstover

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Pretreatment of Biomass is essential for breaking apart highly ordered and crystalline plant cell walls and loosening the lignin and hemicellulose conjugation to cellulose microfibrils, thereby facilitating enzyme accessibility and adsorption and reducing costs of downstream saccharification processes. Recent reports1,2 have shown very high yields at very low Enzyme loadings. However, pretreatment still remains one of the most costly steps in lignocellulosic biofuel production. Ionic liquids are novel solvents showing great promise for cellulose solubilization. Instant rejection of dissolved cellulose upon anti-solvent addition shows promise for recyclability in addition to other desired attributes like low volatility, non-flammability and thermal stability. Although shown to be very effective in cellulose solubilization 3,4 , disposition of hemicellulose and lignin are not understood. The aim of this ongoing work is to understand ionic liquid pretreatment by monitoring and analyzing process streams towards gaining better understanding of pretreatment process.

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Poster 3-86

Can endoxylanase application in wet storage preserve dry matter and reduce pretreatment severity?

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Is xylose more valuable as a preservative or as product? Enzymatic processing of lignocellulosics during wet storage could potentially improve dry matter stability and reduce pretreatment severity. Limited hemicellulose hydrolysis using endoxylanase would provide xylo-oligomers for ensiling–improving dry matter stability–and also reduce the amount of hemicellulose hydrolysis needed during subsequent pretreatment. To explore the cost and yield tradeoffs of this approach, a commercial endoxylanase was applied to freshly harvested corn stover at three starting water contents (36%, 51% and 68% wet basis) with and without the addition of a commercial silage amendment (lactic acid bacteria at 10^6 cfu g^{-1} dry matter). Experimental enzyme activities were high (47 to 138 U per g xylan) to identify clearly the effects of treatment. Samples were incubated anaerobically at 37° C for 4 months and were sampled for compositional analyses of both the liquid and solid fractions. Dry matter losses ranged from 12.5 to 15.3 %. pH values were lower and organic acid and total sugar concentrations were higher in the highest (68%) water content samples. Ash content was higher in the low water content samples (36% and 51%), which balanced the difference in organic acid and sugar concentrations relative to total dry matter loss. Near infrared spectrometry indicated greater loss of hemicellulose from solids subjected to a combination of high starting water content, endoxylanase addition and silage inoculant. Ongoing work will determine if this leads to a reduction in pretreatment severity, and if so to what extent relative to untreated stover.

Poster 3-87

Kinetic modeling of glucose reversion reactions

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In this study, we have directly measured glucose reversion reactions and have extracted the kinetics of formation of glucose dimers through modeling. While dehydration reactions are well known acid-catalyzed reactions leading to loss of sugar and the formation of potential fermentation inhibitors, reversion reactions (those that lead to the formation of oligosaccharides) have received much less attention, even though these reactions can account for a significant loss of sugars (up to 12% glucose, 9% xylose). Our goal has been to develop a complete picture of the mechanism of reversion reactions using glucose so as to help elucidate the reversion reactions of xylose which are directly relevant to biomass pretreatment. Identification of individual dimers from xylose reversion reactions is difficult due to a lack of standards, so we have modeled the kinetics of dimer formation using the reversion reactions of glucose.

Reversion reactions of glucose to all possible dimers have been measured as have the kinetics of hydrolysis of several of these dimers. These measurements were made in mildly acidic aqueous solutions that were heated using microwave radiation. From these experiments, we determined the kinetics of the reactions, their activation energies and the equilibrium constants for the reversion reactions by modeling all of the products formed. Included in this model is anhydrosugar formation (i.e., levoglucosan) from glucose. In addition, we discuss concentration effects (acid as well as sugar concentration). The ultimate goal is to develop quantitative kinetic parameters that can be used to design and optimize biomass pretreatment reactors.

Poster 3-88

Xylan Hydrolysis Kinetics

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Experiments and calculations suggest that the intrinsic kinetics of xylan hydrolysis is much faster than xylose degradation. This suggests that high yields (>90%) of xylose during pretreatment are theoretically feasible, but that mass transport limits the hydrolysis of xylan in particles of biomass. To more fully understand mass transport in biomass pretreatment, experiments were conducted to measure the intrinsic kinetics of hydrolysis of xylan and xylooligomers (xylobiose), and to measure the hydrolysis of xylan in realistic samples of corn stover. The results from these experiments are used to develop and test kinetic models that contain empirical equations describing mass transport. These models will be useful for analyzing and optimizing large-scale biomass pretreatment reactors.

Poster 3-89

Pretreatment of *Gelidium amansii* for the Production of Bioethanol

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There has been growing interest in an alternative feedstock for bioethanol production. *Gelidium amansii*, which is a red algae, is composed of carbohydrate like a cellulose. It has been, therefore, considered as a potential feedstock for bioconversion to ethanol. In this study, pretreatment using sulfuric acid was applied to this feedstock and its effectiveness on bioconversion was investigated. Galactose was removed from *Gelidium amansii* using sulfuric acid in a batch reactor. Optimum process conditions such as reaction temperature, reaction time, and sulfuric acid concentration were determined in terms of glucose yield after enzymatic hydrolysis. Enzyme loading was 15 FPU/g-glucan in the hydrolysis experiment. Compositional analysis was carried out by HPLC. The optimum pretreatment conditions within the scope of this study were found to be 130°C of pretreatment temperature, 10 min reaction time, and 0.4274% (w/v) of sulfuric acid concentration. At the optimum pretreatment conditions, 88.8% of ethanol yield was obtained in 72 hr.

Poster 3-90

Pretreatment of *P. densiflora* and *Solidago altissima* L. to Produce Bioethanol

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Since the feedstock cost is one of the major obstacles in commercialization of bioethanol production, various feedstocks have been investigated. In this study, *P. densiflora and Solidago altissima L.* were tested as new alternative feedstocks for the bioethanol production. They are fast growing plants.

P. densiflora and *Solidago altissima L.* were pretreated with sodium hydroxide. The pretreated sample was enzymatically hydolyzed using 15 FPU/g-glucan enzyme loading. Compositional analysis such as sugars and ethanol was carried out by YSI-7100. The pretreatment conditions were optimized in terms of reaction temperature, reaction time, and sodium hydroxide concentration.

Poster 3-91

A novel pre-treatment method for the lignocellulose-to-ethanol route

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Production of cellulosic ethanol is still facing some key hurdles on its way to commercial success. A major hurdle, in regard to the actual ethanol production process, is finding an efficient and inexpensive way to hydrolyze the cellulose and hemicellulose polymers into fermentable sugars (monomers). The two main routes to enable this are acid and enzyme-aided hydrolysis. The latter route is inefficient as such, but can be improved significantly by pre-treating the raw material before the actual enzymatic process step. In this paper, we describe a novel pre-treatment method, which is based on the use of a metal catalyst at alkaline conditions. The novel catalytic wet oxidation method showed especially promising effects, when using wood as the raw material. The paper will discuss both scientific and economic aspects of the new pre-treatment method.

Poster 3-92

Improved One-Step Steam Pretreatment of Softwood with Time-dependent Temperature Profile for Bioethanol Production

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At a time when natural petroleum resources are almost on the verge of depletion and concern about climate change is increasing, the potential of lignocellulosics for production of bioethanol is well recognized. Ethanol can be produced from biomass through enzymatic hydrolysis and fermentation. However, pretreatment of naturally resistant cellulosic material is necessary to achieve a high ethanol yield. Pretreatment, as a crucial step for enzymatic digestibility of biomass, is among the most expensive process steps.

To date, two-step, dilute-acid pretreatment of softwood (using SO_2), with separation and washing of the material between steps, has shown to result in the highest sugar and ethanol yields. In the first step, hemicellulose is hydrolyzed at milder conditions. The breakdown of the recalcitrant structure of cellulose is the aim of the more severe second acid-hydrolysis step. However, in an industrial process, filtration and washing of material between steps are difficult, as they should be performed at high pressure to avoid heat losses. Washing also leads to dilution of sugars. Furthermore, two-step dilute-acid pretreatment with separation and washing is energy demanding and requires higher capital costs.

In the current study, a new pretreatment reactor, with a novel time-dependent temperature profile, combining the two-step process into a one-step pretreatment, is being investigated. The aim is to improve sugar and ethanol yields through enhancing the digestibility of the cellulose chain, lowering the dilution of sugars, and minimizing sugar degradation products. Accordingly, the efficiency of different pretreatment experiments is assessed by running fermentability tests (SSF) on the pretreated slurry.

Poster 3-93

Bioconverting the nutrients in dairy manure for L-lactic acid production by *Rhizopus oryzae*

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In this study, dairy manure as a nitrogen source was evaluated for L-lactic acid fermentation by *Rhizopus oryzae*. $(NH_4)_2SO_4$ in culture media was replaced with the purified crude protein from dairy manure (appropriate nitrogen concentration was 0.42 g/L for *Rhizopus oryzae* based on $(NH_4)_2SO_4$). Six levels of nitrogen were used, 0.21 g/L, 0.42 g/L, 0.84 g/L, 1.68 g/L, 2.52 g/L, and 3.36 g/L and the results showed that the corresponding L-lactic acid yields were 6.48 g/L, 14.73 g/L, 38.33 g/L, 55.7 g/L, 54.9 g/L, and 54.1 g/L, while it was 57g/L for control. To improve the utilization rate, crude protein hydrolysis was also studied. Results showed that the greatest hydrolysis degree (DH, 48.9%) was achieved at 0.06 g Alcalase/g of protein, pH 8.0, 53°C for 240 min. Six levels of diluted hydrolysates (same as above) were experimented and 55.9 g/L L-lactic acid was obtained using the hydrolyzed protein at 0.42 g/L and ≥ DH 33.8%. A uniform design (U_6 (6^2×3)) was applied to optimize seed culture using three factors (nitrogen and spore concentration, treatment durations). It was observed that diameter of the seed pellet was 1.03 ± 0.12 mm after 20 hours incubation with optimal medium containing 1.68 g/L nitrogen and 1 ×10^6 spore/mL. A second uniform design (U_8(8^5)) with five factors (glucose, nitrogen in dairy manure, $ZnSO_4$, KH_2PO_4 and $MgSO_4$) was also experimented to optimize flask culture, showing lactic acid yield was 60.5% by weight, 8% higher than the control.

Poster 3-94

Integrated High Throughput Pretreatment and Enzymatic Hydrolysis in 96 Well Plates

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One of the aims of the BioEnergy Science Center is the engineering of advanced plants with reduced recalcitrance for sugar release. As this requires screening many different natural and genetically modified biomass types to identify those with lower recalcitrance for sugar release, a new high throughput (HTP) tool integrating pretreatment and enzymatic hydrolysis in the same multi-well configuration was developed. Water only or dilute acid pretreatment of 1% biomass slurries is performed in a custom-made 300µL 96 well plate made of metal to withstand heating to temperatures up to 180°C in a steam chamber, as well as to prevent corrosion. Furthermore, our so-called co-hydrolysis approach adds citric acid buffer, sodium azide, and enzyme directly to each well without separating the solid and liquid after pretreatment, with enzyme loadings based on original glucan and xylan content of the raw biomass. Next, the plate is incubated at 50°C for 72 hours, and the release of sugars is quantified by HPLC. To prove the feasibility of this concept, performance of co-hydrolysis was compared to that of conventional washed solids hydrolysis. The standard deviation in total sugar yields was only 4.1% across the 96 wells for combined pretreatment and co-hydrolysis, and yields for co-hydrolysis with the multi-well system were virtually identical to those with standard tube reactors as well as washed solids hydrolysis using standard vessels. Operational testing demonstrated that the custom-made well plates did not leak during pretreatment, and heat-up and cool-down required less than 45 seconds at an operating temperature of 180°C.

Poster 3-95

Effects of Mixing on the Enzymatic Hydrolysis and Simultaneous Saccharification and Fermentation of Pretreated Spruce

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Simultaneous saccharification and fermentation (SSF) has proved to be a promising option for ethanol production from lignocellulosic materials. Separation costs for recovery of ethanol in the fermentation is quite significant, and for this reason a high content of water insoluble solids (WIS) is needed in the SSF. Concentrated fiber suspensions are, however, characterized by both a high viscosity and a non-newtonian flow behavior, which may lead to poor mixing and/or high power consumptions in large-scale operation. It is therefore important both to rheologically characterize the material, and also to study the consequences of mixing in the SSF process.

In the current work, enzymatic hydrolysis (EH) and SSF experiments of pretreated spruce (10% WIS) were carried out in lab-scale reactors at different stirring speeds. As expected, the initial rate of hydrolysis increased with increasing stirring speed. This positive effect was more pronounced in the range of low stirring speeds (<100 rpm). Surprisingly, however, the initial ethanol productivity in the SSF experiments showed the opposite trend. A higher ethanol productivity was observed when the stirring speed was lowered. Possible explanations of these results were investigated by several sets of separate experiments in order to consider different individual effects of mixing in SSF.

Poster 3-96

Distiller's dried grains with solubles (DDGS): An alternative cellulose fermentation media for biofuels production

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Distiller's dried grains with solubles (DDGS) contain many growth nutrients at similar or higher levels compared with media components used in cellulose fermentation for biofuels (ethanol and hydrogen) and organic acids productions. This study investigates the potential to replace expensive and reagent grade growth media (1191) components used in lab scale cellulose fermentation process for ethanol, H2, and organic acids production with DDGS as the major component of the culture medium. Batch cultures of Clostridium thermocellum were cultivated on α-cellulose (2 g/l) using two different concentrations (2 and 5 g/l) of DDGS in sodium-potassium phosphate buffer solution. Cultures of C. thermocellum in 1191 medium with α-cellulose were cultivated simultaneously. Controls consisting of DDGS plus buffer only were used to determine the extent of α-cellulose utilization as substrate. Standard cultures with added DDGS (2 and 5 g/l) were also evaluated to determine the effect of DDGS on product synthesis. By 24 hours, ethanol production in cultures containing 5 g/l DDGS in buffer only plus α-cellulose was equivalent to that of cultures containing 1191 plus α-cellulose, but H2 accumulation in cultures containing 2 or 5 g/l DDGS in buffer only was 58% and 68%, respectively, of the H2 produced by cultures containing 1191 medium plus α-cellulose. DDGS has some buffering capacity, less severe drops in pH were observed in cultures containing DDGS. Although H2 production in cultures containing DDGS is reduced compared to cultures containing 1191 medium, DDGS has potential to replace expensive lab-grade media components with respect to ethanol production.

Poster 3-97

Biological pretreatments of corn stover with filamentous fungi

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Filamentous fungi have been used in the biotechnology industry for several years to produce enzymes. Fungi producing cellulose-degrading and lignin-degrading enzymes can be grown directly on lignocellulosic biomass in solid state fermentation to break lignin bonds and produce glucose from cellulose. The costs involved in enzyme extraction and application can be eliminated through this process. However, some dry matter loss will be observed due to aerobic conditions, which can aggravate further if the treatment is performed in unsterile conditions. To limit the dry matter loss, anaerobic conditions can be introduced through ensilage of biomass. Ensilage enables lactic acid bacteria to produce organic acids and lower the pH to 4.0. The low pH and anaerobic conditions minimize microbial activity, thereby decreasing dry matter loss. In the present research, laccase-producing *Pluerotus ostreatus* and cellulase-producing *Trichoderma reesei* were grown on corn stover prior to a 10-day ensilage treatment. Dry matter losses of 1.27% +/- 0.13 and 2.56% +/- 0.49 were observed after 7 and 14 days of fungal growth, respectively. The control samples which had no fungal inocula showed dry matter losses of 1.4% +/- 0.07 and 2.26% +/- 0.06 for the same time periods. The pH values of the samples ranged between 4.2 +/- 0.02 and 4.75 +/- 0.03 for 7 and 14 days. The combined fungal treatment resulted in significant sugar production and lignin degradation.

Poster 3-98

Selecting microbial production hosts for lignocellulosic feedstock utilization

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Increasingly, lignocellulosic biomass is used to make fermentation processes more cost-effective and, at the same time, more environmentally friendly. Currently, the choice for the production organisms is mostly based on prior experience working with a specific host and not on their suitability to utilize 'real life' feedstocks. Wild-type strains of six industrial relevant microbial production hosts (2 fungi, 2 yeasts and 2 bacteria) were evaluated for their performance on lignocellulosic feedstocks. The renewable feedstocks tested included corn stover, wheat straw, bagasse and willow. The biomass feedstocks were generated in two manners: (1) thermal treatment under mild acid conditions followed by enzymatic hydrolysis and (2) concentrated acid pretreatment and hydrolysis (the Biosulfurol process). Moreover, waste glycerol was included in the study. The six microorganisms were evaluated for 'real life' feedstock and carbon source utilization, resistance against feedstock-related inhibitors, and general fermentation and process characteristics on the 'real life' feedstocks. Large differences in the overall performance of the six tested microbial production host on feedstocks were observed. *Aspergillus niger* and *Pichia stipitis* were found to perform best. This study will be extended with 4 more pretreatment methods: dilute acid, alkali (lime), steam explosion and ammonia fibre explosion, thus creating a lignocellulosic biomass pretreatment platform.

Poster 3-99

Consolidated Bioprocessing (CBP) of AFEX-Pretreated Corn Stover using *Thermoanaerobacterium saccharolyticum* ALK2: A Study Case using AFEX-Pretreated Biomass as the Self-Sustained Nutrient Source for Fermentation

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Nutrient characteristic of a fermentation medium is an important aspect that determines the efficacy of fermentation. Ammonia, amino acids, vitamins and trace elements are the essential categories of nutrients which are generally required by fermenting strains at varying concentrations and these requirements might differ depending on the strain selection. Fermentation using lignocellulosic materials as carbon source offers a promising route for various bio-based chemicals production. However, the nutrient profile of the lignocellulosic hydrolysate remains unclear.

Therefore, we are analyzing important nutrient components present in hydrolysate from AFEX pretreated corn stover, both quantitatively and qualitatively; identifying limiting or excess nutrients to fundamentally understand the fermentability of AFEX hydrolysate from corn stover. Furthermore, we seek to address the issue regarding the source (feedstock, pretreatment and enzymatic hydrolysis) of the nutrients in hydrolysate. This development will enable us to (i) Evaluate the need for further nutrient reformulation of the hydrolysate and (ii) Rationally supplement (or remove) certain nutrients to achieve optimal fermentations for different configurations on bio-mediated processes i.e. Separate Hydrolysis and Fermentation/ Simultaneous Saccharification and Co-fermentation (SHF/SSCF) or Consolidated Bioprocessing (CBP).

More importantly, we seek to investigate the feasibility to ferment AFEX-treated feedstock using *Thermoanaerobacterium saccharolyticum* ALK2, a strain capable of CBP, solely based on nutrients provided through biomass and AFEX pretreatment. Through these evaluations, we intend to establish a foundation for effective lignocellulosic fermentation with little (or no) dependence on external nutrient sources.

Poster 3-100

Fractionation of Corn Stover using Aqueous Ammonia and Hot Water

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Two-stage fractionation of biomass using hot water and aqueous ammonia was previously developed to improve overall biomass utilization. One of the problems encountered in this process is that xylan stream recovered from the first stage is significantly contaminated by solubilized lignin (~40% removal), which complicates xylan recovery in the downstream processing. Advanced technologies are needed to significantly increase the purities of separated components to level that will support economically viable biomass processing.

The "three-stage fractionation process using hot-water and aqueous ammonia" was devised to solve this problem in our laboratory. The three-stage fractionation process consists of (1) low-molecular weight lignin separation using aqueous ammonia at low severity; (2) hemicellulose separation using hot-water at high severity; and (3) high-molecular weight lignin separation using aqueous ammonia at high severity.

In this method, the ammonia steeping (SAA: soaking in aqueous ammonia) at moderate temperature method is introduced to remove lower molecular lignin prior to hot-water hemicellulose extraction step. It removes lignin (50-70%) significantly in the first stage and remains most of hemicellulose (>80%) in the solids so that hot water treatment can produce uncontaminated hemicellulose hydrolysates from the remaining hemicellulose-rich solids in the second stage. The optimal process conditions that achieve highest degree of fractionation were explored. The enzymatic digestibility tests were performed for the cellulose fractionated from corn stover. Other technical aspects pertinent to development of a three-stage pretreatment process are presented.

Poster 3-101

A Comparison of Batch Tube and Microwave Reactors for Water-Only and Dilute Acid Pretreatment of Corn Stover

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Pretreatment is one of the most expensive steps in biological conversion of cellulosic biomass. Advanced pretreatment technologies are needed to significantly reduce costs, improve cellulose digestibility, simplify upstream and downstream operations, and provide revenues from co-products. The ability to target advances in this area has been slowed by limited understanding of pretreatment fundamentals and its effect on other processing operations, while more obvious cost reductions have already been realized. Better knowledge of pretreatment would also accelerate commercial applications by giving practitioners and financial organizations greater confidence in scale-up. Use of microwave heating could address a major challenge in pretreatment research of rapid and uniform heat up of biomass. Thus, we applied conventional batch tubes and microwave reactors to water-only and dilute acid pretreatment of corn stover to develop comparative data on sugar, lignin, and overall mass recovery profiles at identical conditions and found that overall sugar yields from microwave pretreatment were comparable to those from conventional sand bath systems. Solid state CP/MAS NMR and solution NMR were employed to elucidate biomass structural characteristics (i.e. cellulose and lignin) of pretreated corn stover, and the impact of pretreatment reactor choice on sugar recovery from enzymatic hydrolysis was determined. Microwave pretreatment demonstrated faster and more effective alteration of cellulose structural features compared to conventional sand batch pretreatment. This data was used to suggest new mechanisms that explain the different results for batch tube and microwave systems on a consistent basis to aid in applications and advances in pretreatment technology.

Poster 3-102

Sugar Yields from Switchgrass for Dilute Acid and Sulfur Dioxide Pretreatment and Subsequent Enzymatic Hydrolysis

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Switchgrass has been highlighted as a leading herbaceous energy crop to support large scale production of biofuels in the United States. As a part of phase III of a Biomass Refining Consortium for Applied Fundamentals and Innovation (CAFI) Project among leaders in biomass pretreatment, our research focused on optimizing total sugar yields from switchgrass by batch pretreatment with dilute sulfuric acid or sulfur dioxide followed by enzymatic hydrolysis of the pretreated solids. In this work, dilute sulfuric acid and SO_2 were applied at concentrations ranging from 0 to 5% over a temperature range of 140-220°C for times up to 60 minutes, and glucose and xylose yields were measured for the overall operations of pretreatment and subsequent enzymatic hydrolysis. Different combinations of cellulases and hemicellulases were also applied to identify the best pretreatment conditions and enzyme formulations that give the maximum total sugar yield with the lowest possible enzyme mass and cost. These results were directed toward understanding how dilute acid and SO_2 pretreatment impact total sugar yields from switchgrass and to gain new insights that facilitate identification of lower cost processes and facilitate commercialization of cellulosic ethanol technologies.

Poster 3-103

Progress toward Automating Biomass Compositional Analysis

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Improving the understanding of process chemistry will help to reduce financial risk and enable successful commercialization of biomass conversion technology. One approach to this is to improve the precision, accuracy, and speed of component concentration measurements. We describe a robotic workstation used to automate and increase the precision of such measurements. Automating the workflow will dramatically reduce the number of manual measurements required to analyze biomass samples. The robotic system will keep an electronic record of how each sample was processed in a database facilitating data tracking and mining. This report documents our progress toward developing operational procedures to perform automated compositional analysis of biomass feedstock. Opportunities for improving the accuracy of component concentration measurements are discussed.

Poster 3-104

The Effect of Water on Sugar Reactions from *ab initio* Calculations

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It is known that solvent and solvent effect play an important role in many catalytic reactions. Here the effect of water and water structure on acid catalyzed polysaccharide hydrolysis and monomer sugar conversion to furfural and HMF were investigated. *Ab initio* molecular dynamics and metadynamics simulations were used to investigate the energetics of several competing pathways encountered during dilute acid pretreatment. The multi-dimensional free energy surfaces (FES) obtained allows accurate determination of both the reaction free energies and barriers of these reaction processes. Water is found to play a critical role during proton catalyzed sugar reactions. Water and water structure affect both the reaction pathways, reaction free energies and barriers significantly. The thermodynamic equilibrium and the kinetic reaction rate constants were also determined and compared with available experimental data.

Poster 3-105

Recovery of Sugars from Ionic-Liquid Biomass Liquor by Solvent Extraction

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Pretreatment of cellulosic biomass is necessary for its efficient enzymatic conversion into fermentable sugars. Ionic liquid pretreatments have proven to significantly enhance the rate of hydrolysis of cellulose (L. Liu et al 2008), but require large amounts of antisolvent to recover the amorphous cellulose. Furthermore, some sugars may be lost to the antisolvent phase, particularly if water is used. One way to overcome this would be to extract any sugars directly from the ionic liquid prior to the addition of antisolvent. Solvent extraction technology, based on the chemical affinity of boronates to complex sugars, has been shown to successfully remove glucose and other sugars from aqueous solutions (M. Matsumoto 2004). This technique was tested for specific ionic liquids containing xylose, glucose, and cellobiose, using phenylboronic acid and aliquot 336 as an ion-pair extractant. Various pH levels (7-12) and temperatures (30-50°C) were also examined experimentally to determine optimal extraction conditions.

Poster 3-106

Structural Studies of Enzymatic Hydrolysis of Cellulose by Neutron Scattering and Reflectivity

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Improving the efficiency of enzymatic hydrolysis of cellulose is a key technological hurdle in reducing the cost of producing ethanol from lignocellulosic material. Typically, enzymatic hydrolysis proceeds to only a limited extent, high solution-to-solids ratios are required, and the rate of enzymatic hydrolysis typically decreases with time. A range of mechanisms have been proposed to explain these phenomena including product inhibition, denaturation of enzymes, nonproductive binding, and many others. We are studying the interaction of enzymes with cellulose to help unravel these mechanisms. Our studies include UV absorption and circular dichroism of enzymes in solution, small angle neutron and X-ray scattering (SANS, SAXS) of cellulose during hydrolysis, and neutron reflectivity (NR) of enzymes interacting with model cellulose surfaces. Insight from these studies should aid the development of more efficient enzyme systems and pretreatments.

Poster 3-107

SPORL Pretreatment for Cellulose Ethanol Production – An Update

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SPORL (Sulfite Pretreatment to Overcome Recalcitrance of Lignocellulose) is a newly developed pretreatment for cellulose ethanol production. Feedstocks react first with acidic sulfite solution, and then defiberized to fibrous substrate using a disk refiner. The removal of the recalcitrance to enzymatic hydrolysis is achieved by combined effects of dissolution of hemicelluloses, slight depolymerization of cellulose, partial delignification, sulfonation of lignin, and increased surface area by the defiberization. During SPORL pretreatment, most hemicellulose sugars and partial lignin (as lignosulfonate) were dissolved. The former can be further fermented to ethanol, and the later is a high-value co-product that can be directly sold. The resulting substrates are readily digestible. The SPORL is effective to all species of feedstocks, in particular woody biomass, and easy to commercialize by using existing infrastructure and capital equipment in paper industry.

In this report, we update the optimization and fundamental understanding of the SPORL technology. Pretreatment parameters (chemical charge, temperature, catalyst, reaction time) were optimized to maximize sugar recovery and enzymatic hydrolysis. Mass balance of the major components (cellulose, hemicellulose and lignin) during the pretreatment was conducted. Lignin behavior during the pretreatment was investigated using 2-D NMR techniques. Fermentability of hemicellulose sugars from the pretreatment was evaluated using an in vitro gas production method. In addition, comparison study between the SPORL and dilute acid pretreatments indicated that at the same acid charge and temperature, the former gives higher overall sugar recovery, produces more readily digestible substrate, and forms fewer inhibitors to fermentation than the later.

Poster 3-108

Withdrawn

Poster 3-109

Evaluation of Ensiling on Biomass Storage and Bioconversion of Corn Stover

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Ensiling feedstocks is one proposed method for storing biomass for use in biorefineries. Ensiling may reduce storage cost, minimize dry matter loss, inhibit feedstock combustion and increase feedstock processability. While ensiling has been used to increase the digestibility of forage materials for livestock, the potential effect on feedstock processability has not been fully determined. We compared the pretreatment and enzymatic hydrolysis performance of dried or ensiled corn stover with samples of the same material that had been dried or frozen immediately after harvest. These samples consisted of the frozen control material, plus dried and ensiled materials, with and without ensiling additives. Biomass was analyzed for feedstock composition and then pretreated using either dilute sulfuric acid or hot water. Liquors and solids were analyzed, and the solids were enzymatically hydrolyzed using a cellulase enzyme. We observed no significant differences in the combined yields resulting from pretreatment and enzymatic hydrolysis in xylose or glucose, between the frozen, dried or ensiled materials ($0.05<p$). The acid pretreated materials exhibited higher yields across all categories compared to hot-water pretreated material. There were no significant differences ($0.05<p$) between ensiled samples that received ensiling additives, compared to those that didn't receive ensiling additives. While ensiling did not appear to affect corn stover reactivity, we observed losses in structural sugar concentration in the ensiled materials. Glucan losses averaged 11% from the initial glucan while xylan loss averaged 9%. Further investigation is required to fully determine the effects and cost of ensiling on biomass storage and bioconversion.

Poster 3-110

Understanding the effects of reactor design on glucose and xylose recovery from pretreatment and enzymatic hydrolysis

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Pretreatment is a key step in the production of ethanol from cellulosic biomass and is essential to high sugar yields from enzymatic hydrolysis. High temperature water or dilute acid can be employed to primarily solubilize sugars and oligomers from hemicellulose during pretreatment, and cellulase enzymes can access the cellulose left in the pretreated solids and convert it to predominantly glucose. The sugars from both operations can then be fermented to ethanol or other products. Although many different reactor designs, sizes, and heating mechanisms have been applied for water only and dilute acid pretreatment of a number of cellulosic materials, little attention has been given to correlating the results from these different reactor scales and configurations or evaluating the effect of reactor design features on their performance. The goal of this study is to compare results for water only pretreatment of poplar wood in stirred and non-stirred batch reactors heated by either a fluidized sand bath or steam. In addition, the heat transfer performance of each device will be analyzed as a possible source of variations in performance. The resulting correlations will provide new insight into factors controlling pretreatment performance and enable scale up to larger reactors.

Poster 3-111

Investigation on effect and mechanism of different surfactant applied in pretreatment of wheat straw

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Surfactants including Tween, Polyethylene glycol (PEG), Pluronic, Span, Cetyltrimethylammonium bromide (CTAB) and biosurfactant have been applied into enzymatic hydrolysis of lignocellulosic materials, which not only improve hydrolytic efficiency but also lower enzyme loading amount. The major mechanisms being suggested for the enhancement of hydrolytic process are that surfactant can reduce the non-productive adsorption of cellulytic enzymes on lignin and act as enzyme stabilizer and effectors. However, there is little work being done on surfactant's application on pretreatment, in which surfactants may make contribution to improve efficiency of pretreatment and the economics of the entire process. In this study, we hypothesized that surfactant applied simultaneously in the alkaline pretreatment could enhance extracting the hydrolysate of lignin part into another phase and thus improve the efficiency of pretreatment. Results had shown that the effectiveness of surfactants added during pretreatment on the performance of enzymatic hydrolysis depend on pretreatment process, surfactant type and concentration. In the ammonia pretreatment, PEG addition gave rise to the best hydrolytic performance compared with the results with Tween or Pluronic addition, while in the lime pretreatment Pluronic addition improved more sugar release and higher conversion rate compared with other surfactant addition. After the optimization of processes by Response surface methodology (RSM), the performance of hydrolysis and fermentation and the respective mechanism under this condition were investigated and explained via SEM, pyrolysis GC-MS and FTIR experiments.

Poster 3-112

Pretreatment of lignocellulosic biomass using ionic liquids for production of ethanol

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Declining nonrenewable petroleum resources combined with political and environmental concerns over fossil fuels have necessitated the search for alternate energy sources. In this respect, plant (lignocellulosic) biomass is an abundant, inexpensive and sustainable source of organic carbon, and the production of ethanol as a renewable fuel from lignocellulosic biomass seems to be a promising transportation fuel. However, many technical challenges exist in developing commercially viable processes. Pretreatment and enzymatic hydrolysis are critical steps in the production of fuels from biomass and most of the current pretreatment methods do not effectively disrupt the biomass structure, especially the crystallinity of cellulose, which is a major barrier for efficient enzymatic hydrolysis. Ionic liquids being non volatile, non-flammable, recyclable and designer friendly, are gaining wide recognition as green solvents, and their unique solvating properties make them ideal for pretreating lignocellulosic substrates. Enhanced enzymatic hydrolysis yields of glucan to glucose and xylan to xylose are observed for ionic liquid pretreated ligocellulosic substrates like sugarcane bagasse, corn stover and poplar. However, economic viability materializes only when the process solvent is completely recovered and recycled. This work addresses the enhancement of enzymatic hydrolysis of lignocellulosic biomass using suitable ionic liquids and the recovery and recycle of ionic liquids by different approaches.

Poster 4-07

Starch to Oil: Engineering an Efficient Biofuel Currency

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The project aims to understand the interplay between oil and starch biosynthesis during endosperm development in oat seed with the goal of using the gained knowledge to modify starchy energy crops. Cereals and tuberous crops yield significantly more biomass than oilseed crops providing a unique opportunity to generate dual-use energy crops that can provide both a high-energy density biodiesel fraction while preserving the starch component that is an essential feedstock for the starch-based ethanol industry. Oat seed has evolved the unique ability to store the fixed carbons as a commingled fraction of oil and starch within developing endosperm. By using oat seed as a model system, the molecular switches of carbon allocation can be elucidated, thereby opening the possibility to redirect carbon flux from starch to oil in cereals and tubers through genetic engineering. Data discussed will detail carbon portioning between oil and starch at different developmental stages of endosperm, in conjunction with bioinformatics complexities and strategies concerning 454 pyrosequencing of oat endosperm transcripts. The efficacy of a novel method of gene silencing within oat endosperm will also be presented.

Poster 4-08

Random Shear BAC Cloning: An Optimized Genomic Tool for Improving Biomass and Bioenergy Feedstocks

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Bacterial artificial chromosome (BAC) libraries, BAC-based physical mapping, and genome sequencing are critical for understanding the molecular basis of phenotypic variation. This knowledge can be used to accelerate breeding and engineering of new plant varieties, exploiting natural genetic diversity to increase yield and improve resistance to biotic and abiotic stresses. BAC libraries built with conventional vectors and methods are inherently biased. Numerous gaps exist in all of the physical and sequencing maps of eukaryotic genomes, for example Arabidopsis and rice. We have developed techniques to construct unbiased, randomly-sheared BAC libraries with large inserts (>100 kb) as well as a unique transcription-free BAC vector. We have used random shear BAC libraries to close numerous gaps in conventional deep-coverage BAC libraries for many International Genome Projects, including Arabidopsis, rice, potato, tomato, soybean, barley, etc. Efficient whole genome sequencing of biomass/bioenergy plants from a single Random Shear BAC library has been also demonstrated recently (e.g., for oil palm and *Jatropha curcas*). Lucigen's Random Shear BAC service is publicly available (see www.lucigen.com) and provides a novel genomics tool to efficiently characterize and understand plant cell wall synthesis and deconstruction.

Poster 5-07

Limiting Factors of Enzymatic Hydrolysis of Lignocellulosic Biomass at High Solids Loadings

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Cost effective enzymatic hydrolysis is the key for development of economically viable biological processes for lignocellulosic biomass to ethanol conversion. Urgent attention is needed towards an in-depth understanding of the inhibitory mechanisms during enzymatic cellulose digestion especially at low enzyme usage and high solids loadings. This study summarizes our investigations on plausible inhibitory factors such as mixing, pretreatment hydrolysate, and intermediate sugar products that lower the enzymatic digestibility of the water-only and dilute acid pretreated hardwood at 5-100 mg/g glucan plus xylan in raw biomass enzyme loadings and 2-30% solids loadings. Inhibition of enzymatic hydrolysis by pretreatment hydrolysate was examined and washing strategies were evaluated in order to remove detrimental compounds from the preteated solids. A rotating drum with grinding media and roller bottle with baffles were tested at high solid loadings and compared with results from shaker flasks to evaluate the effect of mixing. Combinations of cellulases and beta-glucosidase, xylanase, and beta-xylosidase were employed to identify limiting factors, and enzyme formulations were investigated to maximize total sugar recovery with the lowest possible enzyme loadings. Furthermore, hydrolysis yields and yields of oligosaccharides (cellobiose, low and high DP xylooligomers) were measured over the time course of high solids enzymatic hydrolysis at low and high enzyme loadings. These results provide a better understanding of enzymatic hydrolysis of lignocellulosic biomass and new insights that will facilitate lower cost processes and commercialization of cellulosic ethanol technologies.

Poster 5-08

Cellulolytic enzyme production and response to pH and temperature by *Trichoderma reesei*

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Trichoderma reesei has long been considered to be the most efficient producer of cellulases and it is currently used for production of commercial cellulolytic enzymes. However, further improvements in enzyme production are necessary to reduce costs sufficiently to make second generation bioethanol production economically feasible. *T. reesei* is well characterised on a molecular level and the genome has been sequenced, but little has been done to transfer this knowledge to process relevant conditions. Therefore, a physiological characterisation of *T. reesei* was conducted, focusing on the enzyme profile and levels produced under different process conditions.

T. reesei RutC30 was studied in a series of 4L batch fermentations at different temperatures (from 23°C to 33°C) and different pH values (from 3.0 to 6.0) using Avicel PH-101 (25 g/L) as carbon source. It was observed that germination time and growth rate increased greatly with increasing temperature, and the highest μ_{max} of 0.25h^{-1} was reached at pH 4.5 and 33°C. In contrast, cellulolytic enzyme activity as well as total protein production was increased when the cultivation temperature was lowered. The maximum amount of filter paper activity units (FPU) was 7.36 FPU/ml reached after 180 h of cultivation at pH 4.5 and 23°C. Sporulation was observed in all cultivations at pH 6.0, which also foamed excessively. Analysis of the different fermentations by SDS-PAGE revealed that the same bands were seen for the enzymes secreted into the media, but that the concentrations of them (and thus the profile) were different when pH and temperature were changed.

Poster 5-09

Microfluidic Glycan Assays for Cellulosic Biomass

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High-throughput, rapid, and sensitive approaches for quantitation of carbohydrates are valuable for bioenergy research in the areas of feedstock development and biomass deconstruction. Conventional assays such as Azo-CMC and the DNS assay are high-throughput but don't provide information about the glycans. HPLC-based assays provide precise information about the oligosaccharide content but the technique is low throughput. We are developing microfluidic capillary electrophoresis (μCE) devices for rapid, precise, and high-throughput characterization of glycans for enzyme screening.

We have used μCE to characterize Thermotoga maritima endoglucanase for degradation of cellulosic materials. The enzyme assays were performed on soluble substrates (cellodextrins and CMC) as well as ionic-liquid (IL) pretreated avicel and switchgrass. In μCE, the hydrolyzed glycans are tagged with a charged fluorophore at reducing ends. This enables separation based on charge-to-mass ratio as well as high-sensitivity fluorescence detection. The run-time per sample is around 60 s (> 10X faster than HPLC) and the detection sensitivity is around 1 amol. The cellulase effectively hydrolyzes cellotetrose and higher oligosaccharides. The major hydrolysis products are cellobiose, cellotriose along with small amounts of glucose. Further, results demonstrate μCE is particularly suitable for quantitative analysis of IL pretreated samples since no signal interference, arising from ILs, was observed. Whereas, the HPLC/ELSD data shows a significant overlap between the ionic-liquid and glucose peaks. We are extending the μCE technique to measure enzyme kinetics of various glycosyl hydrolases as well as glycosyltransferases. In addition, we are developing a high-throughput μCE system that integrates seamlessly with the microtiter plate assays.

Poster 5-10

Discovering novel protein families for biomass deconstruction: A domain-based functional classification

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Biological processes have evolved to decompose recalcitrant biomass not only recruiting a variety of related proteins but also maintaining biomass-specific protein repertoire. Recent advances in computational genomics and proteomics have identified potential protein families deconstructing biomass for bioenergy production. The major obstacle to use them is a complication in predicting correct molecular function, which is often caused by domain multiplicity occurring in protein families. In order to overcome this barrier, we retrieved protein families related to biomass deconstruction from carbohydrate active enzyme database (CAZY) and parsed them into domains. We then constructed a protein domain network from which we deduced interactions and relationships among domains. Based on our analysis, we suggest new classification system for proteins related to biomass deconstruction and annotation procedure for their contextual molecular functions. In specific, our domain-based approach is advantageous for functional prediction of proteins because a protein domain behaves as a functional unit or module during evolutionary history. The algorithm is semi-automatic and we designated the system as protein domains for biomass deconstruction (PDBD) database. The new classification system assists to discover novel protein domains and thus provides a design principle for novel catalysts in biomass deconstruction process for bioenergy production.

Poster 5-11

Effect of the carbon source consumption rate on cellulase production by *Trichoderma reesei* in fed-batch cultures

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One of the most effective industrial micro-organism used for the production of the cellulolytic enzyme cocktail suitable for the hydrolysis of ligno-cellulosic biomass is the imperfect fungus *Trichoderma reesei*, mainly thanks to its high secretion capacity. One option to decrease the cellulase production cost is increasing the cellulase secretion productivity which is a function of cell density and specific production rate. From a process optimisation standpoint, maximizing this rate requires understanding the dynamics of growth, substrate consumption and enzyme production. The organism ability to grow and produce enzymes is affected by the nature of the carbon source used and by its specific consumption rate. The relationship between the carbon source and the regulation of the cellulase genes in *T. reesei* is already partially characterized but data on specific production rates and activity of the proteins at different physiological states of the cells are scarce. For this purpose, we have investigated the kinetics of growth and cellulase production, in carbon-limited cultures at various specific consumption rates. In the present study, *T. reesei* was cultivated in a bioreactor using a fed-batch mode in order to be in perfectly controlled cultivation conditions, similar to industrial ones. Carbon, nitrogen and redox degree balances were calculated all through the fermentation in order to determine the specific rates, yields, cellulolytic activities and the main parameters likely to affect the cellulase production rate.

Poster 5-12

Beta-glucosidase and its effect on lignocellulosic biomass hydrolysis

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For lignocellulosic biomass hydrolysis to be a cost effective process for producing fuel ethanol, the efficiency of the enzyme mix used in the saccharification step needs to be improved. At least three types of cellulase activities, working synergistically, are necessary for the efficient degradation of cellulose. These enzymes include exoglucanases, which proceed processively along the cellulose chain, releasing cellobiose; endoglucanases which cleave the cellulose polymer anywhere along the chain, creating new chain ends; and beta-glucosidases which convert cellobiose and other oligosaccharides to glucose. In addition to creating a fermentable product, beta-glucosidase also relieves product inhibition for the exo and endoglucanases from cellobiose. While it is well known that small amounts of beta-glucosidase are necessary for efficient cellulose hydrolysis, the type and amount of beta-glucosidase can have a profound effect on the extent of the overall hydrolysis. This talk will focus on the impact of various beta-glucosidases on the specific performance of *T. reesei* cellulase mixtures in the context of lignocellulosic biomass hydrolysis. Kinetic studies of several beta-glucosidases on small molecule substrates will also be discussed.

Poster 5-13

Improving cellulase compositions for lignocellulose hydrolysis

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Lignocellulosic biomass is a renewable feedstock that can be converted to fuels and chemicals through simple sugar intermediates. Despite recent advances in reducing the cost of cellulases for bioethanol production, additional work is required to further reduce the cost by increasing the efficiency of enzymes for converting lignocellulose to fermentable sugars. Earlier this year, Novozymes was awarded a USD 12.3 million contract from the U.S. Department of Energy (DOE). Novozymes will match the DOE funding, bringing the total investment of the research project to USD 25 million. Starting with an advanced biomass enzyme system and using dilute-acid pretreated corn stover (PCS) provided by NREL as a commercially relevant feedstock, Novozymes' objective is to develop a two-fold improved enzyme mixture. This presentation will describe a variety of approaches to reach the goal.

Poster 5-14

Cellulase production, enzymatic hydrolysis and ethanol production on steam-pretreated spruce using Trichoderma atroviride mutants

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The production cost of enzymes represents a significant part in the overall cost of the biomass-to-ethanol process. Therefore, development of more effective cellulase secreting microorganisms and improvement of the hydrolytic properties of the enzyme mixtures are of great importance. *Trichoderma reesei* has been chosen by many researchers and industrial companies to produce commercial cellulases, even though this species practically does not secrete β-glucosidase, which is a key enzyme for the complete hydrolysis of cellulose.

We developed new *Trichoderma atroviride* mutants, which produced high levels of cellulases and β-glucosidases on steam-pretreated spruce (SPS). Due to extracellular β-glucosidases, the *Trichoderma atroviride* supernatants hydrolyzed the SPS more efficiently than the *Trichoderma reesei* supernatants. On the other hand, when the whole fermentation broths were used, i.e. bound enzymes were also present, the hydrolytic capacity of *Trichoderma reesei* was significantly enhanced.
The in-house produced enzyme supernatants and whole fermentation broths were compared with commercial enzymes in the simultaneous saccharification and fermentation (SSF) process to produce ethanol from SPS. The *Trichoderma atroviride* enzyme preparations and the whole broth of *Trichoderma reesei* proved to be as efficient in the SSF as the commercial cellulase mixtures (ethanol yields of 60-75%), while low ethanol yields (< 40%) were obtained with the β-glucosidase deficient *Trichoderma reesei* supernatant.

We presume that the use of new enzymes produced on SPS by a *Trichoderma* strain with good extracellular β-glucosidase level will lead to more cost effective production of bioethanol from pretreated lignocelluloses.

Poster 5-15

Analysis of the white rot degradome by mass spectrometry identifies small molecules correlated with lignin degradation

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White rot fungi efficiently degrade lignin and provide an excellent starting point for deriving biologically inspired strategies for industrial-scale lignin degradation. The breakdown of lignin involves both enzymes and small molecule mediators and cofactors. In this study we explore how the set of small molecules secreted by P. Chrysosporium and released from degraded wood (the degradome) changes as a function of colonization time. P. Chrysosporium was cultivated on a substrate of poplar wafers, initially enriched with potato dextrose broth. The degradome was analyzed using LC-FTMS, following acetone extraction of the wafers. Wafers were harvested after zero, one, two, three and four weeks of culturing, and mass spectrometric profiles were compared to assess changes in the degradome as a function of colonization time. We report our progress in analysis of these profiles and identification of small molecules correlated with lignin degradation.

Poster 5-16

Cloning of cellulase and regulation factor genes in Penicillium decumbens and their expression profile analysis in glucose-repressed and cellulose-induced culture conditions

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Production cost of cellulases is critical for the effective cellulosic ethanol production processes. *Penicillium decumbens* 114 and its catabolite-repression-resistant mutant JU-A10, have been successfully used to produce cellulase preparations and cellulosic ethanol in industrial or pilot scale in China. Therefore, identifying the key cellulase components and elucidating the mechanisms of cellulase gene expression in this strain are very important.

Six cellulase genes (cbh1, cbh2, egl1, egl2, eg5 and bgl1), three regulation factor genes (creA and ace1, encoding repressor of cellulase and xylanase expression; xlnR, a transcriptional activator) and a swollenin gene in *P. decumbens* 114 were cloned by TAIL-PCR and degenerate PCR. Gene expression profiles of these cellulases and regulation factors from *P. decumbens* 114 in different culture conditions (glucose-repressed or cellulose-induced) were assayed. The gene expression level of EG1, EG2, EG5, CBHI and swollenin increased 70-fold, 84-fold, 179-fold, 20-fold and 19-fold respectively, in cellulose-induced culture in comparison with that of in glucose-repressed culture. Gene expression level of creA changed a little, showing creA might require some post-translational modification or interaction with some catabolite to act as a repressor. Gene expression level of acel is down to 7.2% in cellulose-induced culture comparing with that of in glucose-repressed culture, suggesting that ACE I regulates cellulase gene by its auto-regulation. Gene expression profile of catabolite-repression-resistant mutant *P. decumbent* JU-A10 is also under investigation, may find some key factors which influence the difference of gene expression regulation between the two strains.

Poster 5-17

The Potential of Agro-Industrial Residues for Production of Holocellulases from Filamentous Fungi

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Thirty fungal species, including *Aspergillus oryzae, Penicillium citrinum, Fusarium proliferatum* and *Paecilomyces lilacinum*, were isolated from agro-industrial residues. They were screened for their ability to produce holocellulase when cultured in liquid state media containing sugar cane bagasse or other agro-industrial residue as the carbon source. For convenience, cultivation conditions (other than temperature) and enzyme assays were the same for all fungi, i.e., no attempt at optimization of individual was made. The objective of this exercise was to identify fungi and holocellulase (cellulase, hemicellulase and pectinase) activities of academic and as well as of potential commercial application. The pattern of holocellulase induction was influenced by the type of agro-industrial residue present in the medium. They were detected in different incubation periods. Holocellulase activities were very active in extracts of A*spergillus oryzae*. Fractionation of the crude extracts on ultrafiltration, gel filtration and ion-exchange chromatography procedures showed enzyme multiplicity. Some enzyme preparations were more active and stable at determined pH and temperature ranges. While some of the results obtained fall into discernible and expected patterns, the overall picture is one of variety. Some fungi and growth substrates yielded promising results.

Poster 5-18

***Pichia pastoris* as host for the expression of lignocellulolytic enzymes: Expression of *Trichoderma reesei* cellobiohydrolase II (Cel6A) as a model case**

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Lignocellulose provides a globally available, renewable resource for energy and biofuel production. A sustainable exploitation of his natural resource relies on innovative technologies such as the application of robust enzymes for the hydrolysis of lignocellulose. Additionally, competitive industrial processes for bioethanol production require cheap and highly active enzymes to be available in large quantities.

Pichia pastoris is known as a suitable expression host for many different proteins. However, so far efficient production of lignocellulolytic enzymes in *P. pastoris* has only been shown in some cases and expression of such enzymes in *T. reesei* usually gives complex enzyme mixtures. Here we present *Pichia pastoris* as a suitable host for the heterologous expression of eukaryotic cellulases. Our effort to express a whole set of individual (hemi-)cellulolytic enzymes was initiated by the expression of active Cel6A from *T. reesei*. Using proprietary expression technology and a codon-optimized gene of *Tr*Cel6A, *Pichia* transformants were screened in 96-well format applying an adapted reducing sugar assay. Additionally, coexpression of protein disulfide isomerase resulted in a synergistic effect, leading to secretion of *Tr*Cel6A in high yield.

These results show that *P. pastoris*, in combination with our expression technology, can be used as high-level expression host for the production of functional *Tr*Cel6A. The enzyme is now available as a pure enzyme and in unlimited amounts, enabling detailed analysis of the specific properties and capabilities of this isolated enzyme.

Poster 5-19

Multi-mode Spectroscopic High Throughput Screening (HTS) of Phenols and Monolignols

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Phenolic structure is a common motif amongst monolignols. We developed multi-mode, high throughput screening (HTS)-compatible, UV-Vis and fluorescence assays for phenols and monolignols that are executed in optical "turn on" or "turn off" formats and operated under kinetic or endpoint modes. We used *p*-cresol as a model phenol and coniferyl alcohol as a prototype monolignol. We chose *Trametes versicolor* fungal laccase to oxidize *p*-cresol and coniferyl alcohol and thus expanded the spectroscopic tools for analyzing these molecules. Laccase activity toward *p*-cresol was monitored kinetically at pH 4.5 by absorption changes at 250, 274 or 297nm, and in endpoint mode by a bathochromic shift to 326nm. Laccase oxidation of *p*-cresol was also detected by product fluorescence at 425nm after excitation at 262 or 322nm. We optimized the kinetic parameters for *p*-cresol oxidation (pH optimum 4.5-5.1; 37°C; *Km* = 2.2mM) resulting in laccase limits of detection and quantization (LOD, LOQ) of 25 and 75pg/mL, respectively. The *p*-cresol LOD was 8 micromolar. We similarly characterized the spectroscopic properties of coniferyl alcohol. Three isosbestic wavelengths were identified at 240, 242 and 262nm with *S/B* of ~50 for 500 micromolar coniferyl alcohol, establishing assay sensitivity. Coniferyl alcohol excitation spectrum (270 – 335nm) overlapped with its absorption spectrum. Fluorescence emission was between 360 – 500nm with peak at 416nm yielding 1 micromolar detection sensitivity. Unlike *p*-cresol, laccase oxidation of coniferyl alcohol quenched the fluorescence. In conclusion, orthogonal interrogation and ratiometric analysis capabilities of our assay enable high specificity while minimizing interferences during compound library screenings.

Poster 5-20

The Effect of Xylooligomers on Enzymatic Hydrolysis of Cellulose and Pretreated Corn Stover

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High doses of expensive cellulase are required to obtain satisfactory yields from biological conversion of cellulosic biomass, and enzyme costs must be reduced if cellulosic ethanol is to become a reality. Typically, the rate of hydrolysis is fast initially but then slows down more rapidly than can be explained by just consumption of substrate. Thus, factors such as enzyme inhibition, loss of activity, a drop in substrate reactivity, or nonproductive binding of enzyme to lignin could be responsible for this loss of effectiveness. Although glucose and cellobiose are known to inhibit enzymatic hydrolysis, we recently reported that xylose, xylan, and xylooligomers also appear to dramatically decrease conversion rates and yields. In this study, addition of xylan and various xylooligomers dramatically reduced the rates and yields for Avicel hydrolysis at low enzyme loadings and had a greater effect than adding equal amounts of xylose derived from these materials or when added separately. In addition, the effectiveness of combinations of cellulase, beta-glucosidase, xylanase, and beta-xylosidase in relieving inhibition of hydrolysis for Avicel and pretreated corn stover was evaluated. Kinetic models were then developed based on this data to characterize the inhibition constants of the different inhibitors and clarify their relative importance in slowing conversion rates.

Poster 5-21

Fundamentals of Enzymatic Hydrolysis of Cellulose through a Restart Approach

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To better understand the mechanism of enzymatic hydrolysis of cellulose, we applied a restart protocol to explore how cellulose reactivity changed and the interaction of cellulose with major cellulase components over the course of enzymatic hydrolysis. This approach allowed us to accurately monitor the hydrolysis rate of partially converted cellulose over the time course of substrate digestion excluding the impact of cellulase changes. In this study, the effect of enzyme-substrate interactions on reaction rates was investigated using purified key cellulase components (i.e., CBHI, EGI, and/or EGII) from wild type *Trichoderma reesei*. The synergism of these key components was studied by comparing the interrupted enzymatic hydrolysis of pure cellulose using the restart protocol with uninterrupted hydrolysis by individual key cellulase components and their mixtures. For the first time, our results showed the dynamic profiles of adsorption, sugar release rates, and oligosaccharide (DP up to 7) yields from hydrolysis by individual enzyme components and their synergism over the hydrolysis time course during interrupted enzymatic hydrolysis of Avicel. A new model of enzymatic hydrolysis of cellulose regarding cellulose reactivity, oligomer distribution, effective enzyme binding capacity, and equilibration of depolymerization limiting factors will also be discussed.

Poster 5-22

Aspartic Protease from *Trichoderma reesei*: Crystal SStructure and Statistical Coupling Analysis

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Trichoderma reesei is an industrially important cellulolytic filamentous fungus, capable of secreting large amounts of several cellulose-degrading enzymes, which has been extensively studied with the aim to produce low-cost enzymes for the conversion of plant biomass materials into industrially useful bioproducts, such as sugars and bioethanol. Fungal aspartic proteases have been shown to participate in the processing of secreted enzymes and, as a rule, to act as regulatory enzymes. Here we present crystal structures of an aspartic protease from *Trichoderma reesei* (TrAsP) and its complex with a competitive inhibitor, pepstatin A, solved and refined at 1.85 Å resolution. The three-dimensional structure of TrAsP is folded in a predominantly β-sheet bilobal structure with the N-terminal and C-terminal domains of about the same size, that undergo a rigid body movement upon inhibitor binding, tightly enclosing the inhibitor. The structures of TrAsP were used as a template for performing statistical coupling analysis (SCA) of the aspartic protease family. This approach permitted, for the first time, identification of a network of structurally linked residues putatively mediating conformational changes relevant to the function of this family of enzymes. SCA reveals co-evolved continuous clusters of amino acid residues which extend from the active site into the hydrophobic cores of each of the two domains and also include amino acid residues from the flap regions, highlighting the importance of these parts of the protein for its enzymatic activity, extending our comprehension of the protease action and opening new possibilities for its modulation.

Poster 5-23

Directed evolution of a thermophilic beta-glucosidase for cellulosic bioethanol production

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Characteristics that would make enzymes more desirable for industrial applications can by improved using Directed Evolution. This process allows evolution of enzymes in the laboratory via iterative cycles of mutagenesis and screening for superior properties. We developed a directed evolution technique called Random Drift Mutagenesis (RNDM). Mutant populations are screened and all functional mutants are collected and put forward into the next round of mutagenesis and screening. The goal of this technique is to evolve enzymes by rapidly accumulating mutations and exploring a greater sequence space by providing minimal selection pressure and high throughput screening. Our target enzyme is a beta-glucosidase isolated from the thermophilic bacterium, Caldicellulosiruptor saccarolyticus that is not end-product inhibited and cleaves cellobiosde resulting from endoglucanase hydrolysis. Our screening method is Fluorescence Activated Cell Sorting (FACS). FACS is an attractive method for assaying mutant enzyme libraries because individual cells can be screened, sorted into distinct populations and collected very rapidly. It is possible to screen 10,000 mutants per second using the FACSAriaTM system. However, FACS screening poses several challenges, in particular, maintaining the link between genotype and phenotype because most enzyme substrates do not remain associated with the cells. We employed a technique called In Vivo compartmentalization (IVC) where whole cells can be encapsulated in cell-like structures along with the enzyme substrate. We present how we have used RNDM, in combination with IVC, to create and screen mutant beta-glucosidase libraries which then can be further improved by a gene shuffling technique such as Degenerate Oligonucelotide Gene Shuffling.

Poster 5-24

High Throughput Mass Spectrometry Based Enzymatic Assays for Biofuels Development

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Mass spectrometry's ability to efficiently generate intact biomolecular ions in the gas phase has led to a wide range of biological applications and is recently being applied for global metabolite profiling ('metabolomics') primarily though liquid chromatography coupled to electrospray mass spectrometry. However the complexity and relatively low throughput of this approach has limited application for high throughput enzymatic assays. To overcome this, we have developed the Nanostructure-Initiator Mass Spectrometry enzymatic (Nimzyme1) assay where enzyme substrates are immobilized on the mass spectrometry surface using fluorous phase interactions. This 'soft' immobilization allows efficient desorption/ionization while also allowing surface washing to reduce signal suppression from complex biological samples as a result of the preferential retention of the tagged products and reactants. We have also shown that Nimzyme can detect multiple and competing enzymatic activities and screen for optimal pH, temperature, and enzyme inhibition from crude cell lysates and a hot springs microbial community. This approach is being implemented at the DOE Joint BioEnergy Institute for high throughput functional characterization of both enzyme libraries and environmental samples. Specifically, we are constructing a complete set of glucose polysaccharides (cellobiose to cellihexose) for screening glucohydralase and glucotransferase activities and a p-coumaryl alcohol substrate for characterization of laccase activity. Together these assays will help to identify and optimize the conversion of lignocellulose into biofuels.

Poster 5-25

Second generation bioethanol from sugarcane bagasse: SSF operative conditions and flowsheeting implementation

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It is a general opinion that future expansion in bioethanol production has to be based not only on bioethanol from starch and sugar, but also from lignocellulosic materials, i.e. second-generation bioethanol. A promising option is to integrate sugarcane bagasse, a lignocellulosic and abundant feedstock, with sugarcane molasses. The main purpose of this study was to investigate the enzymatic process for ethanol production using sugarcane bagasse as raw material at different Simultaneous Saccharification and Fermentation (SSF) conditions and subsequently implement a plant flowsheet in order to study the energy efficiencies in the bioethanol production process. After steam explosion pretreatment with catalyst addition, the material was then simultaneously hydrolysed by a cellulase enzyme mixture and fermented by ordinary baker's yeast in batch mode. The screened SSF conditions regarded mainly the variation of the most important parameters such as nutrients, bagasse load (WIS), and molasses addition, in order to check if the SSF ethanol yield can be improved. Experimental data are used as input data for simulation of the integrated bagasse-to-ethanol process using ApenPlus. The plant flowsheeting model also comprises co-products from the bioethanol production, which still have useful heating value, such as pellet from the solid fraction (mainly lignin) and methane from the anaerobic digestion of the stillage stream. The simulations combined with a sensitivity analysis on the whole plant products (ethanol, pellet and methane) are used to determine the energy efficiency, which is starting data to evaluate the environmental performances in biofuels production. Results from this study will be presented.

Poster 5-26

Enhancement of Cellulase Production from Kraft Paper Mill Sludge by *Trichoderma Reesei* Rut C-30

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Paper mill sludge is a waste material generated from the pulping and paper making process. High-glucan/low-lignin content, well-dispersed structure and low cost make the Kraft paper mill sludge a promising feedstock for bioconversion into value-added products. However, it has high ash content which is harmful to cell growth and other cellular reactions. In this study, the sludges from Kraft paper mill was partially de-ashed and treated additionally with sulfuric acid and sodium hydroxide in sequence to further reduce the ash content. It was then used as the substrate for cellulase production and bioconversion to ethanol. The cellulase enzyme produced from de-ashed sludge exhibited cellulase activity as high as 8 FPU/mL. The cellulase was further characterized in terms of specific activity, and activities of three major components in cellulase and xylanase. It was also found that the particle size has a significant effect on cellulase production. It appears that increased surface area of the substrate enhances availability of the solid substrate and oxygen mass transfer, consequently the cell growth as well as cellulase production. The efficiency of the cellulase enzyme was further evaluated by enzymatic hydrolysis and simultaneous saccharification and fermentation (SSF) using untreated primary sludge as the feedstock. Ethanol yield of 71% of theoretical maximum and 2.8% (w/v) ethanol concentration were achieved in straight batch SSF experiment using *Sacharomyces cerevisiae*. The ethanol concentration was increased to 6.0% (w/v) when the SSF was operated in fed-batch mode. The results are comparable to those of the SSF using commercial cellulases.

Poster 5-27

Production of biodiesel via enzymatic ethanolysis of sunflower and soybean oils: Modeling

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Biodiesel has become attractive due to its environmental benefits compared to conventional diesel. Although the enzymatic synthesis of biodiesel requires low thermal energy, low conversions of enzymatic transesterification with ethanol (ethanolysis) of oils to produce biodiesel are reported as a result of deactivation of the enzyme depending on the reaction conditions. The synthesis of biodiesel via enzymatic ethanolysis of sunflower and soybean oils was investigated. Kinetic parameters for the overall reactions were fitted to experimental data available in the literature with the Ping Pong Bi-Bi mechanism including the inhibition effect of the ethanol on the activity of lipase Novozyme® 435. The model was applied to a batch reactor and the concentration profile of the reactants and products were determined. The modeling of a semi-batch reactor with continuous feeding of ethanol was also performed and the results showed a reduction of roughly three hours in the reaction time compared with the batch wise operation.

Poster 5-28

A cellulase related dehydrogenase of *Hypocrea jecorina* (*Trichoderma reesei*) specifically responsive to soluble inducing compounds

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Hypocrea jecorina is nowadays the most important industrial producer of cellulase and hemicellulase enzymes, which are used for pretreatment of cellulosic biomass for biofuel production. This fungus expresses high levels of cellulases on cellulose, lactose and upon induction by sophorose. However, the mechanisms regulating cellulase gene expression on these carbon sources are different. In a study aimed at elucidation of the distinct signaling pathways, we identified a putative short chain dehydrogenase to be expressed upon induction by sophorose and on lactose, but not during growth on cellulose. *ccd1* (cellulase correlated dehydrogenase 1) thereby specifically responds to inducing conditions with a larger transcript, while on non inducing carbon sources such as glucose or glycerol only a small transcript was observed. This smaller transcript is also present in mutants, which do not express cellulases under conditions leading to cellulase transcription in the wild-type. We therefore conclude that expression of CCD1 is inducer-dependent or may alternatively be involved in inducer formation. Analysis of growth patterns of a *ccd1* deletion strain on 96 carbon souces (BIOLOG) reveals several changes in carbon source utilization.

Poster 5-29

Mechanistic Study of Enzymatic Cellulolysis Inhibitions

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Enzymatic conversion of cellulosic biomass materials to useful chemicals is prone to various interferences from non-cellulosic substances derived from biomass feedstock or upstream treatments. Understanding these cellulolysis-inhibitory or cellulase-inactivating reactions is of importance for the development of economically viable biorefinery technology. In this study, we investigated the mechanism under which oligomeric phenolics might inhibit cellulolysis. It was found that tannic acid, a representative oligomeric phenolics mimicking solubilized lignin, inactivated cellulases by reversibly complexing them. Individual cellulases showed different susceptibilities toward these inhibitions. Polyethylene glycol and tannase could bind and degrade tannic acid, respectively, and by doing so mitigate tannic acid's inhibition on cellulolysis. We also investigated the mechanism under which redox-active metal ions might inhibit cellulolysis. A correlation between oxidation potential and inhibition efficacy indicated the oxidative nature of the inhibition. Strong iron ion chelators and polyethylene glycols might be used for effectively mitigation of the inhibition. Potential implication of the observed effect from the inhibitors and their prevention/mitigation in biomass research was discussed.

Poster 5-30

The effect of substrate properties on enzyme adsorption during hydrolysis of ethanol organosolv pretreated hardwoods and softwoods: Potential for enzyme recycling

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In times of energy and oil crisis new ways of producing liquid biofuels are evaluated. Bioethanol from lignocellulosics is one of those biofuels that is predicted to be used as a renewable transportation energy source. The major steps for bioconversion of lignocellulosics into bioethanol are pretreatment, enzymatic hydrolysis and fermentation. The enzymatic hydrolysis is a crucial but still costly step. To decrease the direct costs of this process, means to reuse the robust enzymes in subsequent hydrolysis reactions are under investigation. Strong adsorption onto the pretreated substrate for hydrolysis and then release back to the liquid phase is highly advantageous in many recycling processes. Hybrid poplar (*Populus nigra x P. maximowiczii*) and mountain pine beetle (*Dendroctonus ponderosae*) killed Lodgepole pine (*Pinus contorta*) chips were pretreated at various conditions using an ethanol organosolv process. This library of pretreated substrates were used to determine the influence substrate characteristics have for the ability of a commercial enzyme preparation to adsorb to the substrate and efficiently hydrolyze it without showing non-productive adsorption to the residual substrate. The lignocellulosic substrates produced exhibited different monomeric sugar compositions and also different properties. Substrate properties such as hydrophobicity, pore size, sugar and lignin ratio were correlated against the enzymatic adsorption and hydrolysis, unraveling the most crucial properties responsible for the degree of enzymatic hydrolysis and adsorption. The work presented here will describe chosen methodology used to evaluate those substrate factors. The properties which are important for efficient adsorption, hydrolysis and minimal non-productive adsorption will be presented and highlighted.

Poster 5-31

JBEI Computational Biology Core

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Background: The Computational Biology Core group in the Technology Division of the Joint BioEnergy Institute (JBEI) is responsible for data integration and comparative, evolutionary, and functional genomic analysis for the purpose of enabling metabolic engineering for biofuel production. Leveraging the VIMSS MicrobesOnline website and database (http://www.microbesonline.org) for comparative and evolutionary genomics and analysis of microarray, proteomic, and metabolomic data sets, we are extending and integrating these capabilities to allow for pursuit of questions specific to biofuels challenges.

The MicrobesOnline Database: We have extended the MicrobesOnline database to include eukaryotic microbes that may be useful for understanding the process of biologically-mediated degradation of plant cell walls. Building a more complete picture of the enzymes nature employs for breaking down plant biomass is essential for developing industrial processes for biofuel production, and as such we are developing computational tools for metagenomics to permit searching for and comparative analysis of such enzymes in natural microbial communities. We are also working to combine computational structural biology with evolutionary analysis to grasp the mechanistic details of such "deconstruction" enzymes. This will permit prediction and engineering of novel enzymes with enhanced activities and custom specificities with an eye toward building a library of parts for metabolic engineering. Our efforts also include the study of the regulation of the expression and activity of lignocellulose deconstruction enzymes. Finally, we are extending the visualization and analysis tools in MicrobesOnline to provide a pathway-based view of systems to permit integrated analysis of systems biology data to facilitate metabolic engineering.

Poster 5-32

Cellulase Enzymatic Complex Production in Solid State Fermentation Using Tropical Amazon Agro Industrial Wastes

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Enzymes production via fermentation process on solid substrates, using filamentous fungi, has an important application in ecology, nutrition and industrial microbiology. The application of this technique to bioconversion and valorization of agro industrial wastes is the best process. In the Amazon region, mainly in the north of Brazil, the economy is based on agro business. A great amount of wastes is therefore produced but they could be commercialized with a higher aggregated value. This work has the objective of applying a solid state fermentation process to regional agro industrial wastes in order to obtain cellulase enzyme. *Trichoderma harzianum* strains, obtained at FIOCRUZ – Rio de Janeiro, Brazil, were tested. The results obtained with these fungi strains permitted the selection of regional fruit waste, taperebá (*Spondias mombin* L) as the solid substrate. Taperebá is a typical Amazon fruit with high commercialized value. After the selection of one fungi strain and the solid media, the most important process variables were studied using a factorial design experiment, 2^{4-1} with IV resolution, which allowed us to conclude that the celullolytic activity at this substrate were influenced by both the initial substrate temperature and humidity during the beginning of the fermentation process. Finally, it was observed that after 4 days of fermentation, the enzyme begins to loose its activity and furthermore the mathematical model could not describe satisfactorily the experiments after this period for a 90 % confidence interval

Poster 5-33

Immobilization and stabilization of xylanase by multipoint covalent attachment on glyoxyl agarose support

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Xylanases have important applications in industry, such as the bioconversion of lignocellulosic biomass to xylose and other fermentable sugars for the production of ethanol and xylitol. Immobilization and stabilization of enzymes may allow their reuse in many cycles of the reaction, decreasing the hydrolysis process costs. This work proposes the use of a rational approach to obtain immobilized commercial xylanase biocatalysts with optimized features. Xylanases NS50014 from Novozymes was characterized and immobilized on glyoxyl-agarose support. The activation of agarose was performed by etherification with glycidol, followed by oxidation with sodium periodate, to produce linear aldehyde groups in the support. When immobilizing xylanases (25°C, pH 10.05) on this activated support, only 5% of yield immobilization was reached after 24 hours and no significant stabilization of the immobilized enzyme was observed. The immobilization reaction occurs between aldehyde groups of the support and amine groups of the protein. Therefore, the low concentration of lysine groups in the enzyme molecule, measured by acid hydrolysis followed by amino acid analysis, could explain these poor results. In order to increase the concentration of amine groups on the enzyme surface, the protein was chemically modified with ethylenediamine (EDA). The modified enzyme was then immobilized on glyoxyl-agarose. The new enzyme derivatives were 20-fold more stable than the soluble, aminated and dialyzed enzyme (70°C, pH 7), with 100% of immobilization yield. Therefore, the increase of the number of amine groups in the enzyme surface was confirmed to be a good strategy to improve the properties of immobilized xylanases.

Poster 5-34

Effect of ammonia pretreatment on switchgrass for production of cellulase using *Trichoderma reesei* Rut C-30

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Switchgrass was milled to 3 different particle sizes i.e. 0.5-1mm, 1-2mm and 2-10mm and pretreated with 15% ammonia hydroxide compared with non-pretreated switchgrass. Shaker flask studies were conducted using *Trichoderma reesei* Rut C-30 for production of cellulases for the selection of particle size. Scaleup tests were then tested using 2%, 5% and 10% switchgrass loading in a 5 liter fermenter. Corresponding mass transfer coefficient (K_La) were determined and enzyme activity were analyzed using HPLC and DNS methods

Poster 5-35

Conversion of *Thermobifida fusca* free exoglucanases into cellulosomal components: Comparative impact on cellulose-degrading activity

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Cellulosomes are multi-enzyme complexes produced by certain anaerobic bacteria that exhibit efficient degradation of plant cell wall polysaccharides. To understand their enhanced levels of hydrolysis, we are investigating the effects of converting a free-cellulase system into a cellulosomal one. To achieve this end, we are replacing the cellulose-binding module of the native cellulases, produced by the *aerobic* bacterium *Thermobifida fusca,* with a cellulosome-derived dockerin module of established specificity, to allow their incorporation into defined "designer cellulosomes". In this communication, we have attached divergent dockerins to the two exoglucanases produced by *T. fusca* exoglucanase, Cel6B and Cel48A. The resultant fusion proteins were shown to bind efficiently and specifically to their matching cohesins, and their activities on several different cellulose substrates were compared. The lack of a cellulose-binding module in Cel6B had a deleterious effect on its activity on crystalline substrates. In contrast, the dockerin-bearing family-48 exoglucanase showed increased levels of hydrolytic activity on carboxymethyl cellulose and on both crystalline substrates tested, compared to the wild-type enzyme. The marked difference in the response of the two exoglucanases to incorporation into a cellulosome, suggests that the family-48 cellulase is more appropriate than the family-6 enzyme as a designer cellulosome component.

Poster 5-36

Biodiesel Synthesis from Babassu Oil Catalysed by Immobilized Lipases on Poly-(Hydroxybutyrate)

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Transesterification of vegetable oils to obtain biodiesel consists in replacing the glycerol of triglycerides by a short chain alcohol in the presence of a catalyst that can be basic, acidic or enzymatic. Recent studies show that biodiesel produced by lipases are more attractive, since the glycerol can be easily recovered and the biodiesel purification process is simpler than in the chemical route. Six lipase preparations from *Thermomyces lanuginosa* (TLL), *Candida antarctica* (CALB), *Bacillus thermocatenulatus* (BTL2), porcine pancreas (PPL), Lipex® 100L and *Pseudomonas fluorescens* (PFL) were immobilized by physical adsorption on granular and powder poly-hydroxybutyrate (PHB) for biodiesel synthesis through transesterification of babassu oil with ethanol. Transesterification reactions were carried out in solvent-free medium at fixed molar ratio babassu oil to ethanol (1:9), employing 10% w/w of biocatalysts in relation to the total weight of reactants under agitation, at 45ºC. PPL and BTL2 led to the lowest conversions of babassu oil into FAEE. The reactions catalyzed by most of the lipases immobilized on granular PHB reached the highest transesterification yields at 24 hours, while most of lipases immobilized on powder PHB reached maximum transesterification yields at 48 hours. Using powder support, the highest reaction yields were 78.5, 75.1 and 70.3% for Lipex® 100L, CALB and TLL derivatives, respectively. The maximum conversion into FAEE (97%) was reached with Lipex® 100L immobilized on powder support at 120 hours of reaction.

Poster 5-37

Isolation and Biochemical Characterization of Novel Esterases for Transesterification of Hemicellulose and Production of Value-added Fiber

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High-value bioproducts from biomass is important to off-set the cost of second-generation biofuels. For instance, biopolymers with increased surface hydrophobicity or reactivity could increase their performance in plastic-displacing materials and biocomposites. However, the extensive representation of hydroxyl functional groups on biomass-derived polysaccharides complicates reproducible alteration of these polymers using chemical catalysts. By contrast, microbial enzymes have evolved to specifically target precise positions on these polysaccharides and catalyze stereospecific reactions. Over 170 putative lipase and esterase encoding genes from more than 15 microorganisms were expressed in Escherichia coli. Functionally expressed enzymes will be evaluated for ability to catalyze the transesterification of hemicellulose and phenolic or aliphatic compounds. Here, biochemical data for two novel esterases are reported. SAV_Est1 and RP_Est1 from Streptomyces avermitilis and Rhodopseudomonas palustris, respectively, were cloned from the corresponding genomes, and purified using affinity chromatography. While RP_Est1 hydrolyzed substrates ranging from p-nitrophenyl (pNP)-acetate to pNP-palmitate and pNP benzoate, SAV_Est1 hydrolyzed pNP-acetate and pNP-benzoate. RP_Est1 also hydrolyzed olive oil. These date suggest that RP_Est1 is a lipase, whereas SAV_Est1 is an aryl esterase. The optimum pH for SAVest1 and RPest1 was 8 and 7, respectively. SAVest1 was stable from pH 7 to pH 10 whereas RPest1 is stable from pH 4 to pH 9. The half life of SAVest1 and RPest1 at 50°C is 2 h and 5 h, respectively. Both enzymes exhibited stability in the present of detergents, organic solvents, and ionic liquids. These data will be used to develop optimal reaction conditions for transesterification of biomass-derived hemicellulose.

Poster 5-38

Biochemical Characterization and Performance Testing of Family 48 Exocellulases

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Clostridium thermocellum displays the ability to produce plant cell wall degrading enzymes and ferment the resulting sugars to ethanol and other products. *C. thermocellum* utilizes cellulosomes to degrade biomass and genomic studies have confirmed that this organism produces no exocellulases from glycosyl hyrolase families 6 or 7. The only enzymes confirmed to be exoglucanses in this system are from GH family 48, although several members of GH family 9, thought to be processive endoglucanases, probably assist the GH48 enzymes in hydrolyzing crystalline cellulose. Therefore, the deconstruction of cellulose depends heavily upon the action of the GH48 enzymes, which are produced by *C. thermocellum* in both a cellulosomal (GH48; contains a dockerin domain) and non cellulosomal (CelY; contains a CBD domain) configuration. We are building a library of GH48 enzymes from a diversity of sources for biochemical characterization and activity testing. We will report results from initial studies of available enzymes using both wild type and chimeric constructs. Understanding the mechanism of action of these enzymes is critical to the challenge of improving the wild type performance of this microbial cellulose degrader.

Poster 5-39

Characterization of cellulases and hemicellulases produced by *Thermoascus aurantiacus* in hydrolysis experiments

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Cellulase and hemicellulase enzymes were produced by *Thermoascus aurantiacus* on sugar cane bagasse, sugar cane straw, wheat straw and corn stover. The enzymes produced were characterized by protein and various enzyme activity measurements. On sugar cane straw and sugar cane bagasse higher cellulolytic enzyme activities were reached, while the activities obtained on wheat straw and corn stover were considerably lower. Elevated levels of endoglucanase and b-glucosidase activities were produced simultaneously and both enzymes exhibited significant thermostability, with half-lives of 42 and 18 min, respectively, at 80 C. The produced and two commercial cellulases (Celluclast 1.5 L and novozymes) were also characterized in hydrolysis experiments. They were dosed on the basis of filter paper activity in order to ensure constant enzyme/substrate ratio in all experiments. Sugarcane bagasse was treated with hydrogen peroxide in an alkaline media in different conditions: temperature (20 and 60°C), hydrogen peroxide concentration (2 and 6 % w/v), reaction time (4 and 16 h) and magnesium sulfate concentration (0 and 0.5 % w/w). The results showed that at the best condition it was recovered more than 96 % of the original polysaccharide and also separated more than 88% of lignin. On bagasse pulp obtained from alkaline peroxide process composed of 5.9% lignin, 61% cellulose and 16% hemicellulose, the performance of commercial enzyme was 63%. As a result, our experiments demonstrated that sugarcane bagasse is a good substrate both for enzyme production and hydrolysis, since high cellulolytic activities could be reached using it as carbon source.

Poster 5-40

A Study of Cellulases Production by Filamentous Fungi Strains using Experimental Design

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Ethanol is recognized as one of the most important renewable fuels due to the economic and environmental benefits of its use. Agro-industrial residues and forests are the most promising raw materials to be converted in ethanol. This transformation is mediated by an enzymatic complex entitled cellulases. There are theories suggesting that three cellulases act synergistically hydrolyzing the cellulose of pretreated lignocellulosic materials into glucose that is used for ethanol production. Embrapa Food Technology team is studying the screening of filamentous fungi strains isolated from different biomes with the aim of identify the best cellulase producers. Two filamentous fungi: 3T5B8, a mutant strain and PIP, a wild strain, were selected. In this work, the effects of process variables on cellulose production by two strains have been studied in aerated column by experimental design. The fractional factorial method using the Plackett-Burman design was studied investigating six variables: temperature, aeration, humidity and the concentration of cellobiose, nitrogen and inoculum. Fifteen grams of sterilized medium using wheat brain and the composition designed by the method were inoculated in each aerated column. After 48 h of fermentation, each column was taken for enzyme extraction with citrate buffer pH 4.8. The best results in enzymatic activity were obtained for PIP strain: Carboxymethylcellulase (326 U/gds), FPase (8 U/gds) and beta-glucosidase (409 U/gds). A new experiment has been carried out with a third selected strain of filamentous fungus. The study of the significance of the available variables in the cellulases production will still be concluded.

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Poster 5-41

Enzyme Characterization for Hydrolysis of Lignocellulosic Biomass and their Conversion to Ethanol

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Genencor, a Danisco Division, is committed to supplying cellulosic ethanol projects with solutions through its merchant enzyme business. Genencor launched the first commercially available cellulase product, Accellerase® 1000, specifically developed for lignocellulosic biomass hydrolysis in October 2007. Accellerase® 1000 is an unclarified, minimally formulated product designed to facilitate the commercialization of lignocellulosic biomass conversion.

This poster will present recent lignocellulosic biomass hydrolysis and simultaneous saccharification and fermentation (SSF) results. New enzyme activities that constitute potential candidate for incorporation into future generation of Accellerase® will be identified. Effects of enzyme dosage and biomass solids loading on enzyme performance on different biomass substrates will be discussed.

Poster 5-42

Pipeline for Novel Biomass Degrading Enzymes

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Having the correct enzymes for biomass degradation, at a low enough price to be affordable, is a major goal of biofuels research. Currently, the biomass-degrading enzyme products that are commercially available are too expensive for practical use in the production of biofuels. The discovery of new high specific activity biomass active enzymes for evaluation in degradation studies is the focus of this research. An improved pipeline for enzyme discovery specific to the problems unique to this field was developed and validated, and a number of new carbohydrases were produced. Endo- and exo-cellulases and hemicellulases were discovered with high specific activity and broad specificity. We have over expressed, purified and characterized a number of unique cellulytic enzymes and will present data on representative examples. The next step is to develop a minimal set of biomass active enzymes that eliminates the bottleneck in cellulose degradation, in conjunction with research scientists at the Great Lakes Bioenergy Research Center.

Poster 5-43

Coarse-grained modeling of cellulose 1β identifies processive movement of family 1 carbohydrate-binding modules on a broken-chain surface

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A coarse-grained model for the simulation of cellulose 1β was derived, in which each β-D-glucose unit is represented by three beads. The bonded interactions are derived from atomistic simulation of crystalline cellulose 1β in water, whereas the non-bonded interactions are based on modification of an existing coarse-grained model for malto-oligosaccharides to incorporate directionality into this model. When used to study the interaction of the family 1 carbohydrate-binding module (CBM1) with this cellulose surface model, the CBM "opens" as in earlier atomistic simulations. Furthermore, this cellulose 1β model produces simulations in which the CBM translates along a broken cellodextrin chain. This processive motion of CBH I has long been suggested by experimental studies, but has never before been observed in computer simulations.

Poster 5-44

Comparison of the hydrolytic activity of different cellulase to develop simultaneous saccharification and fermentation process of pretreated rice straw

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Rice straw is the most abundantly agricultural waste in Asia and the potential amounts are estimated to be 670 million tons one year. To date, most of study in the development of cellulase was concentrated on the saccharification of pretreated corn stover; less research was paid attention to understand the influence of cellulase composition on enzymatic hydrolysis of pretreated rice straw. This study was aimed to develop a simultaneous saccharification and fermentation process for pretreated rice straw by means of specifying the saccharification ability of different industrial cellulase. The pretreated rice straw was prepared by dilute sulfuric acid pretreatment with a pressurized reactor. The results of saccharification were indicated β-glucosidase activity determined with salicin as the substrate was proposed to be above 50U/ml under 15FPU/g cellulose of enzymatic loading. This is useful to avoid the accumulation of cellobiose during hydrolytic reaction. The level in activity of exoglucanase was more significant than that of endoglucanase for enzymatic hydrolysis of pretreated rice straw. The enhancement in the sugar yield was obvious when the exoglucanase activity of cellulase was more. The maximal glucose yield from enzymatic hydrolysis at 50°C was achieved to be 80-85%. Moreover, the highest ethanol yield from SSF process at 38°C was found to be about 75%, where conversion from saccharification and fermentation was nearly 80% and 94%, respectively. Consequently, the ethanol conversion from SSF process of pretreated rice straw was suggested to be under industrial interest and then shown potential for further scale-up experiments.

Poster 5-45

Investigating the expression, secretion and enzymatic activities of the cellulolytic machinery of the filamentous ascomycete fungus, *Neurospora crassa*

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The model filamentous ascomycete fungus, *Neurospora crassa*, can be easily isolated in nature from the stems of tropical grasses and has the capability to degrade both lignin and cellulose. Our lab has used transcriptional profiling combined with proteomics research to characterize proteins/enzymes involved in plant cell wall degradation. To further understand how fungal enzymes attack the substrate and how they work synergistically to degrade the components of Miscanthus cell walls, we generated two versions of vectors to epitope-tag candidate genes that encode proteins identified by mass spec analysis of secreted *N. crassa* proteins. These constructs will be used for investigating the expression, secretion, and enzymatic activities of cellulases induced by cellulose using the native promoter and constructs under the regulation of a promoter expressed in minimal medium in *N. crassa*. Biochemical subtraction experiments will be used to evaluate changes in biochemical activity of depleted enzyme mixtures to identify known protein function and perhaps unknown proteins that have crucial roles in cellulose and plant cell wall degradation.

Poster 5-46

Cellulase enzymes production from rice straw pretreated without sulfuric acid by *Acremonium cellulolyticus*

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Lignocellulosic biomass is an abundant renewable resource that can serve as a substrate for the production of alternative fuels, such as ethanol. Recently, rice straw which can be a potential source for ethanol production has been attracted a lot of interest in Asian countries. The digestibility of lignocellulosic biomass by enzymatic saccharification and the cost of cellulase enzymes are important factors for ethanol production. We have been studied the wet disk-milling pretreatment without sulfuric acid, and proposed that as an economical pretreatment for enzymatic hydrolysis of rice straw. In this study, we investigated the cellulase enzymes production using the pretreated rice straw as substrates to produce cellulase enzymes at a lower cost. The rice straw cut out to less than 3 mm by the cutter mill was pretreated by the wet disk milling, the ball milling and the hot compressed water treatment. These pretreated rice straw and Solka floc (80% crystalline cellulose) were used as carbon sources for cellulase enzymes production by the fungus *Acremonium cellulolyticus*. The enzyme activities in the supernatant of these cultures as crude enzyme preparations were measured. The FPase activities in ball milled and disk milled samples were as much high as that in Solka floc. On the other hand, the xylanase and xylosidase activities in ball milled and disk milled samples were higher than them in Solka floc. This work was supported by the Resional Biomass Energy Project, Ministry of Agriculture, Forestry and Fisheries, Japan.

Poster 5-47

The potential of ethanol production from the organic fraction of municipal solid wastes

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The organic fraction of municipal solid wastes (MSW), which represents about 44% of garbage, is an easily recovered fraction that is an especially appealing feedstock for ethanol, because cellulosic materials comprise about 60% of the dry weight of a typical MSW stream. In 2007, Spanish households generated 24.000 tons of MSW of which a significant fraction was landfilled. The fact that the organic fraction of MSW has a relatively high carbohydrate content and the need to find a solution to its disposal problem has encouraged the research about its utilization for the production of fuel-ethanol. Further, unlike other cellulosic feedstocks, MSW has an already well-established collection system, and is available at a negative cost.

A number of process to convert MSW into biofuels are being developed using different production pathways such us combined thermo-biochemical process and concentrated acid hydrolysis technology. However, due to the heterogeneous nature of household cellulosic waste the enzymatic conversion route has not been well investigated.

In this work, results of laboratory experiments aimed to evaluate the performance of municipal organic solid wastes as substrate for ethanol production are shown. The process comprises a hydrothermal pre-treatment and the bioconversion of the pretreated substrate to ethanol by simultaneous saccharification and fermentation. The analysis of ethanol yields attained in the different laboratory conditions (substrate and enzyme loading, process time, etc) will be presented.

Poster 5-48

Determination of Product Inhibition of CBH1, CBH2 and EG1 Using a Novel Cellulase Activity Assay

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Along with other factors, product inhibition limits lignocellulosic biomass hydrolysis. For this reason, we are interested in engineering our cellulases to resist product inhibition. The direct measurement of product is difficult in the presence of large amounts of added product. Instead, we have developed a method of measuring a decrease in substrate, taking advantage of the fluorescent properties of a calcoflour dye. The selected calcoflour dye has greater fluorescence when in the presence of intact cellulose. The hydrolysis of phosphoric acid swollen cellulose by endoglucanases and cellobiohydrolases results in a decrease in fluorescence of the calcoflour dye. We find that, while cellobiohydrolase 1 is strongly inhibited by cellobiose, cellobiohydrolase 2 and endoglucanase 1 are not significantly inhibited.

Poster 5-49

The Improved Cellulosome: Computational Modeling to Minisomes

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The first coarse grained model to study the formation and function of the cellulosome was developed within CHARMM. Some of the binding constants between cohesins and dockerins were derived from all-atom simulations. This study aims at understanding the mechanisms involved in the sequential binding of the cellulosomal enzymes on the scaffold of *C. thermocellum*. Individual subdomains were also studied with CHARMM and Amber on cellulose surfaces or with individual cellulose chains. These domains include, catalytic domains, carbohydrate binding domains, and fibronectins. All five cellulosomal fibronectins (Fn3) of *C. thermocellum* have been identified, overexpressed and purified, the crystal structure of one of them has been solved, and this provided experimental structure for the computational modeling of cellulosomal Fn3 function.

Poster 5-50

The use of calorimetry to monitor inhibition during biomass hydrolysis

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Enzymatic hydrolysis of lignocellulosic biomass is a complex process due to insoluble substrates, synergy effects between different enzymes and adsorption/inhibition of non-cellulosic components such as lignin or components derived from lignin during pretreatment. This complex process is hard to analyze and to address this problem the enzymatic activity have been monitored through the heat produced during hydrolysis using calorimetry, rather than the disappearance of substrate or the formation of products. With this technique it is possible to get real-time information of the enzymatic activity at high resolution in complex mixtures of lignocellulosic biomass containing non dissolved cellulose and lignin as well as non reacting components. This has previously been hard to obtain with other assay techniques. In this study the effect of lignin inhibition has been studied by titration with inhibitors to reacting mixtures of cellulose and cellulases and titration with cellulases into a mixture of inhibitors and cellulose. Changes in the heat produced as a result of addition of inhibitory compounds were there after evaluated. The inhibitors were both dissolvable and non-dissolvable lignin model compounds as well dissolvable and non-dissolvable ligniceous residues. Using this system it has been possible to compare the role of different functional groups as well as the role of total amount of different functional groups. Other factors that might influence the hydrolysis are high concentrations of sugars such as xylose and arabinose and a high ionic strength, which both are present after pretreatment of corn stover. These concerns were therefore also studied.

Poster 5-51

Thermochemical screening of cellulolytic enzymes for second generation bioethanol production

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The study of cellulolytic enzymes has traditionally been carried out using a variety of assays and techniques with both advantages and disadvantages. Typically, assays are specific to a particular enzyme type (specific substrates) or all encompassing (sugar production)[1]. Specific substrates may not reflect the situation during the breakdown of a complex biomass substrate, whilst all encompassing assays such as HPLC determination of sugars are limited to end product monitoring.

Thermochemical methods using I*sothermal* T*itration* C*alorimetry* (ITC) or T*hermal* A*ctivity* M*onitoring* (TAM) using power compensated or passive heat conduction measurements respectively, yield data where the primary observable is heat production; this in turn may be converted directly to the rate of reaction [2]. This means thermochemical measurements performed on such complex substrates as a variety of biomass types as well as model substrates monitor *real time* hydrolysis.

The current poster is a summary of the systematic benchmarking to use thermochemical screening for cellulolytic enzymes. We outline the possibilities for future use as well as some advantages and disadvantages of the investigated methods.

1 Ghose, T. K. (1987). "Measurement of cellulase activities." *Pure and Applied Chemistry* 59(2): 257-268.

2 Todd, M. J. and J. Gomez (2001). "Enzyme kinetics determined using calorimetry: A general assay for enzyme activity?" *Analytical Biochemistry* 296(2): 179-187.

Poster 5-52

Enzymatic hydrolysis of lignocellulosic biomass: Modeling and simulation of CSTR's in series

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Enzymatic hydrolysis of biomass has gained considerable interest in the past decades because it can provide glucose, which serves as a raw material for alcohol and other chemical products. The bioreactor in which enzymatic hydrolysis occurs is a logical focal point in pursuing cost reduction for ethanol production from lignocellulosic biomass. Considerable efforts have been focused on understanding the mechanism of heterogeneous and interfacial hydrolysis, but there are no major studies on the configuration and performance of bioreactors for enzymatic hydrolysis.

The purpose of this study is to model glucose production from lignocellulosic biomass in a well mixed and at steady state series of CSTRs with continuous substrate and enzyme addition. The kinetic model for enzymatic hydrolysis assumes a pseudo-homogeneous Michaelis-Menten mechanism. Glucose and cellobiose profiles during hydrolysis of delignified sugarcane bagasse are fitted to a kinetic model for an initial substrate concentration of 5% W/V.

Two case studies are considered: a series of n CSTRs of optimal volumes and a series of n CSTRs of equal volumes, both with and without recycle. The reaction of particulate biomass in a CSTR is considered equivalent to segregated micromixing with respect to the substrate and complete micromixing with respect to the aqueous phase and the conversion is predicted using a particle population model in conjunction with the batch kinetics. Finally, a discussion is made about other reaction systems and modes of operation with respect to enzyme and substrate in order to reach the practical realization of the technology.

Poster 5-53

Understanding dynamics of cellulase adsorption on AFEX treated corn stover during the course of enzymatic hydrolysis

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The adsorption of cellulases to the substrate is a prerequisite step for enzymatic hydrolysis of lignocellulosics. However, the mechanism of enzyme adsorption on crystalline cellulose is better understood than native lignocellulosics. In this study, high-throughput '*Fast Protein Liquid Chromatography*' (FPLC) based enzyme separation and quantification based methods were developed. Based on different isoelectric points of various cellulases such as cellobiohydrolase I (CBH I), cellobiohydrolase II (CBH II) and endo-glucanase I (EG I), individual enzymes could be eluted out separately from the column applying a linear conductivity gradient. Protein concentration could be correlated to the eluted peak area detected by UV absorption at 280 nm. Because this technique is non-destructive, eluted enzyme activity could also be measured.

Ammonia Fiber Expansion (AFEX) pretreated biomass has been found to improve enzyme accessibility. However, very little is understood about absorption dynamics of various cellulases and hemicellulases on AFEX treated biomass. In this study, AFEX treated corn stover was selected as the adsorption substrate. Cellulase (i.e. CBH I, CBH II, EG I) adsorption onto AFEX corn stover was studied and compared to untreated corn stover. Enzyme adsorption to lignin extracted from AFEX treated corn stover was also studied. The differential binding capacity of individual and multiple hydrolytic enzymes onto AFEX treated biomass was explored.

Investigating the enzyme adsorption behavior could help better understand the limiting factors affecting enzymatic hydrolysis. This work should also help in the rational design of synergistic enzyme cocktails that would maximize hydrolysis yields while reducing enzyme loading costs.

Poster 5-54

Induction and repression of ß-xylanase by different strains of *Thermomyces lanuginosus*

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Fuel ethanol that is renewable energy was demanded in the future. It was better if we could utilize agricultural wastes (straws and bagasse) as sources for ethanol production. These biomass sources were developed by enzymes such as xylanase. This enzyme could break the plant cell wall down into xylan. Then xylan was saccharified hydrosylate as xylose that could be fermented to ethanol by yeast. Thus, we interested to isolate xylanase producing *Thermomyes lanuginosus*. Our result showed that isolated *T. lanuginosus* separated to 2 groups depending on their xylose induced xylanase formation. Moreover, it was found a group of strains produced high xylanase either in the xylan or xylose medium. Addition of xylose to the xylan medium did not decrease xylanase production by *T. lanuginosus* THKU-11 and THKU-25 that were members of this group. In contrast, there was another group producing high xylanase only in the xylan medium. Addition of xylose to the xylan medium resulted decreasing of xylanase formation in *T. lanuginosus* TISTR 3465 and THKU-85 that were belonged to this group. Phylogenetic analysis obtained from random amplified polymorphic DNA (RAPD) pattern using one primer UBC 241 point to greater diversity of high and low xylanase producing strains using xylose as a carbon source.

Poster 5-55

Optimization of extracellular catalase production from *Aspergillus phoenicis K30* by Plackett-Burman design and linear regression using date flour as single carbon source and purification of the enzyme

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Aspergillus phoenicis K30 is the selected mutant which produces an amount of extracellular catalase. To amplify the extracellular catalase production by the strain, a fermentation optimisation was performed. To select the factors affecting the production, nine active variables (factors) were analyzed by Plackett-Burman design consisting of 12 experiments. Each variable was tested at two levels, a higher and a lower level. The calculation of the effect of each variable and the establishment of a correlation between the response of enzyme activity and variables, have revealed that the link is a multiple regression form. The optimization was carried out through a simplex algorithm. The amount of extracellular catalase produced by the strain in the optimised medium was about four times higher than that obtained in nonoptimised medium corresponding to 3820 mg/L of extracellular proteins including 59500 U/L of extracellular catalase activity after 96 h of fermentation. The steps of purification was allowed to improve enzyme activity by 305-fold. From an analytical gel electrophoresis under native conditions, an apparent molecular mass of 158 kDa was determined suggesting that the enzyme is a dimer. The isoelectric point of the protein was found to be 5 ± 0.1 as determined with a Pharmacia Phast-system

Poster 5-56

Dose Response Modelling of Enzymatic Biomass Hydrolysis

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Process modelling of enzymatic hydrolysis is useful as a tool for better scientific understanding of the process, as well as for predicting the effect of process changes in connection with optimizing the process for commercial use.

Two approaches will be shown. The first one is an empirical model, a.k.a. "curve fitting". This approach is used for comparing enzyme blends or substrates pairwise, and for assessing the economic feasibility of the process. The second approach is a kinetic model based on numerical integration of a set of coupled differential equations. This approach is used for predicting process performance for different enzyme doses and hydrolysis time. The second approach has the additional advantage that it can possibly relate observations to process mechanisms, in order to gain an enhanced understanding of these, but it also involves more assumptions than the empirical approach.

Poster 5-57

Hemicellulolytic enzymes from the maize endophyte *Acremonium zeae*

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A complex suite of enzymatic activities is required for complete hydrolysis of the hemicellulose fraction of lignocellulosic biomass. *Acremonium zeae* is one of the most prevalent fungal colonists of preharvest maize, producing symptomless infections of seeds and inhabiting the stalks of mature plants. *A. zeae* grows most vigorously on artificial media containing corn cob xylan, suggesting that *A. zeae* might be a source of hemicellulolytic enzymes uniquely adapted for the utilization of corn cell wall components. An examination of *A. zeae* grown on corn fiber found that it possesses a full complement of hemicellulolytic enzymes including xylanases, xylosidases, and arabinofuranosidases capable of releasing greater than 90% of xylose and arabinose from corn cob and wheat arabinoxylans. A 30 kDa arabinofuranosidase (AF30) and a 47 kDa arabinofuranosidase (AF47) were purified from the cell-free culture supernatant by ion-exchange and hydrophobic interaction chromatography. AF30, which does not bind to the anion exchange support at pH 6.5, has a K_m for 4-nitrophenyl-α-L-arabinofuranoside (4NPA) of 8.6 mM and V_{max} of 3.2 U/mg. Temperature and pH optima are 45°C and pH 4.5. TLC analysis of the hydrolysis products indicated that AF30 releases primarily arabinose and some xylobiose from corn fiber arabinoxylan. AF47, which binds to the anion-exchange support, has a K_m for 4NPA of 4.4 mM and V_{max} of 1.7 U/mg. Temperature and pH optima are 32°C and pH 6.0. The enzymes produced by *A. zeae* may have industrial application for the hydrolysis of recalcitrant lignocellulosic feedstocks such as corn fiber.

Poster 5-58

***Saccharophagus degradans* 2-40 Utilizes a Novel Group of Processive Endoglucanases to Degrade Cellulose**

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The marine bacterium *Saccharophagus degradans* 2-40 produces a multi-component cellulolytic system proposed to consist of 10 annotated GH5 endoglucanases, two GH9 endoglucanases, one cellobiohydrolase, five glucosidases and two phosphorylases. The *S. degradans* system is unusual in the abundance of GH5-containing cellulases and an apparent deficiency of processive enzymes. Each of the 10 GH5 containing cellulases were cloned into pET28b and expressed in *E. Coli* Rossetta™. After purification to near homogeneity, selected biochemical properties were assessed. For each enzyme either the full-length enzyme or a degradation product sufficient to carry the catalytic domain exhibited cellulase activity, thus confirming their annotation as endoglucanases. One cellulase, Cel5H, showed significantly greater activity on several cellulose substrates. The activity of this enzyme primarily released cellobiose during short digestions and the ratio of soluble to insoluble products was greater than 4 irrespective of the length of digestion, consistent with processivity. This activity resided with the catalytic domain and was found to be specific to amorphous cellulose. The processivity coupled with viscosity reduction of Carboxymethyl cellulose solutions and synergisms with known cellulases argues that Cel5H is a processive endoglucanase. Phylogenetic analyses indicated that Cel5H is a member of a separate clade of GH5-containing enzymes that also included Cel5G and Cel5J. These enzymes were also found to be processive endoglucanses whereas the other GH5 cellulases of *S. degradans* were classical endoglucanases. Thus the *S. degradans* cellulolytic system utilizes novel GH5-containing processive endoglucanases to degrade cellulose.

Poster 5-59

Kinetics and Synergy of *Trichoderma reesei* Cellulases on Ionic Liquid Pretreated Avicel and *Miscanthus giganticus*

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A number of lignocellulose pretreatment methods have been explored to increase the susceptibility of lignocellulose to cellulase action. Certain ionic liquids can dissolve lignocellulose under mild conditions and have been shown to disrupt the hydrogen bonding network of crystalline cellulose. We have compared the activities of purified *T. reesei* cellulases CBHI, CBHII, and EGI on untreated and ionic-liquid pretreated cellulosic and lignocellulosic substrates. Excess beta-glucosidase was included to reduce potential inhibition by cellobiose. To assess the impact of pretreatment methods on the potential synergistic action of cellulases, the rates of each individual enzyme on pretreated and untreated substrates were compared to those of ternary enzyme mixtures.

Poster 5-60

Cost Driver Influences on a Biomass to Ethanol Process Model

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In 2002, NREL published a report with data on a comprehensive theoretical study into a biomass to ethanol process using dilute acid pretreatment and enzymes for corn stover hydrolysis (NREL/TP-510-32438). This study suggested such a process could be economically viable given cost optimizations in several key areas of the process, and has become a baseline for much work in academia, industry and technology development.

Research and Development organizations have focused efforts within portions of the process where they own technical expertise. However, having met particular development targets, it can be difficult to assimilate these changes into the holistic cost picture in "today's dollars" to evaluate true economic viability. A model, based upon the NREL 2002 study, is suggested which incorporates process flow correlations using AspenPlus as well as statistical techniques to overcome assumption uncertainties in a plug-in and out format. A sensitivity analysis is presented which illustrates major cost drivers in the overall production cost based upon this model.

Poster 5-61

Characterization of the specific activities and hydrolytic properties of the cell-wall degrading enzymes produced by *Trichoderma reesei* RUT C30 on different carbon sources

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Conversion of lignocellulosic substrates to biofuels requires efficient cell wall degrading enzymes at reasonable price, and among the possibilities are enzymes produced in situ on available lignocellulosic substrates. Cellulases and hemicellulases were produced by fermentation of *Trichoderma reesei* RUT-C30 on different carbon sources: steam pretreated corn stover (SPCS), Solka Floc 200 (SF) and lactose. The produced enzymes were characterized by their specific enzyme activities. Enzymes produced on Solka Floc and SPCS had similar FPU activities and protein content, and were rich in xylanase, while enzymes produced on lactose had significantly lower cellulase activity per unit volume. Substrates containing xylan led to an increased secretion of xylanase and ß-xylosidase, whereas enzymes produced on lactose had highest specific β-glucosidase and CBH I activities.

The hydrolytic properties of the enzymes were compared with the commercial Celluclast 1.5L on four substrates: SPCS, steam pretreated spruce (SPS), SF and Avicel using a standard FPU loading. Enzymes produced on the lignocellulosic substrates performed generally better than the commercial one. As expected, the substrates containing xylan were clearly hydrolyzed more efficiently with enzymes rich in xylanases, whereas the cellulosic substrates depended more on the cellulase enzyme pattern. Hydrolysis of SPCS and SPS was also performed with various enzyme preparations (Celluclast and the enzymes produced on SPCS and SF) by adjusting with purified enzyme components the xylanase and ß-glucosidase levels to reach similar activity levels. The results lead to further understanding of the hydrolytic mechanisms and the factors determining the performance of different mixtures of *T. reesei* enzymes.

Poster 5-62

Packet bed reactor running on babassu oil and glycerol to produce monoglycerides by enzymatic route using immobilized *Burkholderia cepacia* lipase

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From the different methods available for the enzymatic synthesis of monoglycerides, glycerolysis reactions seem to be more advantageous than the other reactions due to its high yield and productivity. In addition, glycerolysis route has additional relevance in the present worldwide context, due to the sharply increase on the glycerol supplied in the market as a primary by-product from biodiesel plants. Therefore, converting glycerol into value-added products provides an alternative for glycerol disposal and for its surplus problems. Previous work carried out in our lab has identified that Burkholderia cepacia lipase (Lipase PS-Amano) is a potential enzyme source to produce monoglycerides from the glycerolysis of babassu oil.

Pursing our interested in developing a feasible enzymatic process an attempted was made to perform the process under continuous mode. For this, packet bed reactor (PBRs) configuration was selected based on its suitability to mediate typical lipase catalyzed reactions. Prior to the continuous experiments, the optimization of molar ratio glycerol to oil (15:1) and reaction temperature (50°C) was carried out batchwise via response surface methodology.

The reactor was packed with 6.70g of lipase PS immobilized on SiO2-PVA and feeding with substrate at a flow volumetric rate of 0.028 mL/min. The inert atmosphere was guaranteed by sparking N2 in the feed medium storage. The PBR operated continuously for 22 days and monoglycerides concentration were between 25 and 29%wt. The biocatalyst stability was found to be high and during the first 16 days no significant decrease on the initial lipase activity was observed.

Keywords: glycerolysis, lipase, babassu

Poster 5-63

Withdrawn

Poster 5-64

Evaluation of oxalate decarboxylase and oxalate oxidase for industrial applications

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Increased recirculation of industrial process water can result in problems with calcium oxalate precipitation. Calcium oxalate precipitation (scaling) is a well known problem in the pulp and paper industry, where it gives rise to calcium oxalate incrusts on process equipment. The oxalic acid comes from the raw material and is formed in reactions with strong oxidizing agents. The potential in using oxalate decarboxylase from *Aspergillus niger* for oxalic acid removal in industrial bleaching plant filtrates containing oxalic acid was examined and compared with barley oxalate oxidase. Ten different filtrates from chemical pulp mills were selected for the evaluation. Oxalate decarboxylase degraded oxalic acid faster than oxalate oxidase in eight of the filtrates, while oxalate oxidase performed better in one filtrate. One of the filtrates inhibited both enzymes. The potential inhibitory effect of various compounds in industrial pulp mill process waters on the enzymatic activity was tested. Oxalate decarboxylase was more sensitive than oxalate oxidase to hydrogen peroxide. Oxalate decarboxylase was not as sensitive to chlorate and chlorite as oxalate oxidase. Up to 4 mM chlorate ions, the highest concentration tested, had no inhibitory effect on oxalate decarboxylase. Analysis of the filtrates suggests that high concentrations of chlorate present in some of the filtrates were responsible for the higher sensitivity of oxalate oxidase in these filtrates. Oxalate decarboxylase was thus a better choice than oxalate oxidase for treatment of filtrates from chlorine dioxide bleaching.

Poster 5-65

The Effects of High Solids Conditions on Specific Enzymatic Activities Involved in the Saccharification of Lignocellulosic Biomass

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Increasing political and economic pressures worldwide have spawned growing interest in recent years towards bringing the production of lignocellulose based fuels to the industrial scale. While research in this field has progressed greatly over the past thirty years, there are still a few fundamental problems that have so far remained unresolved and will prove crucial to the economic viability of lignocellulosic conversions. One of these is related to running the enzymatic saccharification/fermentation processes at high levels of dry solids (~ 20% dry matter) which is necessar for a cost effective process. To date a number of studies have reported depressed conversions with increasing solids concentrations compared to the levels typically used for bench scale studies (usually less than < 5 % dry matter). While a handful of studies have begun to reveal potential causes of this phenomenon, most often the studies are conducted using commercial cellulase preparations which are composed of a significant number of different enzyme activities and individual proteins. In recent studies we have attempted to determine if this effect is consistent across all enzyme activity types or if the adverse phenomenon can be isolated to certain groups of enzymes. We have investigated these high solids conditions with respect to a range of different commercial enzyme preparations in addition to a handful of purified enzymes representing activities that are known to be crucial to the overall conversions process. We believe that work of this nature will provide valuable insight to researchers investigating possible mechanisms behind this detrimental phenomenon.

Poster 5-66

Effects of analysis error on the results of enzymatic hydrolysis of acid pretreated bagasse

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Lab scale enzymatic hydrolysis of cellulosic biomass is usually carried out at predetermined percentage of substrates loading and dosage of enzymes. For the saccharification of acid pretreated sugarcane bagasse (APB), glucan content and moisture content of APB are the two key factors that influence the amount of substrate loading, while total protein content of cellulase, if enzyme is dosed on total protein, is the factor that affects the amount of enzyme used in a particular experiment. It is obvious that saccharification results will be influenced by the analysis error of the aforementioned three key factors. In this poster, we will present the results of a Design of Experiment we conducted, and demonstrate how different the APB saccharification results will be if a small error (i.e., 5%) in glucan, moisture, or total protein content analysis, is introduced.

Poster 5-67

Production and characterization of recombinant cell wall-active enzymes from *Aspergillus nidulans* for use in plant biomass utilization

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A comprehensive set of 74 fungal genes encoding enzyme types active towards all known carbohydrate linkages within plant cell wall polysaccharides were recently cloned from the saprophytic filamentous fungus *Aspergillus nidulans* and made publically available (Bauer et al., PNAS 103:11417–11422, 2006). Recombinant production of these enzymes provide monocomponent activity preparations (i.e., having no major competing or distinguishable side activities) and eliminate expensive labor-intensive purification from heterogeneous enzyme mixtures. Monocomponent enzymes can be used as a high resolution analytical tool to decipher polysaccharide fine structure and as a specific biochemical tool to modify and tune functional properties of polysaccharides with commercial value. Such enzyme preparations can also be used to evaluate and optimize complementary enzyme blends for efficient hydrolysis of plant cell walls through synergistic action. Hemicelluloses, such as glucuronoarabinoxylans, and pectins are complex structural polysaccharides present in cell walls due to their extensive side-branching and accessory functional groups. Selective modification or saccharification therefore requires a broad set of complementary enzymes. We are filling our enzyme toolbox with hemicellulases and pectinases, and we will present our progress on the *in vitro* production and biochemical characterization of these enzymes. Hemicellulases under investigation include endo-β-xylanases, β-xylosidases, α-arabinofuranosidases acetylxylan esterases, α-glucuronidase, and ferulic acid esterase. Pectinases include endo- and exo-polygalacturonases, pectin methylesterase, rhamnogalacturonase, and rhamnogalacturonan acetylesterase. Determination of substrate specificity of the xylanases will be highlighted. These enzymes may find use in generating renewable polysaccharide-based bioproducts or for more efficient biomass conversion to liquid biofuels.

Poster 5-68

Withdrawn

Poster 5-69

Changes in Pleurotus ostreatus laccase isoenzyme pattern in cocultivation with Trichoderma viride

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Laccases are phenoloxidases involved in aromatic compound transformation but also in stress response towards antagonist species such as Trichoderma sp. Increase laccase production during interespecific Trichoderma-basidiomycetes interactions is an environmentally safe biological alternative to chemical induction, which has received considerable research efforts in the last years. Pleurotus ostreatus was cultivated in liquid media containing malt extract. In the control cultures a sharp decreased on the laccase volumetric activity was observed, however the cocultures maintained 57 % of their highest laccase activity obtained at 72 hours after inoculation of T viride. During the cocultures T viride induced changes in the laccase isoenzyme pattern as a result of the alteration of laccases secreted by P ostreatus, modifying lcs1 to lcs3. The Km values of lcs3 for two phenolic substrates were lower than those of lcs1 indicating that with the processing to which the isoenzyme was subjected increased the affinity to certain substrates.

Poster 5-70

Analysis of cellulase hyper-producing mutants derived from the fungus Trichoderma reesei QM9414

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The fungus Trichoderma reesei QM9414 secretes a large amount of cellulase. Furthermore, QM9414 was treated with mutagens such as ultraviolet radiation and chemical compounds, and cellulase hyper-producing mutants were obtained. We investigated cellulase production mechanism of 3 mutants by analyzing the amounts of secreted protein, cellulase activity, and transcription of cellulase genes. The analyzed mutants were T. reesei X31, T. reesei PC-1-4, and T. reesei PC-3-7. We used Avicel and lactose as carbon sources. When we cultured these strains for 7 days using Avicel as carbon source, the amounts of secreted protein and Filter Paper enzyme activity of these mutants were 2.3 to 2.7-fold and 3.0 to 4.3-fold, respectively, higher than those of QM9414. In addition, RT-PCR analysis revealed that the transcription of cellobiohydrolase I gene and endoglucanase I gene of these mutants were increased compared with those of QM9414. Similar results were obtained using lactose as carbon source. These results suggested that mutations of these mutants were affected cellulase production at transcriptional level.

Poster 5-71

A Thermodynamic Study of the Carbohydrate Binding Modules [CBMs] from Trichoderma reesei Cellobiohydrolase I and II

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Trichoderma reesei produces 2 processive cellobiohydrolases, CBHI and CBHII. The former is specific to the reducing-end of cellulose whereas the latter is a nonreducing-end specific enzyme. It is known that these enzymes act in concert when degrading crystalline cellulose, and therefore increase the rate of conversion when used simultaneously in an enzyme cocktail. Both enzymes consist of a carbohydrate-binding module [CBM] and a large catalytic domain connected by O-glycosylated linker peptides. We use molecular simulation techniques to investigate the binding and action of these CBMs on a crystalline cellulose substrate. The results from these simulations aid in understanding the specificity of these CBMs and their roles in processivity. Where possible, we use these computational results in concert with traditional experimental biochemistry to understand the function of each enzyme sub-domain.

Poster 5-72

Enzymatic conversion of butyric acid to butyl butyrate in a packed bed reactor

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Continuous enzymatic synthesis of butyl butyrate by esterification has studied in a packed bed reactor (PBR) using Novozym 435 (Candida antarctica lipase B immobilized on macroporous polyacrylate resin). Several solvents such as toluene, heptane, hexane, 1,4 dioxane and tetrahydrofuran were screened for the esterification and heptane showed the highest conversion. The optimum temperature for butyl butyrate synthesis was founded in batch reactor (50 ml) to be 45 °C. The maximum esterification reaction was obtained when the molar ratio of butyric acid to butanol is 0.5 or less. For continuous butyl butyrate production, PBR system was developed and fluid residence time was determined.

Poster 5-73

Raffinose and lactose induce α-galactosidase and β-galactosidase activity from Lactobacillus reuteri

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α-Galactosidase and β-galactosidase have major applications in food industry. Over production of α-/β-galactosidase from food grade microorganisms might lead to incorporation of these microorganisms in food products. The objective of this experiment was to investigate the over production of both enzymes in six strains (CF2-7F, DSM20016, MF14-C, MM2-3, MM7 and SD2112) of Lactobacillus reuteri (L. reuteri) by the inclusion of different sugars in media. L. reuteri strains were cultured on media with different sugars (dextrose, raffinose, galactose, lactose, sucrose, and melibiose). Activity of α/β-galactosidase activity were tested on ρ-nitrophenyl-α-D-galactopyranoside and o-nitrophenyl β-D-galactopyranoside, respectively. Raffinose was the best sugar to produce α-galactosidase in MF14C, SD2112, and CF2-7F (15-13 Gal U/ml). Lactose and galactose enhanced β-galactosidase production in CF2-7F (82 Gal U/ml). Results suggest that MF14C, CF2-7F, and SD2112 might be used in fermented soy products to eliminate flatulence. CF2-7F could be used as natural additives in milk for lactose intolerant individuals.

Poster 5-74

Insights into the Structural Basis for the Thermostability of a Glycosyl Hydrolase Family 12 Endoglucanase from Acidothermus cellulolyticus

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A glycosyl hydrolase family 12 endoglucanase from Acidothermus cellulolyticus is one of the most thermostable endoglucanases known. It has been demonstrated to have activity on a number of substrates including beta-glucan, arabinoxylan, xylan, and xyloglucan, making it an important enzyme for biomass conversion. To better understand the structural basis for its thermotolerance, the crystal structure was determined at high resolution and evaluated in regard to known parameters conferring thermotolerance. Using CHARMM, we also conducted molecular dynamics simulations at ambient conditions to build a wild type native contact map of the protein in solution. Melting simulations were conducted at high temperature to identify the structural features of the enzyme susceptible to thermal unfolding. A library of mutants was then screened with these computational methods and the most promising mutants were produced experimentally for thermostability and activity measurements. Correlations between in-silico and in-vitro results will be discussed.

Poster 5-75

Detection of a Dihidro-dihydroxy-naphthalendiol dehydrogenase in a strain of Mucor circinelloides isolated from petroleum-contaminated soil

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The second step in aromatic hydrocarbon biodegradation pathway is catalyzed by the activity of a dihydroxy-di-hidrodiol dehydrogenase (DHD) enzyme that uses NAD as cofactor. There exist only few reports about this enzyme in filamentous fungus. Previously, we were capable to detect an activity band using electrophoretic zymograms revealed with phenanthrene-diol as substrate. In this work, we are interested in the detection and characterization of naphthalene diol-dehydrogenases that could be the enzymes that catalyze the second step in the aromatic hydrocarbon biodegradation pathway. The results suggest the presence of different DHD activities depending of the cofactor NAD or NADP used in the mixture to reveal the enzymatic activity.

Poster 5-76

Desorption of CBH1 from BMCC substrate is a function of enzymatic activity

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Desorption of CBH1 from BMCC substrate was studied here as a function of enzymatic activity. CBH1 was first separated from Spezyme CP cellulases by ion exchange chromatography. The maximum adsorption capacity of CBH1 was about 4 μmol/g BMCC. No desorption of CBH1 was detected following 1-hour incubation after dilution at 0 oC (in an ice-water bath). At an enzyme loading of 2.1 μmol/g BMCC, almost all CBH1 adsorbed onto the substrate. The CBH1 and BMCC were incubated together for two days. Free enzyme in the solution was monitored during this period. The CBH1 showed different desorption behavior depending on enzymatic activity. Desorption was quantified by measuring CBH1 content in the buffer solution using the Bradford Assay. About 30% desorption of CBH1 was observed at 0 oC (with end-over-end mixing); nearly 100% desorption of CBH1 occurred at 50 oC and 150 rpm on a shaker table; 35% desorption of CBH1 occurred at 50 oC and 300 rpm (enzymatic activity was lower than at 150 rpm). With the addition of K2PdCl6, which denatured the catalytic domain of the CBH1, desorption decreased from ~100% to ~40% after a two-day incubation period at 50 oC and 150 rpm. PNPC assay was used in each of these cases as a means to determine enzymatic activity. The amount of CBH1 desorption was seen to decrease as a function of decreasing enzymatic activity, whether the reduced activity was due to the denaturing agent, low incubation temperature, or difference in the shear environment.

Poster 5-77

Screening and production study of xylanase producer microorganisms from the Brazilian Cerrado

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Hemicelluloses are polysaccharides of low molecular weight including xylan, mannan, galactan arabinan and arabinaxylan. Xylan is the most common hemicellulosic polysaccharide in cell walls of land plants, comprising a backbone of xylose residues linked by β-1,4-glycosidic bonds. Xylanolytic enzymes from microorganism have attracted a great deal of attention in the last decade, because of their biotechnological in various industrial processes, such as food, feed, ethanol and pulp and paper industries. A microbial screening of xylanase producer was carried out in Brazilian Cerrado area from Selviria, Mato Grosso do Sul State, Brazil. About fifty bacterial strains and fifteen fungi strains were isolated from soil sample using a medium composed by 10.0 g.L-1 of corn husk, 5.0 g.L-1 of meat extract, 0.20 g.L-1 of peptone and 5.0 g.L-1 of Na2CO3 added separately at 45 ºC. The bacterial named P5B1 was cultivated on submerged fermentation using as substrate xylan, wheat bran, corn husk, corn cob and crushed sugar-cane. Corn husk and crushed sugar-cane show a good xylanase activity after 72 hours of fermentation. Crude xylanase was characterized and the optimum pH was 5.5; and it was stable in the pH range 5.0-10.0. The optimum temperature was 60°C at pH 6.5; and it was thermally stable up to 50 ºC. A fungus named P2D16 was cultivated on solid state fermentation using as substrate source wheat bran, wheat bran plus sawdust, corn husk, corn cob and crushed sugar-cane. Wheat bran and corn cobs show the better xylanase production after 72 and 96 hours of fermentation, respectively.

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Poster 5-78

Development of continuous process on biodiesel production by immobilizeed and co-immobilized lipases

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In this study, transesterification and esterification were investigated in a packed-bed reactor using immobilized and co-immobilized *Candida rugosa* and *Rhizopus oryzae* lipases. In a circulation process, optimal conditions were investigated to increase the reaction rate and conversion yield. The optimal flow rate was 0.8 mL/min. When immobilized and co-immobilized lipases were used in the circulation process, the conversion yield of biodiesel reached 94.56 % at 3 h and 99.45 % at 2 h, respectively. In the continuous process, optimal conditions of batch and circulation processes were also investigated. Under optimized reaction conditions (45 °C, 0.8 mL/min flow rate and 10 % water contents), the conversion yield of immobilized and co-immobilized lipases exceeded over 80% for 120 h.

Poster 5-79

Adsorption of cellulases on cellulolytic enzyme lignin from Lodgepole pine

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Enzymatic hydrolysis of lignocellulosic materials is significantly affected by the cellulase adsorption onto the cellulosic substrates and lignin. The presence of lignin plays an important role in the lignocellulosic hydrolysis and enzyme recycling. Three cellulase preparations (Celluclast, Spezyme CP and MSUBC) were evaluated to determine their adsorption onto cellulolytic enzyme lignin (CEL) from steam exploded Lodgepole line (SELP) and ethanol (organosolv) pretreated Lodgepole pine (EPLP). The adsorption affinity of cellulase (Celluclast) onto isolated lignin (CEL-EPLP and CEL-SELP) was slightly higher than that from corresponding EPLP and SELP substrates based on the Langmuir constants. Effect of temperature, ionic strength and surfactant on cellulase adsorption on isolated lignin was also explored in this study. The thermodynamic analysis of enzyme adsorption onto isolated lignin (Gibbs free energy change $\Delta G \approx -30$ kJ/mol) indicated this adsorption was a spontaneous process. The addition of surfactant (0.2% w/v) could reduce the adsorption of cellulase onto CEL-SELP by 60%. Two types of adsorption isotherm were compared for cellulase adsorption onto isolated lignin. Langmuir adsorption isotherm showed better fit for the experimental data than that from Freundlich adsorption isotherm

Poster 5-80

Porcine pancreatic lipase purification on poly(ethylene glycol)-potassium phosphate aqueous two-phase system

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Lipase (EC.3.1.1.3) catalyses the hydrolysis of triglycerides at the oil-water interface, synthesis of esters and transesterification in microaqueous conditions. For the application is necessary its purification, which requires delicate enough steps to preserve the biological activity. This study examined the application of the aqueous two-phase system (ATPS) to purify porcine pancreatic lipase, studying the influence of molecular weight and concentration of poly(ethylene)glycol (PEG), tie line length (TLL), potassium phosphate concentration, NaCl addition and temperature in the partition. The enzyme was further purified in PEG-8000, presenting more recoveries at the top phase to the lowest TLL, concentrations of PEG and potassium phosphate (purification factor of 2.8-fold), the increase of these variables represses the purification. The addition of NaCl did not promote the purification of the enzyme and temperatures of 14.5°C were more effective in the purification (PF = 4.0-fold). This study demonstrated that aqueous two-phase system is a well suitable methodology for lipase purification.

Poster 5-81

Production and characterization of a thermostable cellulase from *Geobacillus* sp.

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Thermostable cellulases are known to provide better stability and reaction rates at higher temperatures (≥60°C) which could benefit the enzymatic hydrolysis of cellulose for bioethanol production. A thermophilic cellulolytic facultative anaerobe was isolated from soil samples collected from the 4850 ft level below the surface of the Homestake gold mine in Lead, SD, now known as the NSF Deep Underground Science and Engineering Laboratory (DUSEL). The strain showed high 16S rDNA sequence homology (99%) with the genus *Geobacillus* and was named *Geobacillus* sp. R7. It produced extracellular cellulase activity when grown in a medium containing cellulose as a sole carbon source at 60°C. The growth of the R7 culture was studied at different pH and temperature in a 2L bioreactor with automated control. The crude cellulase had a pH optimum of 5 and was most active at a temperature of 80°C. It retained about 50% and 70% of its initial activity after incubation for 7 days at 70°C and 50°C, respectively. The cellulase induction ability of different substrates - avicel, lactose, hardwood acid hydrolyzate, corn stover and prairie cord grass - were investigated. The last two substrates were used before and after thermo-mechanical pretreatment (extrusion at high temperature). The nutritional factors were examined for their effect on cell growth and cellulase production. Both cellulase productivity and activity were evaluated during the course of the batch fermentations. The results obtained warrant further investigations to evaluate the potential of the *Geobacillus* sp. R7 enzymes in the development of a simultaneous saccharification and fermentation process.

Poster 5-82

Biochemical Characterization of Bacterial and Fungal Hemicellulases and Heterologous Expression *in planta*

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Transgenic expression of microbial carbohydrate-active enzymes will be evaluated as a means to decrease the load and cost of enzymes used to refine pulp fibre for the production of value-added biomaterials, or hydrolyze plant biomass to platform sugars. Over twenty bacterial gene targets have been isolated from fourteen commercially available bacterial genomes. The corresponding enzyme activities included: α-arabinofuranosidase, α-glucuronidase, α-galactosidase, α-fucosidase, and mannanase. Purified enzymes will be biochemically characterized to measure pH optimum, temperature stability, and substrate specificity using both synthetic and natural substrates. In addition to bacterial genes, a glucuronoyl esterase gene from the white-rot fungus *Phanerochaete carnosa* (*PcGE*1) will be included in our studies. *PcGE*1 was recombinantly expressed in *Pichia pastoris*, and the purified enzyme will be biochemically characterized *in vitro* using 4-O-methyl-D-glucopyranuronate as the substrate. The transient expression of these microbial enzymes in tobacco leaves were validated using green fluorescence protein. These data may reveal correlations between microbial gene features, such as codon usage or AT content, and functional enzyme expression in plants. Transiently expressed microbial genes will then be targeted for transgenic expression in Arabidopsis. Initial experiments will constitutively express the microbial genes in Arabidopsis using the 35S promoter. We are also developing a transcription activation system based on the LhGR/pOp vector pair, which will facilitate tissue-specific and time-dependent expression of microbial genes in Arabidopsis. Resulting transgenic plants will be characterized in terms of morphology, cell wall structure, and total sugar composition.

Poster 5-83

Nitrogen source optimization for cellulase production by *Penicillium funiculosum* using experimental planning

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Cellulases are enzymes able to hydrolyze β-1,4 glycosidic bonds of celullose and have many applications in food and textiles industries and in biofuels production. For this purpose these enzymes can be produced by using agricultural and agroindustrial wastes as nutrients sources, carbon particularly. Sugar cane bagasse is one of the most abundant wastes in Brazil which cellulose composition is between 34 and 37% on dry weight. However, this waste is poor in nitrogen, one of the most important nutrients for the microorganism that is used for the synthesis of structural and catalytic proteins. Thus, this work aimed at evaluating and optimizing different nitrogen sources for cellulases production by submerged fermentation of sugar cane bagasse. The nitrogen sources, urea, ammonium sulfate, peptone and yeast extract ,were analyzed in two levels with three repetition of the central point. The results were evaluated by analysis of effects (T test), ANOVA and optimized using response surface methodology. The filamentous fungus *Penicillium funiculosum* was used in enzymes production, and fermentation was carried out in Erlenmeyer flasks containing sugar cane bagasse added of the corresponding nitrogen source. The fermentation process was carried out for 72 hours at 30 °C and stirred at 200 rpm. The crude extract was obtained by centrifugation and then the filter paper activity, endoglucanase and β-glucosidase were determinated. Urea and yeast extract presented significance to cellulase production among the evaluated sources. Desirability function indicated that the optimum condition of urea and yeast extract was 0.424 and 0.884 (coded values), respectively.

Poster 5-84

Development of a High Solids Enzymatic Saccharification Method for Lignocellulosic Biomass

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The ability to screen new biomass pretreatments and advanced enzyme systems at process relevant conditions is key to developing economically viable lignocellulosic ethanol. While much research is being invested in developing pretreatment technology and enzyme systems that will more efficiently convert cellulosic biomass to sugars, there currently is no standard screening method for enzymatic saccharification of cellulosic biomass at high solids conditions. Shake flasks are the established reactor vessels used for small-scale enzymatic saccharification reactions; however, at high solids concentrations, shake flasks do not provide adequate mixing. In this work, a small-scale high solids saccharification reaction vessel was identified and a method was developed for use in screening both pretreated biomass and enzyme systems at process relevant conditions. This new method addresses mixing issues seen in high solids saccharifications. In addition, yield calculations from sugar concentrations on a mass basis were used to account for the two-phase nature of the saccharification slurry, which eliminates discontinuities in comparing high solids to low solids saccharifications that occur when using concentrations on a volume basis. In the development of this method, three small-scale vessels with various mixing modes were evaluated for their efficiency and consistency in converting high solids loadings of biomass. The method was tested and compared at bench and floor scales to determine the scalability of the reactor system.

Poster 5-85

Over-expression and purification of *Trichoderma reesei* glycosyl hydrolases in Pichia and in *T. reesei*

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The high cost of degradative enzymes is a limiting factor in the economical conversion of lignocellulose to fermentable sugars. All current biomass-degrading commercial enzyme mixtures are derived from a handful of Ascomyceteous fungi, especially *Trichoderma reesei* and *Aspergillus niger*, but the enzymatic activities and relative proportions of current enzyme mixtures are poorly defined. The goal of this project is to produce a defined enzyme mixture that is optimized for pretreated corn stover. On the basis of abundance and predicted importance, twenty enzymes from a proteomics analysis of *T. reesei* were selected to be expressed either in a heterologous host (*Pichia pastoris*) or in *T. reesei* itself. Several expression vectors for *T. reesei* have been constructed that use either a strong constitutive promoter or a strong cellulose-inducible promoter from *T. reesei*. The long-term goal is to develop an optimized set of enzymes that has higher specific activity on real lignocellulosic materials than current industrial enzymes. These experiments will also help us identify the key enzymes that should be targets of improvement and further research.

Poster 5-86

Production of hemicellulolytic enzymes by a thermophilic Aspergillus strain isolated from sugarcane bagasse

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Degradation of lignocellulosic biomass by microbial enzymes has emerged as an important technology to obtain many products of industrial interest, among which bioethanol stands out. In Brazil, sugarcane bagasse represents an attractive material for this process, being an agro-industrial waste widely available. The production of hemicellulolytic enzymes able to efficiently degrade sugarcane bagasse, aiming to obtain fermentable sugars for ethanol production, is the focus of this work. The strain *Aspergillus* M 7.3, isolated from piles of sugarcane bagasse, was initially cultivated in a mixture of sugarcane bagasse and wheat bran (1:1 w/w), at 45oC, for up to 336h. The production of cellulase (FPase) and xylanase were evaluated, which yielded peaks at 48 h (1.0 U/g) and 192 h (1239 U/g), respectively. Afterwards, other agricultural wastes were evaluated as substrates to cultivate the fungus. Higher xylanase productions were obtained using mixtures (9:1 w/w) of corn straw and wheat bran (4944 U/g at 192 h) and corn straw and barley (4080 U/g at 240 h). The microorganism cultivation in a mixture of corn straw and wheat bran (9:1 w/w) provided a CMCase production of 43.4 U/g (192 h) and the highest beta-glucosidase production was observed during cultivation in wheat bran (26 U/g at 336 h). The results indicate that *Aspergillus* M 7.3 is a promising strain concerning the production of enzymes for sugarcane bagasse hydrolysis. Thus, its enzymes will be subsequently physical-chemically characterized and tested concerning their bagasse hydrolysis performance.

Key words: *Aspergillus,* cellulases, xylanase, sugarcane bagasse, agricultural residues

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Poster 5-87

Production of cellulolytic enzymes by fungi *Acrophialophora nainiana* and *Ceratocystis paradoxa* using different carbon sources

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Global warming has been related to greenhouse gases, such as carbon dioxide accumulation. The need to decrease fossil fuels uses is pushing towards "zero-emission" fuels such as ethanol, that can be produced from agricultural and forestry residues.

The production of bioethanol involves biomass pretreatment and the enzymatic hydrolysis of its polysaccharides into fermentable sugars. The necessary enzymes (exoglucanases, endoglucanase, β-glucosidases, xylanases, plus accessory enzymes) are produced by fungi nevertheless there are differences concerning the types of enzymes that are produced and its excretion levels.

This works evaluated the enzymes produced by *Acrophialophora nainiana* and *Ceratocystis paradoxa*. Activity profiles were compared to that of *Trichoderma reseei* Rut-C30. The growth medium was optimized for carbon (lactose, wheat bran and steam pretreated sugarcane bagasse), and nitrogen sources (corn steep liquor and yeast extract). Shaken flasks cultures were incubated at 30-40ºC and 200 rpm. FPA, CMCase, β-glucosidase and xylanases activities were measured in daily supernatant samples.

The lactose medium was efficient for *A.nainiana,* resulting in the accumulation of (IU/L) 2016 CMCases, 144 FPA, 9 β-glicosidase and 2200 xylanase. For *C.paradoxa* wheat bran medium allowed the production of (IU/L) 570 CMCase, 70 FPA, 890 β-glucosidase and 12620 xylanase. *T.reseei* Rut-C30 produced (IU/L) 25000 CMCase, 1000 FPA, 600 β-glucosidase and 23000 xylanase. *C.paradoxa* showed to be a strong xilanase producer. Nevertheless the overall CMCase, FPA and xylanase activities for *A.nainiana* were 10 fold lower in comparison to *T.reseei*, this microorganism could be a candidate for genetic improvement as their CMCase/FPA and xilanase/FPA ratio are balanced.

Poster 5-88

Directed Evolution of Hyperthemophilic Endoglucanase, Cel5A, from *Thermotoga maritma* MSB8

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Biomass conversion to biofuels is a four-step process – 1) biomass diminution and physicochemical pretreatment; 2) enzymatic hydrolysis of (hemi-)cellulosic biomass to sugars; 3) conversion of sugars to fuels and 4) finally, recovery and blending of the fuels. Enzymatic hydrolysis of cellulose to glucose is carried out by the action of enzymes known as cellulases, which include endoglucanases (EC 3.1.2.4), exoglucanases (EC 3.1.2.91) and beta-glucosidases (EC 3.1.2.21). Enzymes from extremophiles, organisms that live under extreme conditions of pH, salt, temperature etc., are adapted to work under the conditions for chemical and physical conditions in the pretreatment steps and are thus chosen as targets for improvement of enzymatic hydrolysis.

Hyperthermophilic endo-β-1,4-glucanase, Cel5A from Thermotoga maritime MSB8, was chosen as our initial target for directed evolution to improve the hydrolysis efficiency under high temperature. Codon-optimized cel5A gene was cloned into pCDF2 Ek/LIC vector (Novagen) and highly soluble expressed in E. coli strain, BL21 (DE3). It has an optimal pH and temperature at 4.8 (citric acid buffer) and 81°C, respectively. Error-prone PCR was used to generate a low error-rate library. Thousands of colonies were screened for activity improvement by DNS assay compared with wild type cel5A. About 10 hits with 30-50% improvements were further investigated. After expression and purification by Ni-NTA columns, these mutants were assayed for specific activity (U/mg protein). 5 mutant cel5A genes with 20-30% improvement were confirmed by DNA sequencing.

Poster 5-89

Towards the Development of Cellulases compatible with Ionic Liquid Pretreatment for Saccharification of Cellulosic Biomass

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Enzymatic hydrolysis is the rate-limiting step in the fermentation of biomass to sugars. The main barrier is the highly crystalline structure of cellulose that limits accessibility to enzyme adsorption sites and slows the hydrolysis of cellulose to sugars in aqueous media. To increase enzyme accessibility, a combination of high temperature and extremes of pH are used during common pretreatment steps like dilute acid or ammonia fiber explosion. We are exploring the use of Ionic liquids (IL), a new class of environment friendly, non-volatile solvents, in the pretreatment of cellulosic biomass. IL's have been shown to dissolve cellulose, which can be recovered in the amorphous form by the addition of antisolvents like water. However, significant decreases in cellulase activity in the presence of trace amounts of IL's have been reported in literature, necessitating extensive processing to remove residual IL's from the regenerated cellulose. To simplify the entire process, it is necessary to develop cellulases that are stable and active in the presence of trace amounts of IL's. Towards that goal, we are investigating the stability of extremophilic enzymes, for use with the IL, 1-Ethyl-3-methylimidazolium acetate (EMIM acetate). The endoglucanase from the hyperthermophilic bacterium Thermatoga maritima (Tma cellulase) was purified by affinity chromatography and the enzymatic hydrolysis activity was measured in the presence of varying concentrations of EMIM acetate. Herein, we show a comparison of the enzymatic efficiency between the commercially available T.viride cellulase from Sigma and the Tma cellulase and the differences related to biochemical properties.

Poster 5-90

Effect of Cellulose-Binding Module Choice on Catalytic Activity of Cellulases

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Biofuel production from biomass can be divided into four major steps - biomass generation, pretreatment to recover cellulose, cellulose hydrolysis to simpler sugars like glucose, and production from glucose. Enzymatic hydrolysis of cellulose, which is the rate-limiting step in the process, is catalyzed by glycosyl hydrolases. Within the glycosyl hydrolase family of enzymes, cellulases catalyze the breakdown of the β-1,4- linkage in cellulose to smaller oligosaccharides and can be broadly classified into two groups - endo-glucanases and exo-glucanases. These enzymes are found to be modular in architecture where catalytic domain (CD) that hydrolyzes the glycosidic linkage are sometimes found 'joined' together with other accessory non-catalytic domains like carbohydrate binding modules (CBMs) through a flexible linker sequence. While CD hydrolyzes the glycosidic bond, the primary function of the CBMs is binding to the crystalline or amorphous cellulose as an accessory module; CBMs thus present a model system for understanding the structure-function relationship of not only the CBM with cellulose but also for interactions of the CBM with the catalytic domain. The relationship between CBM structure, CBM binding affinity, and optimal enzyme catalytic efficiency had not previously been well-explored although it will play a key role in achieving the cost-effective breakdown of the cellulose in pretreated biomass. To fill this knowledge gap, we have developed analytical methods to characterize a large set of CBMs from thermophilic cellulolytic enzymes. The properties of these binding modules will be discussed, with an emphasis on their varying affinities for insoluble cellulosic substrates and how this affects catalytic activities.

Poster 5-91

Probing the Function of N-Terminal Ig Domain in the Crystal Structure of Endoglucanase Cel9A from the Thermoacidophilic *Alicyclobacillus acidocaldarius* Using Computational Modeling

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Members of the GH family demonstrate a modular architecture composed of one or two catalytic modules connected to several kinds of accessory modules. In order to further engineer enzymes for industrial hydrolysis of cellulose, it is important to understand the structure and functional relationship of cellulases and the accessory domains. The non-processive endoglucanase Aa_Cel9A from thermoacidophilic bacterium Alicyclobacillus acidocaldarius belongs to the subfamily E1 of family 9 of glycoside hydrolases, members of which have an N-terminal immunoglobulin (Ig)-like domain followed by the catalytic domain (CD). The function of Ig-like module has not been determined but its presence is required for activity. Deletion of the Ig-domain promotes complete loss of enzymatic activity in a related cellobiohydrolase, CbhA from Clostridium thermocellum. While there is kinetic and structural information of the Aa_ Cel9A, molecular dynamic simulations (MD) provide a method to piece together the activity, kinetic, biophysical and structural information to offer insights into domain motion, domain interactions and rate-limiting conformation to help piece together the dynamic view of enzymatic hydrolysis of cellulose. The present work takes a special case with newly resolved crystal structure of endoglucanase Cel9A from thermoacidophilic alicyclobacillus acidocaldarius (CelA) and explores the function of N-terminal Ig domain by simulation approaches. Molecular dynamics simulations (MD) combined with simplified model were performed on the structures of CelA with and without Ig domain. Umbrella Sampling /free energy perturbation (UM/FEP) are also performed to obtain unfolding free energy landscapes for both cases. Both methods show that Ig domain stabilized the structures of catalytic domain.

Poster 5-92

Enzyme Engineering of Glycoside Hydrolase-5 Endoglucanases Enzymes for Consolidated Bioprocessing

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The enzymatic hydrolysis of cellulose into simpler sugars starts with the endoglucanases (3.2.1.4) and exoglucanases (3.2.1.91), which hydrolyze insoluble cellulosic polymers into smaller soluble sugars with degree of polymerization <6. Our initial effort is focused on improving the activity of the endoglucanases; specifically, the endoglucanases that can be either used in consolidated bioprocessing by the addition of the enzymes into the pretreatment mix, or as a component of the 'parts list' for engineering into the three hosts- Saccahromyces cerevisiae, Escherichia coli and Sulfolobus acidocaldarius- for fuels production at JBEI. Therefore the selection of the endoglucanases for directed evolution is guided by the characteristics of the enzymes mapped onto the characteristics of the pretreatment method or the JBEI fuel synthesis host. Acidothermus cellulolyticus and Sulfolobus solfataricus enzymes were selected based on their activity, actual or predicted, in dilute acid pretreatment method and as an engineered part for S. acidocaldarius. The endoglucanases from Thermotoga maritima and the three Pyrococcus species were selected based on their predicted stability in temperatures > 80 oC, which would make these compatible with hydrothermolysis pretreatment method. Among these initial target genes, Pyrococcus horikoshii endoglucanase (Pho-EG) was the first candidate to be tested for directed evolution work, because our preliminary work have shown that Pho-EG has highest specific activity among the three Pyrococcus species that we selected. Endoglucanase assay was performed using azo-carboxymethyl cellulose (azoCMC) to determine the pH and temperature optima of the wild-type Pho-EG activity from the cell lysate.

Poster 5-93

Monitoring the rheological properties of pretreated biomass in high-consistency enzymatic liquefaction

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Process to produce fuel ethanol from lignocellulosic biomass needs still to be improved to become economically viable. One of the single most important process parameters with regard to process efficiency is the overall substrate consistency; low carbohydrate levels not only increase the capital cost due to equipment size but they also result in excessive energy requirement of heating, cooling and distillation. Until recently, solid loadings of 10% or less were the standard applied. When aiming at *e.g.* 5% final ethanol concentration in the fermentation broth (considered as a prerequisite for a feasible large-scale distillation technology) the dry matter loading has to be over 20%. High-solid slurries, on the other hand, are difficult to handle because of the insufficient mass transfer conditions caused by the strong, if not complete, adsorption of process water by the pretreated material. One possibility to overcome this problem might be to include a pre-hydrolysis step in the process sequence aimed at the rapid liquefaction of the high-consistency slurry. Because of the more favorable rheological properties at higher temperatures, the use of thermostable enzymes may be of special importance in such technologies.

The goal of the present study was to evaluate the applicability of a short-time liquefaction using various enzyme preparations and purified enzyme components to increase the flowability of slurries at high solid loadings (up to 20% on dry basis). Liquefaction of pretreated spruce, corn stover and wheat straw substrates was followed via measuring the mechanical properties of slurries using a texture analyzer and a viscosimeter.

Poster 5-94

Agarases for red algae biomass deconstruction and saccharification

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Marine algae including red algae (Rhodophyta) have recently been considered as a promising resource complementing land plants for bioenergy production. The major composite of red algal cell wall is an agar polysaccharide. Biological process degrades the agar polysaccharide into monomeric sugars by several steps, which can be a platform for designing biochemical red algal biomass deconstruction process. Various agarases are involved in each step of the decomposition. We collected all known agarase genes from public database and reclassified them using a domain-based network. From our model, we identified candidate agarases from all known sequenced genome. Among selected genes, several were expressed in E. coli to test their predicted activity. Among them, two agarases belonging to glycohydrolase family 50 (GH50) from Saccharophagus degradans were overexpressed as soluble. We deduced active sites from the sequence and structure analysis. The enzymes were identified as exo-type agarase producing neoagarobiose as a reaction product. Although two enzymes show high sequence similarity, the level of activity was much different. We suggest that various agarases collected from our approach can be used in biochemical deconstruction process of red algae biomass.

Poster 5-95

Synergistic enhancement of enzymatic hydrolysis of sugar cane bagasse by *Trichoderma* and *Aspergillus* cellulases and xilanases enzyme pools

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Biomass enzyme blends produced by *Trichoderma reesei* RUT C30 and a selected strain of *Aspergillus awamori,* a high β-glucosidase producer, were pooled and the assessed to hydrolyze sugarcane bagasse, industrially treated with steam to be used as cattle feed. The culture supernatants were individually concentrated by ultrafiltration and blended to obtain preparations with different profiles of exo- and endoglucanases, β-glucosidase, xylanases and feruloyl esterase. Hydrolysis experiments were performed with 25 g/L substrate at 50°C, pH 5,0 and 200 rpm for 72 hours. Glucose, cellobiose and xilose were measured by HPLC. In all *T. reesei* and *A. awamori* enzymes mixtures it was observed a synergistic enhancement of filter paper activity (FPA) that improved hydrolysis yield around 30%. Hydrolysis experiments using different FPA/g of substrate (5, 10, 15 and 20 FPA/g) resulted on a sharp increase on glucose concentration up to 10 FPA/g that leveled off from 10 to 20 FPA/g of steam treated bagasse. Glucose concentrations of 7,5 g/L, in 72 hours experiments, corresponded to 65% cellulose hydrolysis yield. The sugars syrup presented 1g/L xylose, indicating the presence of a substantial hemicelulose amount in the steam pretreated material. A sharp increase in glucose and xilose concentration was observed for all enzyme loads up to 6 hours of hydrolysis. It was not observed accumulation of cellobiose (inhibitory sugar) throughout the hydrolysis experiments that presented a β-glucosidase/FPA ratio of 5. The feruloyl esterase activity produced by *Aspergillus* (7 UI/L) seemed to increase the effectiveness of the enzymatic hydrolysis of the studied substrate.

Poster 5-96

Comparative production and characterization of cellulolitic enzymes from thermophilic fungi Thermoascus aurantiacus CBMAI756 and *Thermomyces lanuginosus*

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The thermophilics fungos *Thermoascus aurantiacus* CBMAI756 and *Thermomyces lanuginosus* were cultivated in a mixture of sugar cane bagasse and corncob on solid state fermentation for cellulases (endoglucanase, exoglucanase, b-glucosidase) production. The maximum production of endoglucanase, 922 ± 17 U/g, from *T. aurantiacus* occurred at 120 h of fermentation. The enzyme exhibited optimum pH at 4.0, optimum temperature at 80 °C and remained stable for 24 h at pH range of 4.0 - 9.5 and for 1 h at 60 °C (remaining 75 % of its original activity). This enzyme was not produced by *T. lanuginosus*. Exoglucanase was not produced by both fungi. The maximum production of b-glucosidase, 77,6 ± 6,0 U/g, by *T. aurantiacus* occurred at 120 h of fermentation. The enzyme exhibited optimum pH at 4.0, optimum temperature at 75 °C and remained stable for 24 h at pH range 5.0 - 9.0 and for 1 h at 70 °C (remaining 75 % of its original activity). The maximum production of this enzyme, 83,0 ± 1,2 U/g, by *T. lanuginosus* occurred at 216 h of fermentation. The enzyme exhibited optimum pH at 5.0 - 5.5, optimum temperature at 60 °C and remained stable for 24 h at pH range 5.0 - 10.0 and for 1 h at 50 °C (remaining 75 % of its original activity). These results indicate that the consortiation of these enzyme preparations acting together may be a good strategy to improve hydrolyses of lignocellulosic material in the area of bioenergy.

Poster 5-98

Induction of α-and β-Galactosidases in *Lactobacillus reuteri* by Different Metal Ions

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Probiotics are food grade bacteria that could be used safely and directly as food supplements. Probiotics have many health benefits to the host such as better digestibility of sugars mainly lactose and raffinose, antimicrobial activity and decrease blood cholesterol of the host. Health benefits of probiotics are strain specific and *L. reuteri* has been shown to have good potential for the production of digestive enzymes (α-and β-galactosidases). Bacterial media could be an important factor in over producing these enzymes. The induction of α-and β-galactosidases in probiotic bacteria is one of the interesting areas in food science. The objective of was test the induction of α-and β-galactosidases by metal ions. 0.1 M of six metal ions (Na^{+}, K^{+}, Fe^{+2}, Cu^{+2}, Mn^{+2}, and Mg^{+2} sulfates) were are added to the growth media and tested to induce the production of α-and β-galactosidases in six strains (CF2-7F, DSM20016, MF14-C, MM2-3, MM7 and SD2112) of *Lactobacillus reuteri (L. reuteri*). Results showed that Na^{+}, Fe^{+2} and Mn^{+2} lead to up to 3 times induction of α-and β-galactosidase in *L. reuteri* than the control group (MRS media). Media contained Mn^{+2} showed that highest induction capability among metal ions studied to induce the production and relative activity of α-and β-galactosidase enzymes in *L. reuteri*.

Poster 5-99

Optimization of lipase extraction conditions obtained by solid-state fermentation

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The aim of this work is to select recovery methods for lipase produced by *Penicillium simplicissimum* in solid-state fermentation (SSF), using babassu cake as the culture medium. The parameters for this enzyme recovery were studied using Plackett-Burman (PB) and Central Composite Rotatable (CCRD) experimental designs.

In our observations of lipase recovery kinetics, an increase in enzyme extraction was identified until 20 minutes, after which time there was no improvement in lipase recovery. The following extraction conditions were evaluated: sodium phosphate buffer (100mM, pH: 7.0) with or without NaCl (0.6%(w/v)), Tween 80 (0.1%(w/v)), Triton X-100 (0.5%(w/v)) and glycerol 20%(w/v). Extraction with Tween and NaCl were the best conditions, yielding lipase activity of 85.7U/g and 65.7U/g, respectively.

In the PB experimental design it was observed that pH and Tween had a positive impact on enzyme extraction, while temperature and buffer molarity had a negative effect. NaCl, stirring and volume presented no statistically significant effects, meaning that any value for these parameters could be used (in the studied range). A drop in the buffer molarity could harmfully reduce the buffer capacity, for which reason it was fixed at a minimum value. Later, a CCRD was employed, which yielded a 70% increase in lipase activity in the crude extract. Through the CCRD results analysis, a quadratic model was built, from which it was concluded that the maximum predicted lipase activity (160 U/g) was obtained at 25ºC, Tween 0.5%(w/v), pH 8.0 and extraction medium volume of 7mL per gram of fermented solids.

Poster 5-100

Effect of high-temperature enzymatic pretreatment on saccharification of acid-pretreated corn stover

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Lignocellulosic biomass is an abundant renewable resource that can be used for production of fuels and chemicals. The biological conversion of lignocellulose to ethanol involves a number of steps, including pretreatment to make the cellulose fraction more accessible to cellulases, enzymatic hydrolysis of cellulose to glucose, and fermentation of glucose and other sugars to ethanol. In this study, we evaluated the possibility of reducing the overall enzyme loading by introducing a high-temperature enzymatic pretreatment step immediately before the enzymatic hydrolysis step. High-temperature enzymatic pretreatment of dilute-acid pretreated corn stover (PCS) was performed using different thermostable endoglucanases and endoxylanases at different temperatures and protein loadings. The enzymatic pretreatment was followed by enzymatic saccharification of PCS by cellulolytic enzymes at 50°C. The effect of the enzymatic pretreatment on the overall enzyme dose needed to achieve the certain degree of cellulose conversion will be discussed.

Poster 5-101

Biochemical characterization of cellobiohydrolases from different GH families

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Cellulose is the major structural component of plant cell walls and the most abundant biological polymer on earth. Many cellulolytic fungi degrade native crystalline cellulose using a set of cellulolytic enzymes dominated by two cellobiohydrolases (EC 3.2.1.91), CBHI and CBHII. These two enzymes often work cooperatively (exo-exo synergy), and appear to be the key enzymes for hydrolysis of crystalline cellulose. Cellobiohydrolases are processive enzymes liberating cellobiose from reducing or non-reducing ends of the polymeric cellulose chains. Most of the cellobiohydrolases belong to glycoside hydrolase (GH) families 6 and 7. This paper will compare properties of various cellobiohydrolases from families 6 and 7. Substrate specificity, thermostability, specific activity on selected substrates, and pH and temperature activity profiles will be discussed in relation to performance of the cellobiohydrolases in hydrolysis of dilute-acid pretreated corn stover at various process conditions.

Poster 5-102

Production by solid-state fermentation and structural modeling of a lipase from *Aspergillus parasiticus*

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The lipase from the filamentous fungi *Aspergillus parasiticus* was produced by solid-state fermentation and demonstrated high esterification activity, making it a potentially feasible catalyst for biodiesel and biolubricant production.

The present investigation reports on the production, characterization and lipase modeling of an *Aspergillus parasiticus* strain. Initially, kinetic experiments were carried out using babassu cake as the basal solid medium, moistened with water or sugar cane molasses supplement. The highest activity was reached in the medium without supplement after 72h of fermentation at 30°C. The influence of the inoculum concentration, temperature and moisture on enzyme production was evaluated employing statistical experimental design, and an empirical model was adjusted to the experimental data. The last two variables were found to be the most significant in the process. It was shown that higher lipase activity could be achieved at lower temperatures and moisture levels. Maximum lipase activity was obtained at 28°C and 65% moisture (w/v). The substrate specificity of the crude lipase was determined using several *p*-nitrophenyls as substrates. Higher V_{max} and lower K_m were obtained with *p*-nitrophenyl butyrate, indicating that this enzyme presents a high affinity to short-chain esters.

In order to better characterize the specificity of this enzyme, a structural model was constructed using *Thermomyces lanuginosus* lipase as template. Analyzing the model, we identified the catalytic triad: Ser145, His260 and Asp198. We also had seen that model was considered satisfactory with 97.2% of the residue in regions allowed in the Rammachandran's plot.

Poster 5-103

Synthetic Genes + Design of Experiment = BioEngineering

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DNA2.0 is developing ProteinGPS for systematic engineering of protein functionality, and Codon Optimization for increased expression of recombinant proteins. The technologies take advantage of efficient gene synthesis and advancements in linear and nonlinear systems optimization to map and navigate in sequence space.

The ProteinGPS method calculates the specific location of a protein variant in multidimensional space and places unique information rich variants at important crossroads within the space assessed. The resulting datasets are synthesized and used to map the functional protein hyper space and calculate new protein variant sequences that fulfill the functional constraints needed. Examples of of ProteinGPS based protein engineering are to be presented.

Codon Optimizaion: DNA2.0 has actively studied the relationship of gene design to heterologous expression yield. DNA2.0 has created large systematically varied gene sets for multiple gene types and analyzed expression in several commonly utilized host systems. Striking differences are observed for the dependence of expression on synonymous codon usage in different hosts. In E. coli, protein expression levels varied over two orders of magnitude for each of two gene sets tested. Our data show that codon bias is a strong determinant of expression levels in E. coli; however, the preferred codon bias is distinctly different from that of genes naturally highly expressed in the bacterium and no correlation is seen between expression and the codon adaptiveness index. The correlation between codon bias and protein expression in multiple systems is compared.

Poster 5-104

Biochemical Comparison of a Gycosyl Hydrolase family 7 Library

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A library of Glycosyl Hydrolase family seven (GH7) cellobiohydrolases was assembled using sequences gleaned from the Carbohydrate-Active Enzyme (CAZy) database and NCBI Entrez. The protein sequences identified on the CAZy webserver as GH7 cellobiohydrolases were analyzed by CLUSTAL and PHYLIP to generate an unrooted phylogenetic tree. Representative sequences were selected for expression in *Aspergillus* and purified to homogeneity using column chromatography. The proteins were characterized and compared using a variety of biochemical tests including activity on cellulose and pretreated corn stover. Assays were run in parallel with Cel7A from *Hypocrea jecorina* (anamorph: *Trichoderma reesei*) to permit unbiased comparisons of activity with the best-studied cellobiohydrolase. Representatives were also homology modeled using the software package Modeler™ and structural variability between enzymes compared. These efforts are expected to further the understanding of sequence-structure-function relationships in cellobiohydrolases and to correlate differences in active site topology with differences in activity on model and realistic cellulose substrates.

Poster 5-105

Probing the Effects of Glycosylation on the Flexibility of the *Trichoderma reesei* Cellobiohydrolase I Linker Peptide with Fluorescence Resonance Energy Transfer and Molecular Simulation

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The *Trichoderma reesei* cellobiohydrolase I (Cel7A) is an industrially-relevant, processive cellulase composed of a carbohydrate-binding module and a large catalytic domain connected by an *O*-glycosylated linker peptide of approximately 36 amino acids. Understanding the overall mechanism by which Cel7A is able to decrystallize recalcitrant cellulose and hydrolyze cellodextrins to cellobiose should permit us to determine the rate-limiting steps in the conversion of cellulose by this enzyme. This accomplishment will allow application of rational design strategies to the task of improving cellulase enzyme cocktails for industrial use. Using a bottom-up approach, we apply a suite of experimental and computational techniques to understand the function of each Cel7A sub-domain. In this study, we apply fluorescence resonance energy transfer [FRET] and molecular dynamics [MD] techniques to study the effects of glycosylation on the flexibility and conformations of the *O*-glycosylated Cel7A linker domain. The resulting conformational states that the linker peptide adopts provide clues to the roles that linker flexibility may play in the processivity of the Cel7A enzyme.

Poster 6-07

Simultaneous Saccharification and Fermentation of Switchgrass with *Kluyveromyces marxianus* IMB 3 in pH controlled Bioreactor

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Previous simultaneous saccharification and fermentation (SSF) of switchgrass experiments using the thermotolerant IMB 3 strain of *Kluyveromyces marxianus* suggest that maintaining a pH at or slightly above 5.0 will result in increased ethanol production. Switchgrass was pretreated with pressurized hot water at 200 °C for 10 min. Two 1.5 L SSFs will be performed in a stirred bioreactor. The pH will be automatically controlled at 5.5 and 5.0 by adding 2 M potassium hydroxide and the temperature will be maintained at 45 °C. 15 FPU/g glucan of cellulase enzyme will be used with a beginning glucan concentration of 40 g/L. Samples will be taken every 24 hours for 7 days and analyzed by HPLC for ethanol, sugars, and organic acid concentrations.

Poster 6-08

Simultaneous saccharification and fermentation of xylan with *Kluyveromyces marxianus* IMB2

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A great amount of today's bioethanol production is based on corn starch. Since this substrate stands in conflict with the food industry, researchers are studying alternatives like cellulosic biomass, namely switchgrass. Switchgrass grows naturally in the US and has great potential for utilization. Switchgrass has ~20% hemicellulose with xylan as the main constituent. Since traditional yeast like *Saccharomyces cerevisiae* are unable to utilize xylose, the monomer of xylan, alternative yeast need to be found. *Kluyveromyces marxianus* IMB1-5 strains have shown the ability to utilize xylose at 40-50°C, which has the advantage to work in areas with higher average temperatures and in applications like simultaneous saccharification and fermentation (SSF). SSF includes enzymatic hydrolysis and fermentation in the same reactor. Since enzymes have higher temperature optima, usually 50 to 70°C, a yeast strain with a higher temperature optimum is preferred to accommodate both temperature optima. In this work different commercially available enzyme mixtures were evaluated in terms of xylan degradation. The enzyme mixture that produced the highest xylose concentration was used together with *K. marxianus* IMB2 in SSF with different enzyme concentrations and temperatures. Highest ethanol production was achieved at 40°C but with 3µl/ml enzyme concentration. However, this was only 17% of the theoretical maximum value. The results imply that under these conditions, *Kluyveromyces marxianus* IMB2 is not suitable for ethanol production based on xylose.

Poster 6-09

Effect the Particle Size on MFC Maximum Power Generation, Power Longevity and Coulombic Efficiency

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Microbial fuel cells (MFCs) are a promising technology for bioelectricity generation from a wide variety of organic and inorganic substrates in water, sediments, and wastewaters. However, little is known about the power densities and longevity of power generation in MFCs using particles such as lignocellulose and chitin, which are the two most abundant biomaterials in the world. These substrates degrade slowly over time and therefore can provide a long term source of fuel for MFCs. The effect of particle size on power and longevity was examined using chitin particles sieved to produce three average particle sizes. The length of a power cycle (longevity) in the MFC increased from 9 to 33 days with an increase in the particle diameter from 0.28 mm to 0.78 mm. Coulombic efficiency based on chitin removal increased from 18% for the smallest particles to 56% for the largest ones. The maximum power generation was lower for the largest particles (201 mW/m^2), with higher power densities for the small particles (301 mW/m^2) and 285 mW/m^2 for the medium particles (0.46 mm). The measured lifetimes of these particles scaled with particle diameter to the 1.3 power. Based on modeling particles as spheres or fractals, it was determined that chitin particles were fractal with a three dimension fractal dimension (D_3) between 2 and 2.3.

Poster 6-10

Media Requirements for Aerobic Cultivation of *Saccharomyces cerevisiae* TMB 3400-F30-3 on Softwood Hydrolysate

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The purpose of this study was to develop a low-cost industrial nutrient medium for the aerobic cultivation of a hydrolyzate-adapted *Saccharomyces cerevisiae* TMB3400-FT30-3. This xylose-utilising yeast is a promising ethanologen for converting lignocellulosic biomass sugars to ethanol. Dilute acid pretreated softwood hydrolyzate is a readily available and economic option for a carbon source for cell growth in the fermentation seed tank and has the added advantage of contributing to cell adaptation before the fermentation. The focus of the work was to determine the minimal level of externally-supplied media supplementation in the form of beet molasses and nitrogen in the form of ammonium sulfate that will still result in near-maximal biomass yields during fed-batch aerobic growth. The experimental work showed that it is possible to supply only 10 % of the sugars in the form of beet molasses (or 21 g/L of concentrated molasses) without any significant reduction of the biomass yield from its near maximum value of 0.45 g biomass / g sugar. It was not until the molasses addition was below 9 % that a statistically significant reduction in yield was observed. Reducing the nitrogen from 0.12 to 0.06 g N / g biomass resulted in a statistically significant decrease of the yield.

Poster 6-11

Characterization of extremophilic cellulose-degrading bacteria from the deep subsurface of the Homestake gold mine, Lead, South Dakota, USA

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Ligninocellulosic materials are among the Earth's most abundant renewable resources. The degradation of cellulosic waste materials with greater rates by high potential microbes (e.g., thermophiles) is very important. Therefore, the present study investigated the cultivable mesophilic (37°C) and thermophilic (60°C) cellulose-degrading bacterial diversity in a weathered soil-like sample collected from the deep subsurface (1.5 km depth) of the Homestake gold mine in Lead, South Dakota, USA. Molecular community structures were determined by phylogenetic analysis of 16S rRNA gene sequences retrieved from enrichment cultures growing in presence of microcrystalline cellulose as the sole source of carbon. All phylotypes retrieved from enrichment cultures were affiliated to *Firmicutes*. Cellulose-degrading mesophilic and thermophilic pure cultures belonging to the genera *Brevibacillus*, *Paenibacillus*, *Bacillus*, and *Geobacillus* were isolated from enrichment cultures, and selected cultures were studied for enzyme activities. For a mesophilic isolate (DUSELG12), the optimum pH and temperature for carboxymethyl cellulase (CMCase) were 5.5 and 55°C, while for a thermophilic isolate (DUSELR7) they were 5.0 and 75°C, respectively. Furthermore, DUSELG12 retained about 40% CMCase activity after incubation at 60°C for 8 hours. Most remarkably, thermophilic isolate, DUSELR7 retained 26% CMCase activity at 60°C up to a period of 300 hours. Overall, the present work revealed the presence of different cellulose-degrading bacterial lineages in the unique deep subsurface environment of the mine. The results also have strong implications for biological conversion of cellulosic agricultural and forestry wastes to commodity chemicals including sugars.

Poster 6-12

Microbes derived from marine invertebrates and algae as a source of cellulolytic, chitinolyitic and lipolytic enzymes

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Marine invertebrates such as sponges are known to harbor large microbial populations which may comprise up to 60% of the biomass. The role of these bacteria is largely undefined however it is likely that some play a role in nutrition through the production of hydrolytic enzymes which break down particulate matter captured by the sponge. Such enzymes have potential utility in the degradation of biomass in biofuel generation. The Harbor Branch Marine Microbial Culture Collection (HBMMCC) contains over 17,000 heterotrophic microbial isolates, 70% of which were derived from invertebrates collected at depths between 50 and 1,000 meters. This study was designed to determine whether these microbes produce cellulolytic, chitinolytic or lipolytic enzymes and to compare their taxonomy with that of isolates with similar activities from the shallow water marine environment. Bacterial isolates of unknown taxonomy were selected from the HBMMCC and tested for degradative activities in plate assays. Samples of sediments, algae and invertebrates were collected from near-shore coastal environments and plated on to a series of isolation media using a standard serial dilution technique. After incubation, environmental samples were replica plated onto assay plates allowing selective isolation of microbes producing degradative enzymes. Following strain purification and confirmation of activity, the taxonomy of the isolates was determined through 16S rRNA gene sequence analysis. Isolates producing these activities included members of the Actinobacteria, Firmicutes, Alpha- and Gamma-Proteobacteria. This study suggests that these marine environments may be a productive resource for the discovery of enzymes with activities relevant to biofuels production.

Poster 6-13

Optimization of lactic acid production by pelleted-form of Rhizopus oryzae in 3 L airlift bioreactor using response surface methodology

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Lactic acid production by pelleted-form *Rhizopus oryzae* was optimized in 3 l airlift bioreactor. The effect of pH and volumetric oxygen transfer efficiency (k_La) was evaluated according to the 2^3 central composite designs. Results of regression analysis suggested linear and quadratic terms of pH and k_La were significant affected on lactic acid. Ethanol formation was found that it depends on linear and quadratic terms of pH and only linear term of k_La. Whereas quadratic term of pH and k_La were significant affected on biomass. Interaction between pH and k_La were not influenced on considered variables. From response surface analysis, optimal pH and k_La were 5.85 and of 0.065 s^{-1}, respectively and lactic acid of 72.10 g/l was achieved. Subsequently, successive five repeated batches with average 77.54 g/l of lactic acid were achieved and lactic acid productivity was improved from 0.75 to 0.99 g/l h.

Poster 6-14

Isolation and Cultivation of Cell Wall Decomposing Bacteria from Plant Biomass Decaying Communities

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Natural decomposition of biomass employs hydrolytic enzyme cocktails, often provided by complex microbial communities. The objective of this study is to isolate and cultivate bacteria from lignocellulosic biomass degrading communities that decay poplar chips, corn stover and switch grass biomass, and explore their metabolic potential for cell wall decomposition. From a microbial poplar biomass decay community, both the aerobic and anaerobic biomass decay zones were taken and used to initiate enrichment cultures. Eight bacterial strains were isolated from an aerobic enrichment, and twenty-one strains were isolated directly from the biomass under an anaerobic condition. Strains were further identified by 16S rRNA sequencing and characterized by carbon source utilization. Potential glycosyl hydrolase activity was observed within these isolates: *Bacillus* sp. strain 1046 showed clearing on CMC-plates; *Clostridium* sp. strains 880 and 883, *C. akagii* strains 885 and 887, *C. aciditolerans* strain 886, and *Enterobacter* sp. strain 889 showed growth on CM3-medium with cellulose as sole carbon source. Strain *Clostridium* sp.VIII and the two enriched consortia were able to grow on washed corn stover, thus biomass composition and glycosyl hydrolase were also tested. Xylosidase, cellobiosidase, and arabinosidase activities were detected in these samples. Most interestingly, two isolates from decaying corn stover biomass showed strong clearing zones on plates with Sigmacell as sole carbon source in an anaerobic growth. Both strains belong to the *Clostridium* sp. (16S rRNA sequencing), and characterization of these strains are currently in the process.

Poster 6-15

Ethanol bioproduction from sugarcane bagasse hemicellulosic hydrolysate

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The objective of this study was to evaluate the sugar-to-ethanol bioconversion using *Pichia stipitis* DSM 3651 and sugarcane bagasse hemicellulosic hydrolysate as fermentation medium. A loopful of cells was transferred to 500-mL Erlenmeyer flasks containing 200 mL of medium consisting of (g/L) 30.0 xylose, 3.0 yeast extract, 3.0 malt extract and 5.0 peptone. The cells were then incubated at 30 ºC and 200 rpm. The fermentations were carried out in 250-mL Erlenmeyers flasks containing 100 mL of medium and 3.0 g/L initial cell concentration, at 30 ºC and 200 rpm for 120 h. The medium was composed by the hydrolysate (not treated or treated with ion-exchange resins adsorption or treated with pH alteration and active charcoal adsorption), supplemented with same nutrients described above. Sugars, acetic acid and ethanol concentrations were determined by HPLC and cell concentrations by spectroscopy. As results, 4.9, 6.1 and 7.5 g/L ethanol concentration, 0.2, 0.3 and 0.3 g/g sugar-to-ethanol bioconversion ($Y_{P/S}$) and 0.04, 0.1 and 0.2 g/Lh volumetric productivity (Qp) were obtained in the fermentations that used medium compounded by hydrolysate not treated, treated with pH alteration and active charcoal or treated with resins, respectively. As conclusion, the fermentation that used hydrolysate treated with ion-exchange resins presented the best results in terms of ethanol concentration and Qp, and also presented a good $Y_{P/S}$. As a whole, the present study points out to the fact that the sugarcane bagasse hemicellulosic hydrolysate is a potential fermentation medium to be used for ethanol production. Acknowledgments: Fapesp and CNPq.

Poster 6-16

Microbial contamination of biodiesel

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Biodiesel is a domestically produced, renewable fuel that can be manufactured from vegetable oils, animal fats, or even recycled restaurant greases. B-20 is more specifically a blend of 20% B-100 and 80% diesel and can be used in diesel engines without modification. Biodiesel tends to contain more polar species than petroleum based diesel. This results in a higher dissolved water content leading to more free water for microbial community growth. Microbial growth results in biofilm formation, increased corrosion of fuel system components as well as a reduction in the quality of the fuel. In order to control this growth it is important to understand how the consortium evolves in the fuel/water environment. A year-long study of the microbial contamination found in a single sample of B-20 was conducted using microbiological and molecular techniques. Over a period of one year, the biodiesel sample was recultured and PCR testing was redone to observe consortia changes at five different time points. Initially, growth was prolific on several types of media and several fungal and bacterial species were identified. Fungi were the dominant species during the early tests but bacterial species became the more dominant organisms as time progressed. By the 6 month time point, growth on culture medium had been reduced significantly with no fungi present and only a few bacterial species growing, which were predominately Gram positive cocci. Organisms found in the B-20 sample included Pseudomonas, Burkholderia, Methylobacterium, Sphingomonas, Ralstonia, Citrobacter, Agromyces, Hyphozyma, and Geotrichum.

Poster 6-17

Cellulose degradation: Two potential novel cellulolytic bacteria able to produce thermostable cellulases

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For the purpose of converting natural cellulose to simple sugars, two aerobic and thermophilic cellulose degraders: *Brevibacillus* sp. JXL and *Anoxybacillus* sp. 527 were isolated from swine waste. These two bacteria can grow on crystalline cellulose as sole carbon and energy sources at 57oC. Regarding B. JXL, it has been revealed that: 1) Cellobiose or glucose can induce more than 10 times higher cellulase activities at concentrations of 0.5 or 1% compared to that from cellulose; 2) Glucose is a better inducer compared to cellobiose over longer experimental period; 3) Cellulases have maximal activity at 70oC and at pH 6-8; 4) Cellulases are thermostable with more than 60% of activity retained after incubation at 80oC for 3 hr. Even at denaturing temperature like 100oC, cellulases are still active; and 4) Under SEM, cellulosome-resembling protuberatant structures are abundant when cells are grown with glucose or cellobiose. In terms of A. 527, it has been observed that: 1) the optimal cellulase activities are obtained at pH 6 and 70oC; 2) Under optimized conditions with the presence of Fe3+, Ca2+, and reducing agent, crude enzymes from the culture supernatant have an activity as 10.0 FPU/ml. Hence, these two bacteria possess great potential in the field of cellulose hydrolysis and warrant further investigation.

Poster 6-18

Screening Soil Metagenomic Libraries searching for Novel Lignin and Cellulose Degrading Enzymes

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Alternative fuels from renewable lignin-cellulosic biomass plant stalks, trunks, steams, leaves, and crop biomass waste, aim to reduce our dependence on fossil oil and to provide a source that decreases the environmental impacts of energy use.(1) Although cellulosic ethanol production has been demonstrated to work in countries like Brazil, attaining a cost-effective, commercial-scale cellulosic bio-fuel industry will require new techniques to lower the price of bio-transforming these materials into bio-fuels. Also there is a high interest in the use of "raw materials" without value in our diet so we don't want to use food crops to convert them to bio-fuels instead of that, we want to use waste crop biomass, switch grass, wood chips and other sources of lignin, cellulose and hemicelluloses. Biotechnological research is the key to accelerating the discovery of new organism that produces unknown catalytically active enzymes that can use these "raw materials" as carbon source and transform them into high energetically value products. There is where the importance of metagenomics relies. Of the microbial diversity in different ecosystems in our planet, only 1% of those organisms are able to grow under known laboratory techniques. That means that 99% of those microorganisms with possible new genes encoding for different abilities remains undiscovered. The goal of our task is to screen a Metagenomic Library constructed from a sample of Tropical Forest soil of Puerto Rico (provided by Dr. Carlos Ríos Velázquez Microbial Biotechnology Laboratory; Department of Biology, Mayagüez Campus) searching for enzymes that degrade cellulose, and lignocelluloses.

Poster 6-19

Characterization and Modeling of Ethanol Production from Gluconate

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Conversion of gluconate to ethanol was investigated with the objective of evaluating gluconate as an alternative carbon source for ethanol production via fermentation as compared to glucose. *Escherichia coli* KO11 was used as the ethanologen. The experimental data showed that gluconate can be fermented to ethanol at high efficiency comparable to glucose. On per mole gluconate basis, about 1.5 moles of ethanol and 0.5 mole of acetate were produced as fermentation products as compared to 2 moles of ethanol produced from 1 mole of glucose. When the fermentation started with equal mole of glucose and gluconate, gluconate was utilized simultaneously with glucose, however at a faster speed. More than 40g/L of ethanol can be produced from a glucose and gluconate mixture in 4 days without any pH control in batch culture. Ethanol production is tightly related to cell growth. Cell growth and ethanol production were inhibited by both ethanol and acetate. A kinetic model including substrate and multiple product inhibition was developed to simulate the cell growth and ethanol production using sodium gluconate as substrate.

Key words: gluconate, ethanol, kinetic model, Escherichia coli KO11

Poster 6-20

Defined co-culture approach for biohydrogen production

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The interesting applications of hydrogen as a fuel and in the chemical industry are providing a driving force for developing more efficient, fully sustainable hydrogen-production methods. In recent years, production of "biohydrogen" from carbohydrate-rich substrates via microbial fermentation has received increased attention. Due to biochemical and thermodynamic limitations, a maximum of four moles of hydrogen can be obtained when one mole of glucose is exclusively oxidized to two moles of acetate. However, the majority of hydrogen-producing fermentations reported in literature, which are carried out using mixed cultures, are resulting in much lower hydrogen yields than the theoretical maximum.

We have previously identified *Caldicellulosiruptor* spp. as potential candidates for biohydrogen production since they efficiently produce hydrogen from various carbohydrates at high yields. In the present work, we combined two *Caldicellulosiruptor* spp. in a defined co-culture for biohydrogen production. The population dynamics in a continuous hydrogen-production system under different conditions were followed using quantitative real-time PCR. Interestingly, the two species stably co-existed in the system under both carbon and non-carbon limited conditions. The hydrogen yields obtained by the co-culture at higher residence times were close to the theoretical maximum, as acetate was the main metabolic end product detected. These yields are at least twice as high as those obtained by most previously reported mixed cultures. Carbon limitation improved the sugar conversion efficiency and slightly increased hydrogen yield and productivity. Depending on the substrate, the developed co-culture might offer an attractive alternative to the traditional "undefined" mixed culture for a cost-effective biohydrogen process.

Poster 6-21

Production of cellulases and hemicelulases by *Penicillium viridicatum* RFC3 on solid state fermentation

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he production of ethanol from the enzymatic hydrolysis of lignocellulosic material has been pointed out as being a promising source of alternative energy. This work evaluated, for the first time, the production of cellulases and hemicelulases by the mesophilic fungus *Penicillium viridicatum* RFC3 on solid state fermentation, using different agro-industrial waste. A better enzyme production was obtained from the cultivation on corncob and wheat bran. The β-glucosidase production, 6.0 U/mL, was high compared to those described in current literature. Activities of xylanase and β-xylosidase were 52.3 U/mL and 1.9 U/mL, respectively. CMCase activity was very low and no avicelase activity was detected. Absence of reports in literature, as well as the levels of enzymes found in the fungus *P. viridicatum*, qualify it as a new and potential source of β-glucosidase, xylanase and β-xylosidase for use in the area of bioenergy.

Poster 6-22

Optimization process using experimental design for biosurfactant production

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Biosurfactants are compounds produced by microorganisms. They present a hydrophilic portion (water soluble) and a hydrophobic portion (water insoluble). These molecules are capable of reducing surface and interfacial tensions in both aqueous solutions and hydrocarbon mixtures, which make them potential compounds for enhancing oil recovery and deemulsification processes. Biosurfactant production using a bacterial strain was studied in this work. The microorganism considered was isolated in previous studies and identified in this work as *Serratia* sp. Initially three media were tested for the biosurfactant production, and the medium 1 presented the best results, after being used in an optimization process, which was conducted in erlenmeyer flasks in a shaker through Experimental Design. During the optimization, we studied the concentrations of the medium components, such as peptone and glycerol, also fermentation temperature and initial pH were studied. The optimum values for biosurfactant production using *Serratia* sp. were between 0.17 and 0.4% for peptone and between 2.0 and 3.14% for glycerol, at 35ºC and pH 7.0. Moreover, some properties of the biosurfactant were studied, for example, its emulsifying capacity and stability at different pH values and temperatures.

Poster 6-23

Screening of microbial fuel ethanol producers using xylose from hydrolyzate obtained from dilute acid pretreated cashew apple bagasse

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The lack of industrially suitable microorganisms for converting biomass into fuel ethanol has traditionally been cited as a major technical roadblock to developing a bioethanol industry. The most widely studied ethanologenic microbes prefer to use glucose as a substrate. Even when yeast cells are modified genetically to use xylose, they ferment all glucose before switching to the much slower xylose fermentation. In the state of Ceará (Northeast of Brazil), the cashew agroindustry has an outstanding role in the local economy and the cashew apple bagasse (CAB) appears as an alternative raw material for ethanol production. The aimed of this work was to screen a number of bacteria and yeasts that can efficiently consumed xylose from CAB. Approximately 78 isolates from this study were tested for growth on xylose as a sole carbon source. Isolates were screened on a MRS modified medium containing xylose as only carbon source and the positive strains were subsequently cultivated in hydrolyzed CAB medium. Three isolates clearly outperformed other strains for rapid growth on xylose as a sole carbon source in MRS. These strains also showed potential to ferment high concentrations of xylose from CAB to produce lactate, acetate and ethanol. Different factors that play an important role in the fermentation were temperature and agitation. One of these isolates was identified as *Lactobacillus* by NCBI analyses of the 16S rDNA sequence which showed 98% identity with that of *L. brevis* deposited in the GenBank. These strains present potential applications in CAB biomass conversion for fuel ethanol.

Poster 6-24

Influence of different substrates on the production of a mutant glucoamylase in submerged fermentation

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Glucoamylase (GA) has a great importance for starch and other related oligosaccharides saccharification in fermentation and food industries. GA hydrolyzes α-1,4 and α-1,6 glycosidic bonds from starch resulting in glucose production. The main use of GA is related to glucose production, which is applied as a feedstock in biological fermentations for ethanol or high fructose syrups production. This enzyme has been used in many researches about molecular biology, mainly regarding to stability improvement. However, there are very few studies about the influence of the kind of starchy substrate on the enzyme production. The aim of this study was to produce a mutant thermostable GA of *Aspergillus awamori* expressed by *Saccharomyces cerevisiae*, using different sources of starch as cassava, potato and corn. The best substrate for GA production was the cassava starch, that shown an enzymatic activity of 6.0 U/mL. The commercial soluble starch (control) displayed an enzymatic activity of 5.8 U/mL. Activities with potato starch and corn starch were 3.6 and 3.0 U/mL, respectively. So, these results show a significant difference on GA production regarding to the carbon source employed. The culture supernatant containing GA from cassava starch was concentrated and diafiltrated against a buffer of 0.5 M NaCl/ 0.1 M NaOAc, pH 4.5, using Amicon S1 spiral ultrafiltration cartridge, and purified by acarbose-Sepharose affinity chromatography. The molecular mass of the enzyme was estimated in100 KDa. The mutant GA exhibited optimum activity at pH 4.5, and optimum temperature of 65 °C.

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Poster 6-25

Physiological characterization of a novel member of the genus *Caldicellulosiruptor* and identification of extracellular cellulolytic enzymes using multi-dimensional LC-MS/MS

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Cellulosic ethanol production from renewable biomass is dependent on the efficient enzymatic hydrolysis of cellulose in order to release fermentable sugars. Cellulolytic microorganisms inhabiting thermal environments are known to possess multidomain/multifunctional cellulases and hemicellulases that display increased heat-stability. Through previous discovery efforts aimed at obtaining novel organisms capable of rapid growth on lignocellulosic biomass; we isolated a new member of the genus *Caldicellulosiruptor* from Obsidian Pool, Yellowstone National Park, Wyoming, USA. The organism, designated *Caldicellulosiruptor sp.* OB47, grows optimally at 80°C and reaches cell densities >10^8 cells/ml on carbon sources such as cellobiose, Avicel (crystalline cellulose), xylan, pectin, filter paper, processed cardboard, and pretreated lignocellulosic biomass (switchgrass and *Populus*). Substrate utilization profiles, growth kinetics, and fermentation end-products were determined and compared against other *Caldicellulosiruptur spp.* and *Clostridium thermocellum*. In order to inventory the proteins involved in cellulose hydrolysis, cultures of OB47 were grown on Whatman #1 filter paper and cellular and extracellular proteins were prepared for proteomics analysis. Multidimensional liquid chromatography mass spectroscopy (LC-MS/MS) was used to identify single components of the major extracellular hydrolytic enzymes expressed by OB47. Throughout biological and technical replicates, roughly 75 non-redundant, extracellular proteins were identified by LC-MS/MS with the most abundant being glycosyl hydrolases, cellobiosidases, solute-binding proteins, as well as S-layer domain-containing proteins.

Poster 6-26

Inhibition of Growth of *Zymomonas mobilis* by Model Compounds Found in Lignocellulosic Hydrolysates

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Fermentation performance in lignocellulosic hydrolysates is substantially inhibited by the presence of toxic compounds which can be formed during pretreatment. The dilute acid pretreatment process gives rise to organic acids, primarily acetic acid, sugar degradation products such as furfural and hydroxymethylfurfural (HMF), phenolics from lignin degradation as well as inorganic salts mainly arising from the pretreatment and conditioning processes. Using a quantitative high-throughput growth assay, a survey of toxicity of the various potential inhibitors including aldehydes, alcohols, organic acids and inorganics was conducted. We will present detailed inhibitory kinetic data on the effect of these compounds, individually and in combination, on growth of *Zymomonas mobilis*. Using this method, we have established a toxicity database for various compounds tested and have begun to prioritize inhibitors based on their relative importance to overall toxicity in hydrolysates for microorganisms.

Poster 6-27

Fractionation of Conditioned Corn Stover Hydrolysates and Inhibition of Ethanologen Growth and Fermentation

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Although many of the inhibitory compounds of pre-treated corn stover hydrolysate have been identified, our understanding of hydrolysate toxicity is still incomplete. By using solid phase extraction techniques to separate hydrolysates into fractions based on their polarity, we tested fractions for toxicity using a quantitative high-throughput microbial growth assay as well as fermentation with *Zymomonas mobilis* 8b as the model organism. Detailed analysis of the chemical composition of the different fractions allows us to differentiate the toxic effects from various compound classes and also provides an opportunity to examine the contribution of unknown compounds to overall toxicity. By using the tools developed in our laboratory for quantifying hydrolysate toxicity under a variety of pretreatment and conditioning processes we may eventually identify those conditions that are favorable for fermentation organisms and provide critical feedback for selecting and optimizing the pretreatment process for biomass to ethanol conversion.

Poster 6-28

Fermentation of syngas produced from gasification of switchgrass by P11

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Syngas fermentation is a thermo-chemical conversion of biomass into liquid biofuels and is carried out in two steps: 1) gasification of feedstocks to produce a mixture of gases which are primarily CO, CO_2 and H_2 called syngas and 2) fermentation of the syngas by microbial catalysts like *Clostridium ljundahlii, Clostridium carboxidivorans* and *Clostridium P11*^{T}. Gas cleaning is an important step in this technology as the contaminants of syngas such as methane, acetylene, ethylene, nitric oxide etc could have deleterious effect on the microbes. The study of the effect of contaminants will help in improving the gas cleaning system thus improving the overall efficiency of the process. In our study, switchgrass syngas was gasified in a fluidized bed reactor to produce syngas with 6.33% H_2, 14.22% CO_2, 15.87% CO, 2.32% CH_4, 0.53 % ethylene, 0.28% ethane, 44.5 ppm NO and the rest N_2. This gas was used as a substrate for growth of bacteria and was compared with a bottled gas mix of 20% CO, 15% CO_2, 5 % H_2 and 60% N_2. There was no growth inhibition due to the presence of contaminants and maximum ethanol concentrations using switchgrass syngas were 2.35 times greater than those using bottled gas. Also, isopropanol was produced at concentrations as high as 3.9 g/l when switchgrass syngas was used. Maximum acetic acid concentrations were found to be 2.75 g/l and 1.69 g/l using control and switchgrass syngas, respectively.

Poster 6-29

Ethanol Production from Sugarcane Bagasse by *Zymomonas mobilis* Using Simultaneous Saccharification and Fermentation (SSF) Process

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During past decades, considerable efforts have been made to utilize agricultural and forest residues as biomass feedstock for the production of bio-ethanol as an alternative fuel. Sugarcane bagasse, composed of 38.1 w% cellulose, 28.4 w% hemicellulose and 18.4 w% lignin, represents the main lignocellulosic material to be considered by most of tropical countries. Compared with *Saccharomyces cerevisiae,* the ethanol yield and specific productivity of *Zymomonas mobilis* are higher, because less biomass is produced and a higher metabolic rate of glucose is maintained through its special Entner–Doudoroff pathway. Fermentation utilizing strains of *Z. mobilis* instead of traditional yeasts and the use of simultaneous saccharification and fermentation (SSF) process has been proposed. Initially, to make easier the accessibility of cellulases to the cellulose microfibrils, the bagasse suffered a pretreatment with diluted acid to extract the hemicellulose sugars fraction and to generate cellulignin. This solid residue was pretread using NaOH (4%), aims at its partial deslignification. Then, the cellulignin suffered the action of a commercial celulolytic preparation, allowing the conversion of cellulose to glucose. This enzymatic pretreatment occurs under temperature of for 12 hours. Thereafter, the temperature was reduced to and the system was inoculated with cells of *Z.mobilis.* Statistical experimental design was used to optimize the conditions of SSF, evaluating solid content, enzymatic load and cell concentration from by submerged fermentation. The optimum conditions were found to be: solid content (30%), enzymatic load (25 FPU/g) and cell concentration (4 g/L), resulting in a maximum ethanol concentration of 60 g/L.

Poster 7-07

Withdrawn

Poster 7-08

Withdrawn

Poster 7-09

MixAlco process: A biorefinery built on the carboxylate platform

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The MixAlco process is a biorefinery that uses the carboxylate platform to process any biodegradable biomass (e.g., municipal solid waste, sewage sludge, manure, agricultural residues, energy crops). It employs the following steps:

1. Pretreatment: To enhance digestibility, the biomass is treated with lime and oxygen.

2. Fermentation: The lime-treated biomass is fed to a mixed culture of acid-forming microorganisms derived from a saline environment. The acids are neutralized with either calcium carbonate or ammonium bicarbonate, thus forming the corresponding carboxylate salts.

3. Dewatering: Using vapor-compression evaporation, the carboxylate salts are concentrated.

4. Chemical Conversion: The carboxylate salts – which are the key intermediates in the carboxylate platform – are chemically converted to a variety of products.

The products include chemicals (e.g., ketones, carboxylic acids, esters, ethers, aldehydes, primary alcohols, secondary alcohols, aromatics, cyclic, olefins) and fuels (gasoline, jet fuel, and diesel).

The advantages of the MixAlco process follow:

· No sterility required in the fermentation

· No genetically modified organisms

· No enzyme costs

· Wide variety of feedstocks can be employed

· Wide variety of fuel and chemical products

· High energy density in fuels

· Low capital cost

· Low product cost

The presentation will describe both pilot and demonstration plants.

Poster 8-07

Uniform-format feedstock supply system for commodity-scale biomass intermediates

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As biorefining conversion technologies become commercial, feedstock availability, supply system logistics and biomass material attributes are emerging as major barriers to the availability of sustainable lignocellulosic feedstocks. The most significant impact these barriers have on a mature lignodellulosic industry is the combined cost associated with harvesting, preprocessing, handling and transporting the material outside of a 50-mile radius footprint. If not addressed, biorefineries will only locate in regions of high biomass production, limiting the participation of land owners in remote or lower production regions where a significant amount of biomass could still be available. In addition, small feedstock supply footprints restrict the size of biorefineries, reduce potential economies-of-scale gains, and increase the risk of supply disruptions due to weather, infrastructure, or competition. To overcome these barriers and decrease supply system risks, an advanced uniform-format feedstock supply system design is proposed that will produce an aerobically stable, mass and energy dense feedstock that meets the specifications of a commodity-scale intermediate product. This uniformly formatted product would then have the attributes necessary to feed a variety of markets including, biochemical fermentation, gasification, pyrolysis, direct combustion, or other conversion technologies. The key to this supply system is a distributed, value add processing facility (Biomass Depot) that takes in low density, unstable biomass and distributes high density, stable commodity products.

Poster 8-08

The effect of wet storage on the value of corn stover as a biofuel feedstock

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As lignocellulosic biofuel technology advances and commercialization is achieved, there will be increasing incentives to characterize and price feedstocks based on downstream processing requirements and potential yield. For biochemical conversion platforms, biomass storage can affect feedstock in two ways: (1) dry matter loss and altered feedstock composition, which will determine the amount of convertible sugars present and (2) the intensity of pretreatment required to optimize fuel yield. The present research investigates wet storage of corn stover and these two effects on feedstock value. Key variables tested included the corn stover collection method, stover moisture content, storage temperature, storage duration, and oxygen level during storage. Feedstock composition before and after storage was determined by Near InfraRed (NIR) spectroscopy. The changes in dry matter and amounts of convertible sugars were quantified and analyzed for impacts on target feedstock price. Reduced –severity pretreatment and fermentation of the samples was used to characterize storage impacts on downstream processing and yield. While structural sugar composition is a major indicator of downstream biofuel potential, inhibitors formed during storage and pretreatment can also interact to influence feedstock value and fuel yield.

Poster 8-09

Potential cost saving strategies for storage of wet biomass feedstocks

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Large quantities of wet agricultural residue feedstock are available for biofuel production, but storage, transportation, and handling costs are prohibitively large. Some potential exists for deriving cost savings through recovery of co-products produced in wet stored feedstocks. And additional savings may be realized as by-products of co-product recovery. This work describes a basic storage cost model and describes laboratory studies that evaluate potential for co-product production in storage. Co-product cost offset estimates based on laboratory results are presented, opportunities for transportation and handling cost savings derived from processing are described, and these potential savings are incorporated into the storage cost model. The analysis suggests wet feedstocks may be competitive with dry cellulosic materials depending on compositional, environmental, and scaling factors.

Poster 8-10

Potential for Biofuel and Animal Feed Production from Native Prairie Grasslands

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Most of the research focusing on the use of native prairie grasses for lignocellulosic ethanol production has been in terms of developing individual species for monoculture, analogous to modern agricultural methods. However, it may be desirable, in certain scenarios, to harvest native mixed-species grasslands for biofuel production. When using minimal agronomic inputs and maintenance, these fields have the potential to provide greater yields than monoculture grasslands, in addition to providing higher value as a wildlife habitat. Additionally, it might be possible to use pretreated biomass produced from these fields as a higher value animal feed.

In order to investigate the feasibility of using native prairie grasses as a biofuel feedstock and animal feed, a number of different samples from fields of differing plant composition were harvested. Ammonia fiber expansion (AFEX) pretreatment followed by either enzymatic hydrolysis or *in vitro* rumen digestibility were performed on these samples in order to determine their value as both a biofuel feedstock and an animal feed compared to untreated material. Because the high diversity in plant species, while environmentally beneficial, may result in widely different optimal pretreatment conditions from one field to the next, a range of pretreatment conditions was tested and the effect on both hydrolysis and digestibility was investigated for each field and compared.

Poster 8-11

The variability of biofuel feedstock availability and delivered price using GIS and the IBSAL dynamic model

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The purpose of this research was to quantify feedstock supply risk over the lifetime of an agricultural residue-based (straw and chaff) biorefinery and to determine the range of delivered prices. The Peace River region of Alberta was used as a case study location, with a geographic information system utilized for data analysis. Inter-year availability of crop residues was highly variable over the 20 year period under study, with the range from 200% of the average availability for the maximum scenario to zero biomass available for the minimum scenario. Biomass availability is a function of grain yield, the biomass to grain ratio, the cropping frequency, and residue retention rate used to ensure future crop productivity. Using minimum, average, and maximum supply scenarios, delivered price was determined using the dynamic (time-dependent) Integrated Biomass Supply Analysis and Logistics (IBSAL) simulation model. Five biorefinery capacities, ranging from 50,000 to 500,000 tonnes of feedstock per year, were analyzed. Since no biomass was available to model in true minimum years, a simulated minimum of half the average availability was used. Delivered cost, including harvest and transportation, for the 50,000 t plant ranged from \$24.01 t^{-1} for the maximum availability scenario at the Sexsmith site to \$42.63 t^{-1} for the simulated minimum scenario at the Fahler site. Since feedstock cost is a large component of total operating cost of a biorefinery, feedstock supply variability and delivered cost inconsistency should be primary decision criteria for any future biorefinery projects.

Poster 8-12

Industrial sustainability of Canada's forest based ethanol industry: Feedstock perspective

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Industrial sustainability of the ethanol industry depends on several factors. Key among the factors is the increasing importance of feedstock type and availability. Most of Canada's ethanol production is obtained from food based feedstock. However, growing concerns on net energy and carbon balances, land use and land use change, water, soil nutrients and biodiversity have led to renewed interest in other feedstock including forest biomass. Although 998 million hectares of Canada is forested, the amount of feedstock available to the ethanol industry is however unclear. In addition to maintaining ecological health and to satisfying societal needs aesthetically, there is additional competition for Canada's forest to supply the raw material for traditional forest industries and the nascent bioenergy sector including lignocellulosic ethanol. Supply of feedstock for the industry is currently achieved through sawmill residues which are highly unpredictable and affected by factors as lumber markets, sawmill closures and competition with the pellet and other industries. This paper employs the material flow analysis methodology to determine the amount of sawmill residue available to the ethanol industry per annum, using input-output modeling. Results obtained indicate that an estimated 1.2 million bone dry tonnes of mill residues are potentially available each year for the forest based ethanol industry. Sustaining the industry from a feedstock perspective will therefore require an effective tracking system of raw materials from sawmill residues and other sustainable-derived additional sources. Suggestions for policy aimed at sustaining the industry without compromising on the ecological health of Canada's forest resources are provided.

Poster 8-13

System Analysis of Integrated Biorefineries

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Among the potential large-scale industrial biorefineries, lignocellulosic feedstock (LCF) biorefinery will most probably be pushed through with the highest success. This study focused on a LCF biorefinery, which integrates biomass conversion processes and equipments to process power, fuels and chemicals. Collected agricultural residues such as corn stover, wheat straw or sugarcane bagasse undergo pretreatment, fermentation and purification to produce ethanol together with organic acids like succinic acid and acetic acid. The advantages of cellulosic feedstocks include a much higher ultimate supply, lower purchase cost, potential reduction of energy input and GHG emissions, and avoidance of competition with food and arable land. By producing multiple products and integrating waste treatment, the biorefinery complex has maximized the values derived from cellulosic feedstocks.

The aim of this study is to quantify the environmental performances of a designed LCF biorefinery mainly focusing on energy use and GHG emissions. The biorefinery is designed using (bio)chemical engineering knowledge and process simulation tools like ASPEN and SuperPro, and then the system is analyzed using LCA tools. Once the designed biorefinery is analyzed successfully, the model system can be expanded to a product-nonspecific framework, in which different production pathways in biorefining are evaluated in order to measure and minimize the energy consumption and GHG emissions. Such a framework provides the opportunities to bridging technical process and product design and environmental analysis, manipulating process and product options to achieve an optimized design, and optimizing biorefineries in terms of technologies, energy efficiency and environmental performances.

Poster 8-14

Barriers and Opportunities for international bioenergy trade – An inventory by IEA Bioenergy Task 40

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This paper presents an up-to-date overview of what major market actors currently perceive as major opportunities and barriers for the current and future development of international bioenergy trade. The work will focus on three internationally-traded bioenergy commodities: 1) bioethanol 2) biodiesel 3) wood pellets. It is based on the results of an online questionnaire and in-depth interviews with mainly industry actors (e.g. producers, traders, consumers and industry associations) and to a lesser extent, the questionnaire will also be sent to policy makers, NGOs and other experts from academia and other institutions. Topics treated include a.o. sustainability certification schemes (and possible impact on trade), logistical barriers, tariff- and non-tariff barriers, and the lack of global classification and clear bioenergy trade statistics. Furthermore, also the role of the food vs. fuel debate and the possible impact of the current economic crisis will be evaluated. The work is a collaboration of IEA Bioenergy Task 40 and UNCTAD.

Poster 8-15

Fuel algae cultivation in municipal wastewater: Some strategic lessons from *Chlorella minutissima*

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For a successful algal biofuel production it is imperative to slash the cost of algal oil to less than $50/bbl. Use of municipal wastewater for algal cultivation could obviate the need for freshwater and the nutrients-N and P. It also adds CO_2 through bacterial activity. Though the wastewater remediation ponds are in use since long but it is essential to determine what makes some algae dominant biomass builders in municipal wastewaters. *Chlorella minutissima* Fott et Nova dominated the entire phycoflora year around and throughout the process of the wastewater treatment at the oxidation pond system of Wazirabad (Delhi) in India. The ability to grow so profusely in such varied and contrasting situations made this alga unique. Besides pollution tolerance, it grew heterotrophically in dark under acidic conditions and as a mixotroph in presence of light over a range of organic C substrates. It utilized both ammoniacal and nitrate nitrogen, survived anaerobicity, 5% NaCl and -10 bar of osmotic stress. *C. minutissima* grew at 4-11 pH and raised the initially set pH (5-8) by 1 to 3 units in 7.5 hours. It showed gigantism, largely kept afloat in presence of utilizable organic carbon, while flocculated in mineral medium and on aging. The alga also possessed potential for biofuel production. The studied parameters indicate why *C. minutissima* was a potential biomass builder in municipal sewage and could be used to determine which other alga(e) may serve the purpose.

Poster 9-07

Withdrawn

Poster 9-08

Membrane based extraction of acetic acid and other compounds from dilute acid pretreated lignocellulosic hydrolysates

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Separations account for 60-80% of the processing costs of most mature chemical processes. However, membrane based separations offer several advantages over conventional technology such as lower energy costs and easy scale up. This technology option was explored as a promising method for removal of acetic acid, sulfuric acid, furfural, HMF and other toxic compounds from dilute acid pretreated biomass hydrolysates. The primary objective was to remove and recover acetic acid, a potent inhibitor of fermentative microorganisms. Experiments were conducted using the liquor fraction of a dilute sulfuric acid pretreated corn stover slurry. Acetic acid, in its protonated form, is extracted into an organic phase consisting of octanol and Alamine 336, a tertiary amine, containing aliphatic chains of 8-10 carbon atoms. Acetic acid removal was most efficient at pH values below 4.8, the pKa of acetic acid. Further, co-extraction of sulfuric acid leads to an increase in hydrolysate pH. The effect of aqueous and organic phase flow rates and temperature on the rate of extraction of acetic acid and sulfuric acid was investigated. Changes in extraction rates may be explained by considering the structure of the acid-Alamine complexes formed in the organic phase. Extraction of furfural, HMF and other toxic compounds was also quantified. Hydrolysates treated by membrane extraction and conventional conditioning technologies were fermented using a glucose-xylose fermenting bacteria to determine the viability of membrane technology to detoxify biomass hydrolysates. Membrane extraction could be a viable hydrolysate detoxification technology because the other conditioning technologies do not remove acetic acid.

Poster 9-09

Microbial Fuel Cells for Removal of Fermentation Inhibitors from Biorefinery Recycle Water

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Microbial fuel cells (MFC) are electrical devices that use microorganisms to convert soluble organic matter into energy in the form of hydrogen and electricity. In this work, MFCs were investigated for their ability to reduce the concentration of known fermentation inhibitors expected from emerging biomass fermentation industry. Specifically, the convesion of furfural, a xylose degradation product, and lignin degradation products such as vanillic acid and 4-hydroxybenzaldehyde was investigated. A new engineering design approach resulted in the high power densities and coulombic efficiencies for the MFC. A 16S rRNA analysis was conducted to determine the composition of the unique exoelectrogenic microbial consortium enriched in the MFC. The consortium demonstrated broad substrate specificity, ability to handle high inhibitor concentrations with near complete removal, while maintaining long-term stability. This approach can lead to: 1) higher ethanol yields at high biomass loading, 2) improved water recycle and 3) electricity production to meet part of the biorefinery power needs.

Poster 9-10

Biodiesel from canola oil using a 1:1 mole mixture of methanol and ethanol

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Canola oil was transesterified using a 1:1 mole mixture of methanol and ethanol (M/E) with potassium hydroxide (KOH) catalyst. Effect of catalyst concentration (0.5 to 1.5 % wt/wt), mole ratio of M/E to canola oil (3:1 to 20:1) and reaction temperature (25 to 75 °C) on the percentage yield measured after 2.5 and 5.0 minutes were optimized using a central composite design. Maximum percentage yield of 98 % was predicted for catalyst concentration of 1.1 wt % and M/E to canola oil mole ratio of 20:1 at 25 °C at 2.5 minutes, whereas a maximum percentage yield of 99 % was predicted for a catalyst concentration of 1.15 wt % and all mole ratios of reactants at 25 °C at 5 minutes. Statistical analysis demonstrated that, increasing catalyst concentration and mole ratio of reactants resulted in curvilinear and linear trends in percentage yield, both at 2.5 minutes and 5 minutes. However, reaction temperature, which affected percentage yield at 2.5 minutes linearly, was insignificant at 5 minutes. The resultant mixed methyl/ethyl canola esters exhibited enhanced low temperature performance and lubricity properties in comparison to neat canola oil methyl esters and also satisfied ASTM D 6751 and EN 14214 standards with respect to oxidation stability, kinematic viscosity, and acid value.

Poster 9-11

Application of Ceramic Membranes to Recover of High Value Hemicellulose from Alkaline Peroxide Pretreated Wheat Straw

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The production of high value co-products will enhance the economic viability of any biorefinery process. Recovery of hemicellulose from biorefinery process streams enables the potential to produce high value chemicals such as water soluble polymers and neutraceuticals like xylitol and arabitol. Alkaline peroxide pretreatment of wheat straw results in a liquid phase comprised of dissolved lignin and hemicellulose polymers as well as a defiberized solid phase. The solid phase can be enzymatically broken down to yield fermentable sugars or is suitable for use in papermaking. The hemicellulose in the liquid phase is still in polymeric form because the mild conditions do not result in further degradation of these polymers to sugars or sugar acids. This stream is an ideal candidate feed stream to produce high value co-products provided the hemicellulose can be recovered in a cost effective fashion. In this project, the potential for use of ceramic membranes to recover the high value hemicelluloses is presented. It is found that good recovery, approximately 85%, of the hemicelluloses polymers can be achieved with 5,000 and 10,000 molecular weight cut-off ceramic membranes. The permeate flux of these membranes is thoroughly assessed as a function of temperature and pressure. The application of membrane back-flushing to enhance the permeate flux is also investigated. In addition, the efficacy of the membranes to remove potential fermentation inhibitors is presented. Finally, results of this study provide data for a techno/economic analysis of the potential for using these membranes in a commercial scale biorefinery.

Poster 9-12

Simple process for production of ethanol from soybean hulls while maintaining protein value

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Soybeans are grown for their protein value and are one of the largest commodity crops in the United States. The soybean hull itself contains cellulose, hemicellulose, pectin, uronic acid and approximately 10% protein by weight. Our research shows the soybean hulls are a source of fermentable carbohydrates for ethanol production. *Saccharomyces cerevisiae* as well as recombinant ethanologenic bacteria readily ferment soybean hulls carbohydrates to ethanol in simultaneous saccharification fermentation mode using commercial cellulase, beta-glucosidase and pectinase enzymes but importantly requiring *no pretreatment*. Bench-scale fermentation process used only non-recombinant *Saccharomyces cerevisiae* yeast and high substrate loading up to 20% w/w. Results showed a significant proportion of carbohydrate fibers are removed by conversion to ethanol while the protein remains intact. Protein and amino acid analysis before and after fermentation revealed up to 2.5X higher concentration of protein with yeast cells accounting for only about 10% of the remaining protein. Among the more than dozen amino acids assayed there was essentially an identical amino acids profile for substrate and product. The U.S., and Brazil plus Argentina each produced about 90 million metric tons of soybeans in 2005, which potentially could yield up to 550 million gallons of ethanol and nearly 3 million ton of higher value protein feed from fermentation of the resulting soybean hulls worldwide, not accounting for the growing market in China. By elimination of the cellulosic fiber matter, the resulting fermentation residues should be suitable for feed for monogastric animals, pigs and chickens, which represent the fastest growing livestock market.

Poster 9-13

Withdrawn

Poster 9-14

Techno-economic evaluation of an integrated biological hydrogen and biogas (BioHythane) production process

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Hythane, a fuel consisting of hydrogen and methane, is an interesting fuel for several reasons. It contributes to lower CO_2 emissions than pure methane due to lower C/H ratio and also improves the combustion, which decreases the fuel consumption. Furthermore it reduces the emission of toxic gases such as NO_x and CO. Moreover, Hythane fits perfectly as a transition step towards the hydrogen economy. However, for it to be a sustainable alternative both hydrogen and methane has to be produced from renewable sources.

One interesting alternative is to combine thermophilic fermentation, producing hydrogen and acetic acid at high yields, with biogas fermentation producing methane from acetic acid. Theoretically 4 mole and 2 mole of hydrogen and methane respectively can be produced per mole glucose. Upstream to the fermentors the saccharides in the raw material (starch based) need to be made available for the thermophilic bacteria, which is done in the pretreatment. The hydrogen and methane rich product gas is purified and concentrated in the gas up-grading through an adsorption-desorption process with diethanolamine.

In this study the whole integrated Hythane from biomass production process is investigated using the commercial flowsheeting software Aspen Plus and the costing tool Aspen Icarus Process Evaluator. The model input was based on literature data as well as data obtained in lab-scale experimental work carried out by partners at Lund University. A what-if study including sensitivity analysis of important process parameters, such as productivities, yields, substrate concentration, retention times and ratio of hydrogen/methane produced will be presented.

Poster 9-15

Oil accumulation from waste via heterotrophic/mixotrophic *Chlorella vulgaris* and *Chlorella protothecoides*

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One of the global issues acquiring significant attention these days is related to energy supply and demand. The irreversible decrease of fossil fuels left to supply energy and the greenhouse gases increase in the atmosphere have led to the utilization of alternate sources of energy, such as oil from microalgae. The aim of this study is to optimize the oil accumulations of *Chlorella protothecoides* and *Chlorella vulgaris* by using carbon sources from waste instead of glucose as the organic carbon source in heterotrophic and mixotrophic culture media. Carbon source concentrations, pH values, temperatures, dissolved oxygen and rotational speeds are studied as factors controlling the growth rate of the microalgae and accumulation of oil. High cell densities achieved from *Chlorella protothecoides* and *Chlorella vulgaris* under heterotrophic-glucose carbon source conditions, 19.7 g L^{-1} and 19.4 g L^{-1}, respectively, are significantly greater than those obtained under autotrophic conditions, 2.07g L^{-1} and 2.15 g L^{-1}, correspondingly. Studies showed superior behavior of the mixotrophic culture of these algae species for the industrial production of liquid fuel than autotrophic culture. Waste materials are applied in this study to partly replace the glucose in order to lower the cost of carbon sources; heterotrophic and mixotrophic microalgae cultures with waste show almost the same cell densities and oil contents as the cultures with glucose.

Poster 9-16

Optimisation of Lipase Production by *Citrobacter freundii*

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Lipases being hydrolases occupy a prominent place among biocatalysts and have a wide array of Biotechnological applications.In the current research the production of Lipase from *Citrobacter freundii* is canvassed. *Citrobacter freundii* was isolated and identified from sewage sample and it was screened for the production of lipase. The organism was inoculated for the production of lipase in the production medium. After the production the broth was subjected to downstream processing and the activity (2, 00,000 U/mg) was computed using precipitation, dialysis & ion exchange chromatography. The enzyme showed optimum activity at pH 5.0, Temp 37°C and with 0.2 mg/ml Activator $MgCl_2$.

Poster 9-17

A simultaneous isomerization and fermentation (SIF) process for efficient co-fermentation of hexose and pentose sugars

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Cost effective production of ethanol from lignocellulose requires full utilization of both hexose and pentose sugars. Glucose is readily fermented to ethanol by *Saccharomyces cerevisiae*, better known as baker's yeast. Xylose, the major pentose sugar released from the hydrolysis of hemicellulose, is not fermented by this same yeast. However, isomerization of xylose to xylulose makes it possible for baker's yeast to convert both the C6 and C5 sugars to ethanol. The xylose isomerization is accomplished using commercially available immobilized xylose isomerase (XI). To accomplish the isomerization under conditions suitable for sugar fermentation, we co-immobilize urease with the XI and add urea to the biomass hydrolysate. In order to reclaim and reuse our co-immobilized enzymes from the yeast fermentation, we have developed a system which incorporates a packed bed reactor for isomerization and a hollow fiber membrane fermentor (HFMF) for sugar fermentation by yeast. The co-immobilized enzyme pellets are placed in the packed bed reactor and yeast cells are loaded on the shell-side of the HFMF. By connecting the two units in series, SIF is achieved under fermentation conditions. This configuration avoids direct contact between the enzyme pellets and the yeast, facilitating recovery and reuse of the enzyme pellets. Furthermore, high yeast density on the shell side is achieved which is crucial for efficient xylulose fermentation.

Poster 9-18

Investigating the changing rheology of high-solids biomass slurries during enzymatic saccharification

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Although exceptionally difficult to transport due to their high viscosities, processing of high-solids slurries will be necessary for the economic operation of lignocellulosic biorefineries. In this work, we examine the changing rheology of high-solids pretreated corn stover (PCS) slurries prior to and during enzymatic saccharification. Prior to saccharification, PCS slurries with fractions of insoluble solids (FIS) ranging from 5-20% (w/w) were found to exhibit rheological properties characteristic of soft solids, including the presence of an apparent yield stress and shear-thinning behavior. Notably, yield stress and viscosity were found to be strong functions of PCS concentration, decreasing roughly ten-fold for each 5% decrease in FIS. During saccharification, FIS drops as glucose and other sugars are liberated by the hydrolysis reaction and move into the liquid phase. As a result, the viscosity and yield stress of PCS slurries within a saccharification reactor diminish rapidly with conversion, by more than an order of magnitude within a few hours. The rheological properties of unsaccharified and saccharified stover agree well when compared at equivalent volume fractions of insoluble particles.

Because the rapid drop in yield stress with conversion is an important design parameter, indicating when a suspension becomes "pourable" or "pumpable", this work will help the process designer identify an optimal residence time during saccharification. In this light, we also present preliminary results suggesting that viscosity-modification / flow-assurance additives may significantly reduce the yield stress and viscosity of PCS slurries, ensuring their downstream processability.

Poster 9-19

Simulation and optimization of an extractive fermentation process for bioethanol production

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Extractive fermentation processes for bioethanol production are advantageous when compared to traditional modes of operation for presenting higher productivity, less water consumption, low vinasse generation and low utilities use for cooling the fermentors.

The process studied in this work consists of a stirred bioreactor linked to a hollow-fiber membrane and a flash vessel under vacuum (for ethanol removal). The high productivity is achieved due to effects of high *S. cerevisiae* and low ethanol concentrations in the system.

The objective of this work is to simulate and optimize the operational conditions of the flash vessel, such as flow rate, temperature and pressure, focusing on the maintenance of the ethanol concentration at 40 Km/m^3 in the bioreactor and on the minimization of water loss in the flash separation. It is well known that at the concentration of 40 Kg/m^3, ethanol does not inhibit *S. cerevisiae* growth and presents an inhibitory effect on fermentation contaminants. On the other hand, the reduction of water content in the top stream of the flash vessel results in a lower fresh water feeding requirement to dilute process medium.

The ASPEN PLUS software will be used to simulate the effect of operational conditions of the flash vessel in the streams and bioreactor compositions.

NRTL (Nonrandom two-liquid) model will be considered in the thermodynamic package of ASPEN PLUS. The complexity of this work is due to the great number of components in the fermentation broth, which change significantly the ethanol-water thermodynamic equilibrium even in low concentrations.

Poster 9-20

A criterion for selecting renewable energy processes

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We propose that minimum incremental cost per unit of greenhouse gas (GHG) reduction, in essence the carbon credit required to economically sustain a renewable energy plant, is the most appropriate social criterion for choosing from a myriad of alternatives. The application of this criterion is illustrated for four processing alternatives for straw/corn stover: production of power by direction combustion and biomass integrated gasification and combined cycle (BIGCC), and production of transportation fuel via lignocellulosic ethanol and Fischer Tropsch (FT) syndiesel. Ethanol requires a lower carbon credit than FT, and direct combustion a lower credit than BIGCC. For comparing processes that make a different form of end use energy, in this study ethanol vs. electrical power via direct combustion, the lowest carbon credit depends on the relative values of the two energy forms. When power is \$70 MWh^{-1}, ethanol production has a lower required carbon credit at oil prices greater than \$80 bbl^{-1}.

Poster 9-21

Integration of fermentation and crystallization for fumaric acid production

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Fumaric acid (FA) is used in industrial polymerization processes. Currently FA is mainly produced from oil-based feedstock. But as oil resources are becoming scarce, renewable feedstock alternatives processes like fermentation, may be required to supply the future demand of this acid [1]. FA fermentation by *Rhizopus oryzae* is inhibited by the presence of FA and its salts that are formed when neutralizing agent is used to control pH. Salts formation lead to excessive downstream processing requirements and high gypsum production [2]. This research aims to produce FA by integration of fermentation and external crystallization. It seeks to avoid the use of neutralizing agents for maintaining pH values at the desired levels and symplifing the number of steps in downstream processesing.

FA fermentation is carried out by *R. oryzae*, via reductive and oxidative pathways. *R. oryzae* can produce 0.9 g FA/(l·h) in stirred tank fermentation at pH 5 [3]. However, for mantaining this pH value neutralizing agent has to be added. In order to avoid neutralizing agent addition, the concept of fermentation at low pH plus product removal by external crystallization of uncharged FA will be developed [4].

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Poster 9-22

High Silica Zeolites as an Alternative to Amine Based Adsorbents in Succinic Acid Recovery

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Succinic acid is one of most attractive renewable C_4 basic chemicals to replace the current chemical building blocks. However, the separation of succinic acid from fermentation broth requires further improvement for sustainable and economical operation. A fundamental improvement would be obtained by selective removal of succinic acid from a low pH fermentation medium (~pH=4) while leaving the succinate salts in the medium. Our approach involves the silicalite molecular sieve adsorbents which adsorb hydrophobic molecules.

Three different powder zeolites, CBV-901, CP811C-300 and CVB 28014, were screened for succinic acid removal from aqueous media. CVB-28014 showed the highest equilibrium loading and was used for the follow up studies. In the presence of Na^+, the succinic acid adsorption dropped in parallel with the succinic acid dissociation and the adsorbent didn't show a significant affinity for charged succinate. At pH ~ 4.4, the adsorbent still performed efficiently. Higher temperatures reduced the equilibrium loadings. But, the effect was too weak to be used for regeneration. Still, it can be used as an auxiliary process condition. In presence of acetic acid at initial concentrations of 25% of that of succinic acid like occurring in fermentation, the equilibrium loading of succinic acid dropped but the succinic acid capacities were still sufficient. Succinic acid in ethanol solution showed a poor adsorption behavior so that regeneration can be achieved by using an adsorption competitive solvent like ethanol. The current results show this type of zeolite is an attractive option for the separation of succinic acid from fermentation media.

Poster 9-23

Withdrawn

Poster 9-24

Reaction kinetics and selective product removal during high-solids enzymatic saccharification

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The rate of enzymatic hydrolysis of cellulose biomass decreases as the reaction progresses, especially when high-solids substrates are used. Due to the high cost of enzyme and need to increase the rate and extent of biomass conversion, it is imperative to identify and attenuate the predominant causes of rate reduction while maximizing enzyme efficiency. Consequently, an experimental design was used to determine the relative effects of four factors: (1) product sugar inhibition, (2) reduced substrate reactivity, (3) unproductive enzyme binding, and (4) enzyme inactivation. Dilute-acid pretreated corn stover (PCS) was used as the lignocellulosic substrate. The effect of product sugar inhibition was determined by using initial rate experiments with initial glucose concentrations ranging from 0 to 50 g/L. Substrate reactivity was estimated by using pre-saccharified, washed PCS at initial cellulose conversions of up to 50%. Unproductive enzyme binding was estimated by comparing enzymatic adsorption and cellulolytic activity on the model substrate Avicel in the presence of commercially-available lignin. Lastly, the effect of enzyme inactivation was investigated by intermittently deactivating the enzyme and reloading fresh enzyme during saccharification. Preliminary results suggest that the rate reduction is predominantly due to glucose inhibition, although diminished substrate reactivity and enzyme inactivation may be significant.

Additionally, a custom ultrafiltration membrane reactor was evaluated for the processing of high-solids biomass. This configuration facilitates the selective removal of inhibitory products from cellulase enzymes and insoluble solids. Finally, the transient product concentration and total mass of eluted sugar from the reactor were modeled using kinetic data.

Poster 9-25

A new low capital process to convert municipal solid waste fiber into ethanol

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Adequate enzymatic hydrolysis of paper from the municipal solid waste has been found by others to require intensive pretreatment which increases capital and operating costs and ultimately the minimum ethanol selling price. We have developed a low cost pretreatment specifically for waste paper which significantly reduces these costs as well as the potential for producing inhibitors to ethanol production. This patent pending approach makes use of differences in physical fiber characteristics to make an 80/20 split of a more hydrolyzable fiber and a smaller less hydrolyzable fraction of fiber that either can be pretreated by itself and added back in, or eliminated from the process entirely depending on economic analysis. A 200 kg mix of waste papers blended to simulate the concentrations expected in the waste stream based on California waste characterization studies was created, pulped, cleaned and fractionated using standard pilot scale sized equipment. Simultaneous saccharification and fermentation (SSF) of the more hydrolyzable fraction using Saccharomyces cerevisiae produced ethanol concentrations of 4% ethanol at 0.29 g/g untreated fiber at 14% solids loading within 68 hours 4% yields of 0.34g ethanol/g wet oxidated clean fiber within 52 hours at 12% solids loading using enzyme additions for both of only 20 FPU/g fiber. The smaller and less hydrolyzable fraction requires more pretreatment. The less hydrolysable fraction has about ½ the conversion in untreated state as more hydrolyzable fraction, but further cleaning (removal of ash) followed by a more concentrated wet oxidation or addition of surfactants before hydrolysis can optimize the overall process economics.

Poster 9-26

Compositional analysis for the 21st century: High throughput methods to evaluate lignocellulosic biomass for bioethanol production

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As efforts to develop environmentally and socially sustainable biofuels intensify, interest in lignocellulosics has increased. While the potential exists for these feedstocks to produce a "greener" biofuel, the number of available types of biomass is overwhelming. In order to optimize land and resource use, the specific biomass chosen for a given area must provide the greatest yield of ethanol per unit area of land with minimal energy inputs. A high throughput screening system is essential to determine which feedstock will provide the highest yield of ethanol. The barriers to development of such a system lie in the fact that while the bioconversion subprocesses of pretreatment, enzymatic hydrolysis and fermentation can be time consuming, the real bottlenecks of the screening process are the analyses of carbohydrates and other by-products.

Conventional carbohydrate analysis by HPLC is lengthy, taking up to an hour per sample. Other methods of analysis cannot simultaneously measure five sugars. Fortunately, our group has developed spectroscopic methods of analysis including Raman and IR that allow complex mixtures of sugars to be rapidly and non-destructively analyzed on line in minutes per sample. In addition, a 15 minute, 5-sugar HPLC method has been developed to validate these new methods. To take full advantage of both rapid analysis techniques, high throughput hydrolysis and fermentation reactions in microwell plates have been developed. The reactants and products are directly measured without the need for lengthy sample preparation. This streamlined system of reactions and analysis allows multiple feedstocks and process conditions to be evaluated simultaneously.

Poster 9-27

Separation of algae cells from the solution using cationic polymers combined with ferric chloride

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Rapid growth in the bio-fuels industry recently has put tremendous pressure on food and animal feed supplies and agricultural land uses. In order for the bio-fuels industry to sustain and continue to grow, new non-food or non-feed biomass feedstock must be implemented and developed. The biomass production and oil content from algae is far superior to that of terrestrial plants such as soybean, corn etc. Because of the small size of algal cells, however, and comparably low dry biomass content of algal suspensions, the regular methods of separating are expensive and time consuming. On account of this, we studied such processes including algae cells in the culture separated with different kinds of polymers combined with Ferric Chloride. The entailed process of an adjustment of culture pH to between 8.50 and 9.50 followed by addition of different kinds of cationic polymers to a final concentration of 0.3 mg L^{-1} combined Ferric Chloride with were investigated. This process was successfully employed to harvest cells of *Chlamydomonas reinhardtii* with efficiencies reached up to 85%. The process was high efficient and relatively cost neutral compared with concentration of algae by other chemical or physical methods. These experiments demonstrated that a commercial application of algae concentrated prepared by flocculation is feasible.

Poster 9-28

Biodiesel production from integration between reaction and separation system: Reactive distillation process

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The purpose of the present work is to develop an efficiency process using reactive distillation columns applied to biodiesel production. Reactive distillation is the simultaneous implementation of reaction and separation within a single unit of column. This combined operation is especially suited for the chemical reaction limited by equilibrium constraints, since one or more of the products of the reaction are continuously separated from the reactants. This work presents the biodiesel production from castor oil and bioethanol by reactive distillation. Different variables affect the conventional biodiesel production process such as: catalyst concentration, reaction temperature, level of agitation, alcohol: vegetable oil molar ratio, reaction time, raw material type. In this study, the experimental design was used to optimize the following process variables: the catalyst concentration (0.5 %wt to 1.5%wt), the castor oil : ethanol molar ratio (3:1 to 6:1), the reactive column reflux ratio (0.5 to 3.5), and the ration time (1 to 5 minutes). The results showed many advantages of the integration processas compared with the conventional biodiesel production such: decrease of the energy requirements, ethanol excess, reaction time, equipments units.

Poster 9-29

Biodiesel production from castor oils: Optimization of alkaline ethanolysis and scale up

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This work presents the biodiesel production from castor oils with bioethanol in the presence of sodium ethoxide and sodium hydroxide as catalyst. The studied variables were reaction temperature, catalyst concentration, and ethanol: castor oil molar ratio. The experimental design was used in the process optimization because this methodology permitted a careful examination of the process variables. A real model that described the biodiesel conversion in terms of temperature, molar ratio ethanol/castor oil was identified. A small scale up was realized with a batch stirrer reactor and these results were compared with the magnetic agitation. A conversion of 99 %wt of ethyl ester was obtained at 30°C with 1%wt of catalyst after 30 minutes of reaction. The kinetic study of the transesterification ration was realized. The apparatus used for the experiment was a 1-L jacketed reactor, equipped with a variable speed agitator. The temperature of the reactor was controlled and maintained at 30, 40, 50, 70 °C. The reaction time was 30 minutes and samples were collected and analyzed in an HPSEC (High performance size-exclusion chromatography). The castor oil molecular weight was determined using the gas chromatography analysis and vapour pressure osmometry technique (VPO).

Poster 9-30

Ethanol Production from Sorghum Grain (*Sorghum bicolor* [L.] Moench): Optimization of the Enzymatic Hydrolysis and Evaluation of the Hydrolysate Fermentability

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Sorghum is a poaceae originally from Africa which is listed as the fifth cereal most cultived in the world. It is cultivated between the soybean harvesting season in some areas of Brazil and has showed great potential in ethanol production besides having wide adaptability, rapid growth (cicle from 90 to 120 days), and it is tolerant to drought, water logging, soil salinity and acidity toxicity. Also it is more resistent to hydric stress than corn. According to CONAB, in 2007 Sorghum production was 1.6 billion tons in Brazil. The main objective of the present work was to study ethanol production from Sorghum grains [*Sorghum bicolor* (L.) Moench]. Initially starch enzymatic hydrolysis was optimized using comercial alpha amylases and glycoamylases, considering particle size, solid:liquid ratio and enzyme load as variables. The hydrolysate, in its optimum conditions, was used to produce ethanol, batchwise in a Biostat B reactor, using an industrial strain of *Saccharomyces cerevisiae* as the fermentative agent. The bioprocess was monitored and its kinetic profile of alcohol production and the consumption of substrate were constructed during 36 hours. The optimum conditions of hydrolysis was as follows: particle size of 1mm, solid:liquid ratio of 1:3 and enzyme load of 20 mcL of alpha amylase and 40 mcL of glycoamylase per g of grain. The maximum ethanol concentration produced was 117 g/L, corresponding to 28 g of ethanol/100 g of Sorghum in 29 hours of fermentation.

Poster 9-31

An intelligent system for multivariate on-line monitoring of a continuous flash fermentation process

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This study presents results from the implementation and testing of a PC based monitoring system for a bioethanol production process using Artificial Neural Network-based software sensors (ANN-SS). The system is based on an array of primary sensor, a communication module and a monitoring and data acquisition subsystem. This integrated framework provides a real-time monitoring solution, which is one of the most important aspects of the decision making in the strategies of optimization and control of bioprocesses. A LabVIEW data acquisition module is implemented to read all influencing variables, which are first used to train the neural networks. The optimal ANN architecture is placed in a LabVIEW based program formula node that monitors the concentration of bioethanol. The ANN-based monitoring system represents thus a robust model-based approach which is expected to contribute for improving the implementation of suitable operating strategies of optimization as well as advanced control to achieve high operational performance.

Poster 9-32

Understanding Mass Transfer in Lignocellulosic Ethanol Using Whole Cell Biocatalyst

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Simultaneous saccharification and fermentation would vastly improve the prospects of large-scale economical production of Lignocellulosic ethanol. One of the approaches to achieve this objective is to use a whole cell biocatalyst that can express clostridium cellulose enzyme system on the surface of ethnogenic *Escherichia coli* LY01. LY01 is capable of co-metabolizing pentoses and hexoses. This approach has been successfully demonstrated in our work with amorphous cellulose and dilute acid pretreated Corn Stover. However, scale-up of such a system would require an understanding of transport phenomena in the heterogeneous hydrolysis of cellulose by such whole cell biocatalyst. In particular, we consider the hydrolysis of dilute acid pretreated Corn Stover by this system in a bioreactor. A model is developed that can account for pore sizes, particle sizes and flow characteristics. The validity of this model will be tested against fermentation data from a bioreactor. The bioreactor allows the control of conditions especially pH which critically affects the enzyme activity. The effect of rate of agitation and particle size distribution are of particular interest. Experiments have also been performed to demonstrate co-metabolism of hexoses and pentoses, which would reduce the entire process to one-step ethanol production

Poster 9-33

Influence of nutrients supplementation on ethanol batch production rates

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Ethanol is a renewable energy source produced through fermentation of sugars unlike the fossil fuels. During fermentation of *Saccharomyces cerevisiae*, the activities of the microorganisms closely respond to changes in the environmental conditions, which are accompanied by variations in the metabolic behavior of the microorganisms. The nutritional necessities of the yeast during alcoholic fermentation influence the cellular growth and the efficiency of ethanol production. The aim of this work was study the influence of nutrients supplementation of the cane molasses and sugar cane musts on the batch ethanol production. Batch fermentation experiments were carried out with sucrose of cane molasses and sugar cane musts as the sole carbon source for *S. cerevisiae*. The nutrients supplementation was done with three commercial nutrients (A, B and C) with different compositions. Fermentation flasks were shaken in the incubator at 200 rpm and 32ºC. The musts were analyzed to determine the amount of each nutrient before the supplementation. Samples were withdrawn and after analysis production rates like fermentative efficiency, ethanol productivity and yield were calculated. For cane molasses must, it was found that concentrations of nutrients that maximize the efficiency and productivity in the fermentation process were: 0.20 g of A/L; 0.50 g of B/L and

0,30 g of C/L of juice. And for sugar cane must these concentrations were: 0.50 g of A/L; 1 g of B/L; and 2 g of C/L. The results consequently provide a better understanding of nutrients supplementation effects on the cell activities for further development of the process.

Poster 9-34

One Step Cellulosic Ethanol Production: Modeling of Enzymatic Hydrolysis of Cellulose by Whole-cell Biocatalyst

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A whole-cell biocatalyst system was successfully developed in our research to directly produce ethanol from amorphous cellulose (Phosphoric Acid Swollen Cellulose, PASC) in a single step. It was also applied for ethanol production from diluted acid pretreated corn stover. For enzymatic hydrolysis of natural biomass, there are several factors determining hydrolysis rate e.g., crystallinity, degree of polymerization, particle size, pore volume, and accessible surface area. The enzymatic hydrolysis mainly occurs on the surface of cellulose. In order to understand the behavior of whole-cell biocatalyst on cellulose, we focus on the relationship between particle size in the sense of accessible surface area of cellulose and hydrolysis rate. The surface morphology of surface of cellulose exposed on the microorganism incorporating with cellulases is observed by SEM. In addition, a model of showing the correlations between the accessible surface area of pretreated corn stover and enzymatic hydrolysis rate is developed. Hence, the quantitative model and the experimental measurement can be applied to develop the hydrolytic capacity of whole-cell biocatalyst with the respect to a particle size of substrate and enzyme.

Poster 9-35

Bioethanol production from germinated grain by inherent enzymes

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The malting in brewing process develops enzymes that are required to hydrolyze the complex starch in grain into simple fermentable sugars. These proceed the three following steps: Steeping encourages germination to start, germination prepares the conversion of the starch to sugars, and kilning stops the germination.

In this study, a method for bioethanol production from rye grain was developed by utilizing the inherent amylase activity from germination of the seed. Grain germination was performed in two steps (steeping, germination) under different conditions, where the effect of temperature, duration and humidity was examined on amylase activity and final ethanol yield. Commercial enzymes were used for reference experiments.

Simultaneous Saccharification and Fermentation (SSF) was performed to reduce end-product inhibition of the amylases during ethanol fermentation. Using cheap nutrient source (cheese whey) minimized the use of water and chemicals with reducing process costs. Whey is a by-product from the dairy industry, which represents a disposal problem. However it containing valuable sugars (lactose) which can be used for bioethanol production. Thermotolerant *Kluyveromyces marxianus* DSMZ 7239 was used in the SSF process since it is able to convert all C6 sugars including lactose to ethanol,

The process was proved successfully. The results showed that germination phase was strongly affecting the final ethanol yield, which could be increased with up to >90% of theoretical. Contaminated grains which can not be used either as food or feed were also involved in our research.

Poster 9-36

The K_La influence on ethanol production by *Pichia stipitis*

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Nowadays, there is a great interest in the development of technologies for ethanol production as an alternative combustible, since it can be used instead of petrol or to blend with petrol, reducing the country's dependence on oil and imported fuel. It is known that the ethanol production by fermentation is influenced by several process conditions such as pH, temperature, medium composition, oxygen availability, among others. Determining the most suitable fermentation conditions is of large importance for the establishment of a successful technology. In the present work, the influence of oxygen transfer volumetric rate (K_La) on xylose to ethanol bioconversion by the yeast *Pichia stipitis* NRRL Y-7124 was evaluated using a semi-defined fermentation medium containing 90 g/l xylose. The assays were carried out in a bioreactor at 30°C, under different aeration conditions (0.5, 1.0, and 1.5 vvm) and stirring rates (200, 300 and 400 rpm) which resulted in K_La values of 2.3, 18.7 and 65.8 h^{-1} respectively. According to the results, the bioconversion was dependent on the aeration rate employed, the highest ethanol production (27.1 g/l) being achieved when using a K_La of 2.3 h^{-1}. The increase of this parameter to 18.7 and 65.8 h^{-1} promoted decreases of 52% and 100% on ethanol production, respectively. By using a K_La of 65.8 h^{-1} the ethanol production was totally deviated to biomass production. Such results are of interest for the development of a suitable technology for ethanol production by *Pichia stipitis*.

Poster 9-37

Ethanol production by fermentation using immobilized cells of *Saccharomyces cerevisae* in cashew apple bagasse

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Cashew apple, source high concentrations of reducing sugars, is a common pseudofruit in the Northeast of Brazil. However, only 12% of the total peduncle is processed and it does not play an important role to the economy of the state. Therefore, ethanol production from cashew apple represents an alternative for this residue. Cell immobilization improves productivity and provides a physical protection to the yeasts. In this work, cashew apple bagasse (CAB) was used for *Saccharomyces cerevisiae* immobilization. The support was prepared through a treatment with a solution of HCl 3% (v/v) at for 2.5 hours. Then this mixture was washed until neutral pH and dried. Deslignification with NaOH 2%(p/v), at 120 rpm, for 24 h was also conducted. Afterwards, the support was washed with water until neutral pH and dried at. Ten consecutive fermentations of cashew apple juice for ethanol production were carried out using immobilized yeasts. Before each cycle, samples were taken before inoculation and after 6 hours of fermentation. After that time, fermentation process was stopped and the support containing immobilized cells was washed with water and reused. A Neubauer-counting chamber was used to quantify the yeasts immobilized in CAB and optical density (OD, λ=660nm), to identify free cells growth. Reducing sugars consumption and ethanol production was analyzed by HPLC. High ethanol productivity was observed from the second fermentation assay until the tenth fermentation. Results showed that cashew apple bagasse was an efficient support for cell immobilization aiming at ethanol production.

Poster 9-38

Modeling temperature variations in a pilot plant thermophilic anaerobic digester

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A model that predicts the changes of temperature in a pilot plant anaerobic digester was developed. The pilot plant, working under thermophilic conditions, was previously described by our research team (Espinosa-Solares *et al*., *App. Biochem. Biotechnol.* (129-132):959-968, 2006). Mass and energy balances under steady and unsteady conditions were applied to develop the model, which was parameterized based on data obtained from bioreactor actual operation routines conducted under different heating strategies. To validate the model new experimental routines were applied for different feed-loading frequencies and also process was simulated by solving equations numerically. The comparable simulated and experimental data allowed accepting the validity of the model. A sensitivity analysis established that the most important causes of the cooling of the mass inside the tank were the heating of the feed and the exit of the effluent.

Keywords: Modeling, Temperature prediction, Mass and energy balances, Overall heat transfer coefficient.

Poster 9-39

Light regime characterization in an airlift photobioreactor for production of microalgae with high starch content

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Biological CO2 fixation and energy production are potential measures expected to mitigate the increase of atmospheric CO2 concentration and to minimize future energy crises. The slow development of microalgal biotechnology stems from the failure in the design of large-scale photobioreactors where light energy is efficiently utilized. Due to the light gradient inside the reactor and depending on the mixing properties, algae are subjected to light/dark cycles where the light period is characterized by a light gradient. These light/dark cycles will determine productivity and biomass yield on light energy. This work reports on the characterization of the light regime in a photobioreactor, based on the airlift principle, that enhances productivity by using the flashing-light effect, determining the time that microalgae spend in the dark and photic zone through the liquid circulation time (10–100 s), which depends on the superficial gas velocity and reactor design (e.g. baffles). The method combines the utilization of particle tracking, signal analysis and optical fiber technology, which altogether give information about temporal and spatial aspects of light patterns. The quality and amount of the light reaching a given point of the photobioreactor were determined and correlated with cell density, light path length and hydrodynamic characteristics of the bioreactor. The importance of this work lays on the fact that it describes the light distribution profile, and therefore the irradiance conditions, more precisely. Moreover, the analysis of the photobioreactor system based on local available light energy presents a valid means of determining the algal cell growth rate.

Poster 9-40

Production of Lactic Acid from Sucrose: Strain Selection, Fermentation and Kinetic Modeling

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Lactic acid is an important product arising from the anaerobic fermentation of sugars. It is used in the pharmaceutical, cosmetic, chemical and food industries as well as for biodegradable polymers and green solvents production. In this work, were used several bacterial strains isolated from industrial alcoholic fermentation, for screening of the best strain to lactic acid production. After of the screening was realized the fermentation with sucrose for lactic acid production. The fermentation was realized in batch system under anaerobic conditions for 48 hours, at a temperature of 37°C, a pH value of 4.5 and 15 g/L of sucrose. Aliquots of the fermented broth were collected every 90 min for determining lactic acid, sucrose, biomass, and byproducts concentration. The pH of the medium was adjusted with sodium hydroxide. A chromatographic system equipped with an Aminex HPX-87H column and an UV and RI detection system was used to analyze sucrose, lactic acid, and byproducts of the fermentative process. To biomass determination liquid samples were centrifuged at 3000 rpm for 15 min and the cell pellets were washed twice with water. The cell were resuspended and dried in the stove at 75°C and dry mass was weighed. A kinetic model has been developed for represented in batch fermentation to produce lactic acid by strains isolated from industrial alcoholic fermentation. The data obtained from the fermentation were used for determining the kinetic parameters. The developed model allows very good predictions for lactic acid production, growth biomass and sugar consumption compared to experimental data.

Poster 9-41

Synthesis of Biodiesel from Non-Edible Oil and Solid Catalyst

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Biodiesel, which has many useful properties such as cetane number, volumetric heating value and flash point, has been used as alternatives of petro-diesel and chemical substitute. Its usage has several advantages, since it may be an eco-friendly, alternative and reversible energy source. Although, biodiesel has become more attractive, the main remaining problem is its production costs. In our previous studies, many factors affect the biodiesel synthesis process such as the free fatty acid and water content of the feedstock, the amount of alcohol, the amount and type of catalyst, the reaction temperature, the mixing strength, and reaction time. In this study, we synthesize the biodiesel from non-edible oil and solid catalyst applying the statistical methodology. For optimization of operation parameters, we applied response surface methodology to delineate the effects of five-level-three-factors and their reciprocal interactions on biodiesel synthesis.

Poster 9-42

Production of acetone-butanol-ethanol (ABE) by direct fermentation of cassava using *Clostridium saccharoperbutylacetonicum* N1-4

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In this research, Acetone – Butanol – Ethanol (ABE) fermentation characteristics of cassava when using *Clostridium saccharoperbutylacetonicum* N1-4 was reported. Cassava is widely grown for its enlarged starch-filled roots, which contain nearly the maximum theoretical concentration of starch on a dry weight basis among food crops. Besides that, cassava is able to grow in poor soils on marginal lands with minimal amounts of fertilizer, pesticides and water. Therefore, cassava is a promising crop for biofuel production from renewable resources. Furthermore, for many Asian countries, cassava is not the main food therefore the food security concerns would be minimized. Fermentation was carried out in batch mode using 1-L fermenter. The obtained results showed that that *Clostridium saccharoperbutylacetonicum* N1-4 fermented cassava mash efficiently to produce ABE under appropriate nutritional and environmental conditions. Batch fermentation of cassava mash resulted in 23 g/L of total ABE production when supplemented with TYA medium nutrients.

Keywords: cassava/tapioca, acetone butanol ethanol (ABE) fermentation, *Clostridium saccharoperbutylacetonicum* N1-4

Poster 9-43

Immobilization of Nitrifier for Nitrogen Removal

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The immobilization of biocatalysts (cells or enzyme) and its application have recently been the subjected of increased interest, which may be defined as the physical confinement or localization of intact cells to a certain defined region of space with the preservation of some desired catalytic activity. Biocatalysts immobilization techniques offer a promising potential for the improvement of the efficiency of bioprocess, the production of useful metabolites and biological wastewater treatment. The immobilization of biocatalysts has also attracted attention due to several advantages, including the easy separation of liquid and solid in a setting tank, a high loading biocatalysts content, the preservation of biocatalysts from the external environment and the prevent of wash-out. Many immobilization methods have been suggested for applications to microorganisms. Several natural materials and synthetic polymeric matrices have been used for cell immobilization. In this study, The objective of this experiment are to immobilize nitrification microorganisms to several support materials and to measure nitrification rate of immobilized carriers.

Poster 9-44

Statistical Optimization of 1,3-propanediol Fermentation Using the Engineered Strain of *Klebsiella pneumoniae*

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1,3-propanediol (1,3-PD) as an important chemical product could be used for synthesis reactions, in particular as a monomer for polycondensations. *Klebsiella pneumoniae* is one of the several well-known microorganisms, which produce 1,3-propanediol (1,3-PD) from glycerol. *K. pneumoniae* is an excellent 1,3-PD producer, but too much by-product (2,3-butandiol, Ethanol, Succinic acid, etc.) yielded greatly reduce the fermentation efficiency of 1,3-PD. We developed mutant strains by genetic engineering the glycerol metabolic pathway. In the present study, we attempted to optimize culture conditions for 1,3-PD by the engineered strain of *K. pneumoniae* using response surface methodology (RSM) based on a 2^5 factorial central composite design (CCD) where the simultaneous effect of five independent variables, glycerol, aeration volume, pH, cultivation temperature and time.

Poster 9-45

Characterization of α-amylases for the removal filter cake on petroleum wall

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Drilling fluid has many functions as carry stones from the hole permitting their separation at sea surface, cool and clean the bit, reduce friction between the drill pipe and wellbore, maintain the stability of the wellbore, prevent the inflow of fluids from the wellbore and form a thin, low-permeable filter cake. The use of drilling fluid reduces wellbore productivity, because it deposit as a filter cake on the wellbore wall.
Laboratory studies have demonstrated that drill-in fluid filter cake can be effectively removed through the application of a newly developed technique incorporating an enzyme-based polymer degradation system. The drill-in fluids are typically comprised of starch, the most important component of the filter-cake. Starch in water-base drilling fluids increases viscosity for friction reduction and lubrication. The amylases are highly efficient to degrade native and chemically modified starches, the α-amylases hydrolyze starch molecules to fine diverse products as dextrin, and progressively smaller polymers composed of glucose units.

Studies herein reported the characterization of four α-amylases and defined the most qualified enzyme to attempt the standards used on this system. Results achieved prove that hydrostatic pressure can been used to increase the activity and stability as well as reported for different enzymes, including thermolysin, in literature.

Poster 9-46

Microalgae production of lipids and starches for bio-fuel production

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Microalgae, fast-growing, efficient converters of carbon dioxide and solar energy offer strong potential for biofuels production at high levels of biomass per unit land area in comparison to other plant materials. As part of an integrated process for converting municipal and industrial wastewater sources to bio-oil and fermentable sugars, current results are presented here from a study of microalgae species used for converting residual nutrients in wastewater. This aspect of the project is focused on determining the feasibility of photosynthetic sugar production by microalgae species in comparison to lipids production as a means of both CO_2 consumption and the coupling of this step with an oleaginous microbial consortium capable of producing triglycerides from these sugars. Results from batch and fed-batch reactors will be presented for raw microalgae isolates and compared with results from *Botryococcus sudeticus (UTEX 2629).*

Poster 9-47

Mixed Culture Acidogenic Fermentation and Acid Extraction from Pre-Pulping Wood Extract

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Pre-pulping extraction is a means of deriving a hemicellulose-rich process stream from the front end of a kraft pulp mill. When the extraction is carried out using green liquor, pulp quality and quantity can be retained while still releasing hemicellulose and acetic acid for recovery as bioprocessing feedstock or chemical products. Acetic acid is naturally present in the extract and its concentration can also be increased through conversion of hemicellulose sugars to organic acids. Either source may provide sufficient value to justify recovery and purification. In this study, mixed culture acidogenic fermentation was done on green liquor pre-pulping liquor and a liquid-liquid extraction method was applied to separate organic acids from the same hardwood extract.

We determined the production levels of carboxylic acids using mesophilic and thermophilic cultures with green liquor salts and calcium carbonate as the buffering agents. Conditions of combined extraction and fermentation conditions were assessed to determine optimal yields of carboxylic acids. The organic acid removal process utilized a solution of trioctylphosphine oxide (TOPO) and un-decane contacted with the wood extract. TOPO has strong hydrogen bonding acceptor properties that induce the carboxylic acid to transfer to the extract phase. The two phases are immiscible and separate gravimetrically. Distillation is used to separate organic acids from the solvent phase, which is recycled back to the extraction. Results present the extraction and recovery efficiencies.

Poster 9-48

Evaluation of Ammonium Hydroxide for Conditioning Dilute Acid Pretreated Corn Stover

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Lignocellulosic biomass pretreated by dilute acid produces monomeric hemicellulosic sugars and enhances the ability of enzymes to hydrolyze cellulose to glucose. However a conditioning process is usually required to remove compounds inhibitory to fermentative microorganisms. Previous work determined that an ammonium hydroxide conditioning (AHC) process decreased fermentable sugar losses and increased ethanol yields compared to a standard overliming process. The goal of this work was to 1) to rigorously quantify fermentable sugar losses (glucose and xylose) that occur during the AHC process, 2) determine the effect of conditioning pH on fermentation performance, and 3) investigate the effects of AHC liquor on enzymatic hydrolysis of dilute acid pretreated corn stover. The results indicate that regardless of the conditioning pH, the combined glucose and xylose loss was less than 1%, well below the 12% and 13% loss of glucose and xylose, respectively, previously determined using an overliming process. Fermentation testing showed that liquors treated at a conditioning pH of 6.8 and 8.0 achieved nearly identical ethanol yields of 70.3% and 71.6%, respectively. The best ethanol yield value of 79% was achieved with liquor conditioned at pH 8.5. At a conditioning pH of 9.0, the ethanol yield was dramatically reduced to 63%. Although ammonia conditioning led to improved fermentation performance, it had a slight inhibitory effect on enzymatic hydrolysis. Enzymatic cellulose conversion of pretreated corn stover solids in the presence of AHC liquor was reduced by 2% to 6% depending on enzyme dosage compared to solids in the presence of overlimed liquor.

Poster 9-49

Improvements to the Analysis for Triglycerides, Diglycerides and Monoglycerides by Liquid Chromatography by Using 2.2-µm C18 Columns with Alternative Solvent Systems

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In the production of ester-type biodiesel fuels, analysis for glycerides has two important functions: one is to characterize the natural lipids for carbon-chain length and unsaturation in the feedstock, and the other is to measure residual glycerides after transesterification. New methods based on liquid chromatography (LC) are displacing earlier methods based on gas chromatography (GC). Even so, there is room for improvement. The well-known ASTM method for residual glycerides in biodiesel by LC requires long run times and a chlorinated solvent because of the type column used in the separation. A variation of the method may be used to get a high-resolution profile of the triglyceride composition of natural lipids. Newer LC columns with particle sizes in the range of 1.5—2.5 µm and the instrument systems that support them permit the optimization of lipid analysis in ways that were previously not available. For these analyses, we demonstrate short run times, reduced solvent consumption and elimination of chlorinated solvent by using 2.2 µm C18 columns, temperature control and replacement of dichloromethane by alcohol or ester solvents.

Poster 9-50

Development of Jatropha Oil Extraction From Biodiesel Feedstocks Using Accelerated Solvent Extraction

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The determination of oil content in biodiesel feed stocks can be performed using several methods, including mechanical press, solvent extraction, and Nuclear Magnetic Resonance. For the feedstock quality control in terms of oil content, it is important that the applied method is universally accepted so as to obtained results that can be compared with those reported from alternate sources. Although the EN (european norm) has specified two methods for the determination of oil content in oil seed crops, i.e. a conventional Soxhlet extraction and NMR imaging, they also have some disadvantages, including time-consuming, intensive labour input, requiring of highly skill labor, significant amount of sample, high cost, as well as being unfriendly to the environment. The accelerated solvent extraction technique developed by Dionex (ASE-100) has great potential to overcome these constraints. Furthermore, it also has a high possibility to be applied with the oil content testing of the third generation of biodiesel feedstocks, i.e. microalgae. In this paper we present the extractraction of oil from Jatropha seeds in compliance with accuracy and reproducibility requirements described in the European standard method. Oil extraction using ASE-100 requires only one to one and a half hours as compared to nine hours consumed by the Soxhlet extraction. In this research, the effects of ASE-100 conditions on the percentage of oil extracted, the optimum conditions for oilseeds extraction and the minimum amount of oilseeds required per test are also investigated.

Poster 9-51

Possible co-products from Sweetgum (*Liquidambar styraciflua* L.)

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Sweetgum (*Liquidambar styraciflua* L.) is an understory, hardwood species that has a widespread distribution in the southeast United States, and is of interest due to its co-existence in forest management areas that are logged for softwoods such as pine. Sweetgum trees must be harvested prior to logging, and represent a residue biomass that can be utilized in the production of cellulosic biofuels. In addition, valuable co-products can be extracted by distillation from the sweetgum leaves, fruit and annual stems. These co-products include flavonoids, such as shikimic acid, which is a component of an antiviral drug, and terpenoids, which are thought to have therapeutic properties similar to tea tree oil. The antimicrobial properties of sweetgum extracts may develop into a use in the food industry. *Listeria monocytogenes,* a gram-positive foodborne pathogenic bacterium, can cause listeriosis, which has a mortality rate of nearly 28%. The objective of this study is to determine if antimicrobial compounds, such as flavonoids and terpenoids, can be extracted from sweetgum prior to its conversion to biofuels. It is important that the compounds are extracted by water distillation so that the extraction step does not hinder the ensuing pretreatment, enzymatic hydrolysis and fermentation steps. Water is an excellent solvent for the extraction of co-products because it does not interfere with biomass conversion to energy by decreasing yields or adding processing steps. Preliminary results indicate that sweetgum water extracts have inhibitory effects against *L. monocytogenes* and *Escherichia coli* O157:H7.

Poster 9-52

Micellar Enhanced Detoxification and Extractive Fermentation in Surfactant Systems for Biofuel Production

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A major obstacle for effective fermentation of monomeric sugars in lignocellulosic hydrolysates is the presence of inhibitory compounds which are toxic to fermenting microorganisms. The toxic compounds can be divided into the following groups: sugar degradation products, lignin degradation products, compounds derived from lignocellulose structures, heavy metal ions, and fermentation end products. In some cases, the end product inhibition is the bottleneck of biofuel production, for example, butanol fermentation. Typically microorganisms can produces less than 20 g/L butanol due to butanol toxicity to the culture. Generally speaking, among all the inhibitory compounds, phenolics are most toxic.

The purpose of this research is to remove inhibitors from hydrolysates or fermentation broth with selected non-toxic surfactant systems. Utilizing the solubilization and uneven partition ability of surfactant micelles (and/or microemulsions), the inhibitory compounds would be extracted into the micelles. The micelles could aggregate and precipitate when the system temperature is above surfactants' cloud point.

Selected phenolic compounds, such as ferulic acid, vanillin and syringaldehyde were completedly removed by 1% nonionic surfactant block copolymer L62 D in a model system. Around 90% *p*-coumaric acid and a small amount (10%) furfural were removed as well. At mild pH 5.5, acetic acid and formic acid, etc in disassociated forms can not be removed. An advantage of the surfactant cloud point extraction systems is no sugar loss. The removal of toxic compounds in hydrolysates can be performed in situ. Fermentation tests in a model system containing butanol showed that the surfactant systems led to better cell growth.

Poster 9-53

Improvement of high-solid slurries mixing for enzymatic hydrolysis of pretreated rice straw

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One of the prerequisites for the efficient utilization of lignocelluloses for ethanol production is to provide a hydrolysate with a high glucose concentration. This was shown significant benefits on capital costs and production cost due to reduction in size of equipment and energy utilization for distillation, respectively. To obtain a hydrolysate with high glucose concentration, high-solid substrate loading for enzymatic hydrolysis is necessary. However, high solid slurries often makes mixing problem associated with the decrease of sugar yield from enzymatic saccharification and causes the power consumption increase in reactors. In this study, a high solid processing approach was developed to provide the enzymatic hydrolysis of pretreated rice straw under a solid content at 25% and above. The pretreated solid residues were prepared by a twin-screw extrusion pretreatment with dilute sulfuric acid. The pretreated lignocellulosic residues were shown lower viscous heterogeneous substrates which sufficiently to perform in high-solids saccharification. The viscosity was in the range of 3500-4000 cp under a 25% of initial solid content. Moreover, a 100L horizontal reactor with a design of oblique-scraped blade was developed for enzymatic hydrolysis. The decrease of sugar yield was below 5% with the increase of initial solid content from 15% to 25%. Nearly 80g/L and above of finial glucose concentration were observed from enzymatic hydrolysis. The power consumption was further compared for enzymatic hydrolysis at batch or fed-batch mode. This was contributed to increase the potential of high-solid enzymatic hydrolysis under industrial interest.

Poster 9-54

Continuous saccharification and fermentation of dilute acid pretreated corn stover and Avicel

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In contrast to batch operations, continuous fermentations are recognized for such advantages as higher volumetric productivity, reduced labor costs, and reduced vessel down time for emptying, cleaning, and filling. Lignocellulosic conversion processes can especially benefit from continuous process designs due to the presence of fermentation inhibitors generated or released during biomass pretreatment. High cell densities and adaptation abilities of the fermenting organism in a continuous setup enable higher productivities and yields than in batch systems and additional detoxification steps can be omitted or at least reduced. However, continuous processing of lignocellulosic materials faces some important challenges such as the handling of insoluble substrate and concerns about organism washout and contamination. Furthermore, although many economic studies assume that continuous saccharification or continuous saccharification and fermentation (cSSF) would be employed commercially, available experience and literature data are extremely limited. Thus, we set up a small scale, multi stage reaction system for continuous saccharification and cSSF of lignocellulosic materials to evaluate the effects of dilution rate, substrate concentration, and enzyme loadings on performance and to determine the potential to reduce enzyme loadings. In addition, data were sought on critical dilution rates for maintaining cell growth without washout. Results for cSSF of Avicel and dilute sulfuric acid pretreated corn stover are compared with kinetic modelling data to predict an optimal process design.

Poster 9-55

Quantifying Livestock Feed Value of AFEX-Treated DDGS and Subsequent Biorefinery Byproducts

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With annual U.S. production of fuel ethanol at nearly 9 billion gallons, coupled with the Renewable Fuels Standard, supplies of coproducts such distillers dried grains with solubles (DDGS) are anticipated to continue to grow for the next several years. DDGS is used as livestock feed. But as supplies of this coproduct grow, it will become increasingly important to investigate opportunities to improve its value and utilization. Ammonia Fiber Expansion (AFEX) is a process that has been shown to improve the nutritional qualities of feedstuffs, and it may play a role in developing corn-based biorefineries using existing ethanol plants. Toward that end, the objective of this study was to investigate the effects of AFEX-treatment and subsequent biorefining on the resulting feed quality of DDGS. During AFEX treatment, samples of DDGS were subjected to ammonia loadings at a rate of 1:1, at 90°C, for 30 min. Portions of both untreated DDGS and AFEX-treated DDGS were then fermented, using SSF, at 20% loading using *Saccharomyces cerevisiae* in order to produce ethanol. Nutritional composition was determined on all samples (untreated DDGS, AFEX-treated DDGS, and fermentation residues) in order to quantify their values as livestock feed. Analyses included proximate compositions (crude protein, lipid, ash), acid detergent and neutral detergent fiber, amino acid and fatty acid profiles, macro/micro mineral compositions, and in-vitro dry matter digestibility. Determining the value of resulting coproducts will be crucial in helping guide the development of biorefineries; their sale and utilization will be key to achieving economic sustainability.

Poster 9-56

Chitooligomers production by *Metarhizium anisopliae*

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Chitinolytic enzymes are produced by microbes, bacteria, fungus and some plants. The products of hydrolysis of chitosan are chitoligosaccharides and these compounds are useful for application in various fields because they have specific biological activities. The aim of this work was to assay the kind of chitooligomers (dimers until hexamers) produced by *Metarhizium anisopliae* and its respective concentration in different times of hydrolysis. *Metarhizium anisopliae* was cultured in liquid medium (containing 0.2%, and chitosan, 0.1% KH_2PO_4, 0.05% $MgSO_4$, 1.4; 0.05% KCl, 0.5; 0.3% yeast extract, 0.5%; bacto-peptone, 0.2% $NaNO_3$, 0.001% $FeSO_4$) using shaken flasks at 25°C and 110 rpm. The chitosanolytic enzyme activity was assayed by measuring the increase in the reducing sugar released from chitosan. The reaction mixture contained 0.5 mL of enzyme solution and 1% chitosan in a final volume of 1.0 mL. After incubation at 55°C for differents times the amount of reducing sugar released was measured by a Dinitrosalicylic acid Method (DNS). The concentrations of chitooligomers, from dimmers to hexamers were determined by high-performace liquid chromatography (HPLC). It was observed that *Metarhizium anisopliae* was able to produce chitoogomers from dimmers to hexamers The highest amounts of oligomers $\underline{(GlcNAc)}_n$ n =2 – 5 were found in 20 minutes. It was observed that hexamers, a chitooligomer with a great pharmaceutics interest, was found in 30 minutes of hydrolysis with a concentration of 0.13 mg/mL.

Poster 9-57

Medium composition effect on the expression of lack antigen of *Leishmania chagasi*

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With the advent of the recombinant DNA technology, the recombinant protein expression becomes an important path in the studies of the structure, function and identification of new proteins, especially ones with therapeutic objectives. *Escherichia Coli* has been the predominant prokaryotic used in the genetic engineering studies due to the abundance of information about its metabolism. Despite the expressive advance of the studies about the molecular biology and immunology of the infections, there is not, actually, any prophylactic drug capable to prevent the *kalazar*. Theferore, there is a real necessity for identifying specific antigens for the development of vaccines and diagnostic *kits* against the *Visceral leishmaniasis*. In this context, this work aimed to study the expression of the LACK recombinant antigen of the *Leishmania chagasi* during the *Escherichia coli* cultivation in shaker. In a set of assays it was carried out an induction procedure using IPTG, in order to observe the influence of the media composition (2xTY, TB) under expression of the LACK recombinant protein. Results showed that LACK recombinant clone expression was able to express the interest protein (LACK), confirmed by electrophoresis. On the other hand, it was observed the high complexity of the TB cultivation medium was limiting in the protein production.

Poster 9-58

Continuous bioethanol production in immobilized cells fermentor coupled with a pervaporation system

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In recent years, the research on bioethanol production from lignocellulosics has been mainly focused on the optimization of single conversion steps while less attention has been devoted to the process integration. In some cases, depending on the specific composition of the biomass processed, the final ethanol concentration in the broth could feebly reach the target of 4 wt% considered acceptable for a subsequent economically feasible distillation. Thus, one possible integration that might be beneficial for the process is the use of immobilized cells bioreactor faced with a continuous ethanol stripping module. This process configuration would offer several advantages: the use of immobilized cell bioreactors yields high productivities and the catalyst entrapment within the bioreactor is more suitable for continuous operations; the ethanol removal guarantees a reduced yeast stress and the production of more concentrated streams prior to the distillation.

In the present paper, the fermentation of glucose by *Saccharomyces cerevisiae* immobilized in alginate beads and the simultaneous pervaporation of the produced ethanol was investigated by using synthetic and real hydrolyzates. Preliminary results indicate that during continuous fermentation with initial cell densities of $2.6\acute{}10^7$ cells/mL, ethanol productivities of 2.5-3 g L^{-1} h^{-1} were achieved using glucose streams of 65-70 g/L. Pervaporation fluxes and selectivities were in the range 0.17-0.26kg $m^{-2}h^{-1}$ and 1.8-2.5.

Poster 9-59

Performances of *Lactobacillus brevis* for producing lactic acid from hydrolysate of lignocellulosics

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Utilizing all forms of sugars derived from lignocellulosic biomass via various pretreatment and hydrolysis process is a primary criterion for selecting a microorganism to produce biofuels and biochemicals. With dilute acid hydrolysis technology, the broad carbon spectra and potential inhibitors such as furan, phenol compounds and weak acid to microorganism are the two major obstacles that limited the application of dilute-acid hydrolysate of lignocellulose in ethanol and lactic acid fermentation. Two strains S3F4 (*Lactobacillus brevis*) and XS1T3-4 (*Lactobacillus plantrum*) isolated from sour cabbage, exhibited the ability to utilize various sugars presented in dilute-acid hydrolysate of lignocellulose. The S3F4 strain also showed strong resistance ability to the potential fermentation inhibitors, ferulic acid and furfural. Shake-flask fermentation indicated that 39.1 g/l of lactic acid could be produced by S3F4 from dilute acid hydrolysates of corn cob with 56.9 g/l total sugars (xylose, 46.4 g/l; glucose, 4.0 g/l, arabinose, 6.5 g/l). The hydrolysate of corn cob could be readily utilized by S3F4 without detoxification, and the lactic acid concentration in the fermentation broth was higher compared to other reports.

Poster 9-60

High-throughput techniques for microalgal biofuels feedstock analysis: Rapid fatty acid fingerprinting using gas chromatography

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There is a need for a rapid, accurate and reliable method for quantitative lipid fingerprinting in the light of current interest in microalgae-based biofuels. It is important to characterize the lipids in algal biomass as a measure to assess its suitability as biofuel feedstock. We are developing fast, high-throughput methods for extraction and profiling of microalgal lipids, using accelerated solvent extractions, rapid derivatization methods and ultra-fast GC. Traditional fatty acid analyses require large amounts of biomass, are slow and not tailored towards the comprehensive lipid analysis needed in algal biofuels research. A high-throughput analytical platform is required to identify candidate algal species with the appropriate lipid composition from a growing collection of largely uncharacterized strains and secondly assess the effect on the lipid profile of a large matrix of environmental variables. We are presenting results showing an automated extraction method optimized for highest lipid yield using different solvents. We are in the process of optimizing an efficient derivatization method for the analysis of fatty acid methyl esters (FAMEs) by gas chromatography. We are presenting results from a comparison between direct biomass derivatization and lipid based derivatization methods with regards to yield and fatty acid profile obtained.

Poster 9-61

Determination of monosaccharides in acid hydrolyzed biocrop feedstock using HPAE-PAD

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Compositional carbohydrate analysis of new biocrop feedstocks is essential to the efficient production of cellulosic ethanol. The analysis is commonly done using HPLC with refractive index (RI) detection, however this method has several limitations. HPLC-RI system robustness can be severely limited by the variation in RI detector response due to changes in temperature, and reversed-phase separations often require long run times and lack sufficient resolution to detect biomass sugars. While high-performance anion-exchange with pulsed amperometric detection (HPAE-PAD) technology permits baseline-resolved separation and detection of biomass sugars in complex samples, its high sensitivity requires dilution prior to analysis, which often introduces error into the measurement. In this poster we present a rapid method (<10 min) for monosaccharide analysis, and determination of composition, based on HPAE-PAD that enables direct injection of corn stover hydrolysates with no dilution. We present our results for performance, including precision (0.2-0.3% retention time and 0.7-1.3% peak area RSDs), accuracy (85-96% spike recovery), limits of detection (approximately 0.002 ppm), and linearity (r2 = 0.9997).

Poster 9-62

Ethanol from xylose using an immobilized enzyme recirculation reactor

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Xylose from straw hydrolyzate and other biomass sources can be converted to ethanol at high yield with a system that recirculates the fermentation broth through a parallel reactor containing immobilized xylose isomerase (EC 5.3.1.5). There are numerous process flow options for this flexible process, but we have chosen to validate the concept on a dedicated xylose fermentation system that occurs after a conventional C-6 Simultaneous Saccharification and Fermentation plus solid-liquid separation. Within the xylose system, several conventional yeasts including *Schizosaccharomyces pombe* are able to ferment the xylulose product created by the isomerization reaction. This yeast requires no genetic modification and the yield, rate, and co-product formation are very good. The engineering controls for the system to ensure optimal performance of the enzyme are straightforward. Economic performance of the system is heavily dependent on the productivity of the enzyme which has been shown to be greater than 3000 kilograms of product per kilogram of enzyme with straw hydrolyzate. Scale-up to a 200 liter system is currently underway.

Poster 9-63

Profiling 32 Low Molecular Mass Organic Acids in Biomass by Ion Chromatography Mass Spectrometry

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Organic acids, especially low molecular mass organic acids (LMMOAs), play crucial roles in plant life, and are associated with fundamental functionalities including energy production, metals absorption, and plant tolerance to environmental stress, supported by increasing experimental evidences. Their importance was recently discovered in biofuel conversion that they account for 7~ 21% dry weight of aqueous extractives from corn stover, and they were also discovered of their inhibitory effects for bioconversion efficiency to ethanol from lignocelluloses. To better understand and optimize the biofuel conversion process, it is paramount to monitor LMMOA in biofuel production. Chromatographic methods with various detection techniques provide the most thorough information to profile and monitor LMMOA. Many reported methods focus on limited number of LMMOAs and are incapable of providing a complete LMMOA profile.

This paper describes an ion chromatography mass spectrometric method for profiling analysis of LMMOAs in biomass. 32 LMMOAs were successfully separated and detected. Coefficient of determination greater than 0.99 was achieved for all analytes through the range from low ppb to 2000 ppb (MS/MS) or 5000 ppb (MS). Sensitive MS detection ensures the detection limit reaches to low ppb level except for formic and acetic acid. This method has been applied for analyzing biomass samples obtained from biofuel production process. Seventeen LMMOAs were confirmed of their presence in biomass. Succinic acid presented as the most prominent LMMOA with concentration greater than 200 ppm. Mucic acid, α-ketoglutaric acid, oxalic acid and citric acid also presented at large amount (> 5 ppm).

Poster 9-64

Analysis of carbohydrates in microalgal biomass samples with HPAEC-MS

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Efficient production of biodiesel from microalgae requires analysis of all cell products, including carbohydrates, lipids and proteins. While lipid profiling is important, a complete characterization of the carbohydrate breakdown products is essential for nutrient recycle in order to determine which sugars are best absorbed by the algae. Carbohydrate analyses of microalgal biomass often contain complex mixtures of C5 and C6 sugars, requiring a high resolution and high sensitivity technique capable of separating and quantifying trace-level carbohydrates of interest in the presence of multiple interfering compounds.

This poster describes an ion chromatography method for profiling carbohydrates in microalgal whole cell lysates. Centrifugation and solid phase extraction are used to remove plant matrix components including chlorophyll in the sample preparation step. Monosaccharides, disaccharides and alditols are separated at high pH via anion exchange prior to desalting using a carbohydrate membrane desalter. Lithium ion is added post-desalter to allow detection of lithium adducts using electrospray mass spectrometry. Carbohydrate profiles are discussed.

Poster 9-65

Ethanol Production from Food Wastes

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Approximately 5 million tons of food wastes are generated annually in South Korea. Most of the food wastes are landfilled or incinerated, causing ground water contamination or emission of noxious gases and dioxins. Hence, food waste management has been important issue for protecting the environment as well as for conservation of resources. Starch and cellulose materials are the major components of the food waste. It also contains some protein materials. The starch and cellulosic components of the food waste can be hydrolyzed to monomeric sugars. The sugars then can be used as substrates in the fermentative production of variety of chemicals, such as ethanol. Bioethanol has been considered as the most promising alternative fuel.

In this work, food wastes was converted to ethanol by simultaneous saccharification with commercial a-amylase and amyloglucosidase preparations, and fermentation by employing a *S. cerevisiae*. The simultaneous saccharification and fermentation was performed in a batch mode. It has been demonstrated that over 40 g/L of ethanol could be produced from 100g/L food waste in less than 36h. The influence of substrate concentration, enzyme loading, and nitrogen supplements on the yield and the rate of ethanol production were evaluated.
Key words: Food waste; bioethanol; SSF; *S. cerevisiae*; amylase.

Poster 9-66

Batch Equilibrium and Kinetic Studies – Biosorption of Uranium by *Sargassum filipendula* Biomass

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Sargassum filipendula biomass was used as biosorbent material for Uranium in batch conditions. Kinetic and equilibrium conditions were studied. Results obtained indicated that *Sargassum filipendula* biomass reached equilibrium after 60 minutes of contact with a 1.0 mg/L and a 100.0 mg/L solution. This is an indication that the concentration of soluble Uranium in solution did not affect the kinetics of the process. The kinetic behavior observed indicated a 20-30% biosorption of Uranium in the first 20 minutes of process followed by a slower uptake up to 38% Uranium biosorption.

The kinetic modeling of the process indicated that the less concentrated Uranium solution fitted well to a second-order kinetic model, while the 100.0 mg/L solution fitted well both the first and second-order kinetic models. Those conclusions were reached based on the high correlation coefficients obtained.

Equilibrium batch tests showed that Uranium biosorption was equivalent, both after 1 hour and 3 hours of contact between the biomass and distinct Uranium solutions. Freundlich and Langmuir models were used to fit the experimental data. The Freundlich model best described Uranium biosorption, in comparison to the Langmuir model, based on higher correlation coefficients obtained.

Poster 9-67

Foaming Tannin from a Cellulose-Tannin Solution

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Foam Fractionation is an inexpensive chemical process in which molecules are separated from a liquid solution by using a gas, such as air, to carry them out of solution and into the collected foam. When that foam is collapsed into a liquid it is called the foamate. Foam fractionation of tea solutions and grape tannin are analyzed in this study, with or without the presence of ethyl cellulose, to see if separation of tannins or tannin-like materials from cellulose can be achieved. It was observed, that a cold (20°C) tea solution produced more foam and at a faster rate than a hot (95°C) tea solution. The foamate and different layers of tea were analyzed for color (mean of the optimal density vs. spectrophotometer wavelength distribution). If tannin can be removed from a tannin-cellulose system by foaming, then perhaps lignin could also be removed by foaming from lignocellulosic solutions. A low cost tannin/lignin removal step could make the generation of sugars from a lignocellulose (and on to ethanol) biofuel production process more economical.

Poster 9-68

Production of Fuel Ethanol from Softwood at High Dry Matter Content

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Bio-ethanol can be produced from cellulose-rich material such as softwood by enzymatic hydrolysis and fermentation. To perform the enzymatic hydrolysis and the fermentation in one single step, so called "simultaneous saccharification and fermentation" (SSF), has proven to result in higher product yields and lower production costs than performing the two steps separately. The raw material is pretreated with steam at high temperature and pressure to break down the hemicellulose and make the cellulose more accessible to the enzymes in the hydrolysis.

Previous studies have shown high ethanol yields for SSF run at 5% WIS (water insoluble solids). It is however important to run SSF at higher dry matter contents in order to achieve higher ethanol concentrations to lower the energy demand in the distillation needed in the post treatment and to reduce the production cost. Previous studies of SSF with higher dry matter contents have however shown a decrease in ethanol yield due to poorer mass transfer and increased inhibition by toxic compounds present in the pretreated material.

SSF at high dry matter content was studied in batch and fed-batch mode. In particular, different feeding strategies for the enzymes used in the process were investigated. Furthermore, since the dry matter contents studied so far (i.e. about 10% WIS) reach a practical limit of stirring capacity in the fermentors used, a new kind of reactor more suited for high viscosity materials was used to further increase the dry matter content. The results from this study will be presented at the conference.

Poster 9-69

Evaluation of Sweet Sorghum for Ethanol Production

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Sweet sorghum as a potential feed stock for ethanol production offers several benefits 1) high fermentable sugar content in the stalk and convertible starch in seed heads, 2) a lower agricultural input than corn for both fertilizers and water, and 3) a wider growing region than sugarcane. Adoption of sweet sorghum as an ethanol feedstock for raw sugar factories/distilleries may have the added benefit of extending mill operations by two months. Sweet sorghum averages 73.7% of stalk (56.8% juice + 16.9% dry solid fiber), 7.5% seed heads and 18.9% leaf matter. Theoretically the ethanol yield would be 5.7g of ethanol (3.8g from juice and l.9g from seed heads)/ 100g of sweet sorghum. Post processed biomass was utilizable for cellulosic ethanol production or fuel. Sweet sorghum as a biofuel crop will be evaluated based on current sciences and economics.

Poster 9-70

Effect of higher culture temperature on the metabolism of Escherichia coli

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Biofuels have been paid recent attention from the view point of energy generation from biomass. Among the biofuel process, simultaneous saccarification and fermentation (SSF) is now being paid more attention. In SSF, the culture temperature has to be increased more than 40°C. *Escherichia coli*, as all kind of organisms, responds to the increase of the temperature by inducing heat shock proteins etc. So the objective is to investigate the effect of higher temperature on the metabolism in *Escherichia coli* from the view point of gene expressions.

The strains used were *E. coli* BW25113. Batch and continuous cultivations were made using 2- L fermentor where the temperature was kept constant either at 37°C or 42°C, and the pH of the culture was maintained at 7.0. Continuous cultivation was performed at the dilution rate of 0.2 h^{-1}. Qiagen RNeasy® Mini Kit was used to isolate total RNA from *E.coli* cells according to the manufacturer's recommendation. After the temperature up-shift the *lpdA* gene which codes for the (lipopolyamide dehydrogenase) subunit of the pyruvate dehydrogenase (PDHc) complex was induced due to the up-regulation of cAMP receptor protein (CRP) .Our results also indicate that global regulator *arcA* as well as heat shock protein genes were up regulated which in turn repressed such genes as *icdA, aceA, sucA, cyoA* while *cydB* were up regulated under higher temperature as compared with the lower temperature. This caused the repression of TCA cycle activity and in turns caused higher acetate production at higher temperature.

Poster 9-71

Extraction and Identification of Julibroside Saponins from the Bark of *Albizia julibrissin*

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The co-generation of value-added products is one way of adding additional value to the biomass used in a biorefinery. One such product, saponins, can be found in the bark of *Albizia julibrissin* (mimosa). Saponins are a natural detergent that can be found in a variety of plants, ranging from *Yucca schildigera* in Mexico to *Panax ginseng* in China. Saponins contain both water-soluble and a fat-soluble components. Saponins have been shown to have antiprotozal activity in ruminals and have been shown to reduce blood cholesterol levels in mammals. Triterpenoidal saponins, julibrosides, are known to be present in *Albizia julibrissin* [1], and have previously been extracted with methanol and other organic solvents, separated by RP-HPLC and analyzed with mass spectrometry. Unfortunately, the julibrosides are not available commercially as reference compounds. Centrifugal partition chromatography (CPC) was used to separate and accumulate fractions of julibrosides for use as reference material. The reference fractions from the CPC were then used to find the most favorable parameters for pressurized hot water extraction of julibrosides from *Albizia* bark. The overall goal of this project was to determine the feasibility of extracting saponins, in a green manner, from mimosa biomass prior to conversion to a liquid fuel.

[1] Zou, K. et al. *Diasteriomeric saponins from Albizia julibrissin*. Carbohydrate Research. 2005, 340, 1329-1334

Poster 9-72

Optimization of biodiesel production by fungus cells immobilized in fibrous supports

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A circulating packed-bed bioreactor system using fibrous nonwoven fabric as the immobilization matrix was suitable for simultaneous cell growth and immobilization of *Rhizopus oryzae* fungus cells. The optimal culture time considering both cell weight and transesterification activity was 72 hr. The immobilized whole cell biocatalyst was suitable for biodiesel production by methanolysis of oil after drying and suitable post-treatment. The best treatment method for immobilized whole cell biocatalyst for biodiesel production was by permeabilizing the cells in acetone for 90 min and incubating the permeabilized cells in soybean oil for 72 hr. Response surface methodology and 5-level-5-factor central composite rotatable design was proved to be a powerful tool for the optimization of methanolysis conditions catalyzed by immobilized *R. oryzae* whole cell biocatalyst. A second-order model could be obtained to describe the relationship between the methyl ester yield and the significant parameters (water content, substrate molar ratio, cell concentration, and reaction time). Under the optimal condition, 10.97% water content (based on oil), 0.64 molar ratio of methanol to oil, 2.25%(w/w) cell weight (based on oil), and 23.3 hr reaction time, the predicted values of methyl ester yield was about 72.6%. Validation experiments with predicted yield of 70.77 ± 2.46% verified the availability and the accuracy of the model. The predicted value was in agreement with the experimental value.

Poster 9-73

Stabilization and Delivery of *Chlorella vulgaris* in Water-in-Oil Emulsions

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Microalgae have considerable promise as a feedstock for biofuel production. Since most microalgae do not store well in aqueous suspensions or as a dry formulations, storage and delivery technologies are needed to facilitate their production. We developed a water-in-refined corn oil (W/O) emulsion formulation of *Chlorella vulgaris* that is physically and biologically stable at room temperature for 6 months. The algae reside in the dispersed water droplets. Emulsion physical stability and algal biological stability is realized by utilizing an oil soluble polymeric surfactant and silica nanoparticles with hydrophobic surface treatment. Light scattering measurements made on emulsions show a slight initial coalescence effect but essentially no change in number average particle size over the span of five months. The excellent physical stability of the emulsions may contribute to the biological stability of the system.

Controlled release studies were conducted to determine the effect of concentration of polymeric surfactant in the W/O emulsion on the release of *Chorella minutissma* upon application to water. As the surfactant concentration in the oil phase was reduced from 3.0 wt% to 0.3 wt%, the percent release of the total cells applied increased from 3.0% to 17%. This result suggests that the rate of coalescence of water droplets within the W/O emulsion and the eventual rupture of the oil film to allow release of algae is governed by the level of surfactant in the oil phase. Therefore the level of surfactant can be used to adjust the release of algae when applied to a water surface.

Poster 9-74

Eco-Ethanol Production from Lignocellulosics with Hot-compressed Water Treatment Followed by Acetic Acid Fermentation and Hydrogenolysis

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<u>*Purpose of the work*</u>: We propose a newly-developed process for bioethanol production from lignocellulosics by applying acetic acid fermentation.

<u>*Approach*</u>: The ethanol production system involving two-step hot-compressed water treatment coupled with acetic acid fermentation followed by the subsequent catalytic hydrogenolysis.

<u>*Scientific innovation and relevance*</u>: Japanese beech flours were firstly treated in two-step hot-compressed water and obtained various mono-saccharides, oligo-saccharides and even uronic acid were found to be converted into acetic acid by *Clostridium thermoaceticum*. The obtained acetic acid was then esterified and converted into ethanol by catalytic hydrogenolysis.

<u>*Results and conclusions*</u>: A new ethanol production process involving hot-compressed water treatment followed by acetic acid fermentation and catalytic hydrogenolysis was proposed. This process does not need any acid as a catalyst, such as sulfuric acid, for hydrolysis of lignocelluloses. The obtained various saccharides, not only hexoses but also pentoses, and uronic acid could be anaerobically fermented to acetic acid without any carbon dioxide emitted. Acetic acid is then esterified to acetate ester which is further converted into ethanol by catalytic hydrogenolysis. Consequently, the high-convertible bioethanol production process with highly-effective CO_2 reduction could be established.

Poster 11-07

Biohydrogen production converted from lignocellulose: A novel source and approach from wood-feeding termites

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In nature, wood-feeding termites possess a great capability to digest lignocellulose and with the unique mechanisms to emit a significant amount of H_2 from their guts into atmosphere. The efficacy of H_2 emission by wood-feeding termites could vary from 122–12348 nmol h^{-1}g of termite body $weight^{-1}$, depending on termite species, food source, and food modification that would affect methanogenesis and other bacteria who uptake H_2 for synthesizing CH_4 or other products. Our recent investigations for three wood-feeding termites, *Reticulitermes flavipes*, *R. virginicus,* and *Coptotermes formosanus* indicated that H_2 emission during 3 h incubation was significant and continuous at the rates of 3076.01 ± 229.47, 12348.84 ± 1130.53, and 1241.48 ± 131.95 nmol h^{-1}g of termite body $weight^{-1}$, respectively. The antibiotic treatments at a proper concentration on termite diets would significantly enhance H_2 production at least 5 times of H_2 production recorded on non-treated diet by *C. formosanus*, which suggested that H_2 production is mainly attributed to the dense population of symbiotic cellulolytic protozoa in termite hindguts. These investigations also represent a novel source of biohydrogen from termites and the unique mechanisms producing hydrogen from cellulosic substrates that showed the highest H_2 conversion rate from lignocellulose among present technologies in biological hydrogen processes (e. g. 12.6655 ± 1.1595 mmol gaseous H_2 (~0.28 liter H_2) when consuming one gram of pine wood by *R. virginicus*). The production of biohydrogen and mechanisms via wood-feeding termites demonstrates a distinct departure from other biological hydrogen routes with low conversion rates.

Poster 11-08

High production of 1,3-propanediol from crude glycerol using suspended and immobilized *Klebsiella pneumoniae*

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In this study, the production of 1,3-propanediol (1,3-PD) was investigated with *Klebsiella pneumoniae* DSM2026 using crude glycerol solution obtained from two different biodiesel production processes. Crude glycerol was utilized without purification to investigate an inhibitory effect of crude glycerol on 1,3-PD production when compared to pure glycerol. In the media containing pure or crude glycerol, *K.pneumoniae* DSM2026 could biosynthesize 1,3-PD with the yield higher than 0.5 g/g. The yields of 1,3-PD production from crude glycerol solutions (90% and 70% glycerol-containing biodiesel wastes) were even higher than those obtained with commercially available glycerol (pure glycerol) in batch and fed-batch cultures. In fed-batch cultures, more than 80 g/L of 1,3-PD was produced with crude glycerol. To our knowledge, this is the highest 1,3-PD concentration reported so far using crude glycerol. Based on the optimum culture conditions obtained, a fed-batch fermentation with the cell immobilization system was performed in a bioreactor to enhance 1,3-PD production. The effect of additional vitamin B12, a co-enzyme for glycerol dehydratase, was also investigated with the cell immobilization system. Cultures utilizing crude glycerol in the immobilization system showed even more effective 1,3-PD production than that with pure glycerol. Further study will be performed to separate 1,3-PD directly from bioreactors to prevent product inhibitory effect.

Poster 11-09

Production of acetone-butanol from wheat straw hemicellulose hydrolyzates

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The cost-effective use of lignocellulosic raw materials requires the integrated production of value-added chemicals, transportation fuels and energy in a biorefinery concept. The pretreatment of wheat straw by steam explosion in mild acidic conditions results in the hydrolysis of hemicelluloses and solubilization of pentoses. Some *Clostridium* strains can utilize pentoses as a carbon source and excrete metabolic products such as acetone, butanol and ethanol (ABE or solvents). However, in addition to C5 sugars, the hemicellulose hydrolyzates obtained by this pretreatment may content toxic compounds, especially furanic and phenolic ones.

In order to define optimum conditions for ABE production from this material, the growth and solventogenic performances of some *Clostridium* strains on different wheat straw hemicellulosic hydrolyzates produced by steam explosion have been determined. Various *C. acetobutylicum* and *C. beijerinckii* strains from collections were compared in order to select the best ones for cultures in lab-scale reactors. Several concentrations of hemicellulose hydrolysates were used and removal of toxic compounds by overliming was sometimes necessary because of the sensitivity of *Clostridium* strains to some of them. In bioreactor, solvent concentration (more than 15 g/L) and productivity close to the performances obtained in a synthetic medium have been achieved.

This work was carried out in the framework of the Biosynergy project granted by the European Commission.

Poster 11-10

Characteristics of biodemulsifier produced by one strain of *Alcaligenes* sp.

S-XJ-1 and its application in emulsion destabilization

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As a kind of biosurfactant, biodemulsifier is highly efficient in breaking industrial emulsions due to their unique functional groups. One biodemulsifier producing strain S-XJ-1 was investigated on its screening methods, its application in model/crude oil emulsion and its effect on the oilfield produced water treatment system. For the identification of efficient demulsifying strains, the proposed screening protocol was set as follows: surface tension level was set at 40 mN/m, then detected strains were further verified in demulsification test. By this screening protocol, one strain named S-XJ-1, was isolated from petroleum-contaminated soil and identified as *Alcaligenes* sp. by *16S* rRNA gene which proved no toxicity by little mouse test. It achieved 96.5% and 49.8% of emulsion breaking ratio in W/O and O/W kerosene emulsion within 24h, respectively. It also showed 93% de-emulsifying efficiency in 150min in W/O Kelamayi crude oil emulsion. S-XJ-1 showed stable demulsifying ability at pH from 3 to 11 while chemical demulsifier doesn't work when pH was 3. Results of its influence on oilfield produced water treatment system showed that the effluent treated by the biological demulsifier had lower COD but higher SS concentrations than that by the chemical demulsifier. In terms of the effluent system, COD in the effluent from coagulation/filtration process was 350mg/L after dehydration by chemical demulsifier. Compared with that, COD was reduced to 150mg/L by biodemulsifier. In terms of reinjection system, the effluent from biodemulsifier treatment had no scaling tendency of S_rSO_4 and very lower coupon corrosion rate than the effluent by chemical demulsifier.

Poster 11-11

Application of High-throughput Methods to Chemical Transformations of Renewable Feedstocks

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Accessible routes from renewable feedstocks to high-value intermediate, commodity chemicals, and fuels are desired as replacements for routes based on petroleum and other fossil based resources. Symyx has built specific capabilities that allow such routes to be discovered and optimized rapidly using high throughput methods. These methods have been used to upgrade diverse feedstocks including sugar streams, syngas, and pyrolysis liquids to higher value molecules. Specific capabilities to be discussed include

1. Synthesis of heterogeneous catalysts such as supported metals, bulk metal oxides, and structured aluminosilicates

2. Synthesis of homogeneous catalysts including organometallic compounds by accessing an extensive ligand archive

3. Screening arrays of catalysts in parallel batch reactors or flow-through reactors utilizing on-line analytics

Poster 11-12

Benefits of real-time analytical monitoring of fermentation processes during development, scale-up and production

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Advanced process analyzers including mass spectrometers, FTIR and FTNIR analyzers have been used to monitor fermentation processes in real-time to help gain a better understanding of reaction progress and kinetics during research, scale-up and production. Mass spectrometers have been used to measure the input and output gases for the primary compounds in fermentation systems including nitrogen, oxygen and carbon dioxide. The measurement of secondary compounds such as ethanol, methanol, acetic acid, ammonia, lactic acid, methane and argon are all also possible. Mass spectrometers routinely make these measurements simultaneously with a virtually instantaneous response time. Measurements have been made over a wide dynamic range for many of these compounds from concentrations in the ppb level through 100%.

FTNIR and FTIR systems have been used to make measurements directly in the fermentation broth typically using direct insertion probes. Real-time measurements of alcohol production, nutrient depletion and the formation of side-products and intermediates have been performed. The determination of biomass, glucose, lactic acid and acetic acids during fermentation processes have been demonstrated using attenuated total reflectance probes.

Real-time monitoring of fermentation processes can be used to lead to a better understanding the effect of real-time changes to reaction systems.

Typical applications will be presented for fermentation systems including implementations in microbial fermentation, batch fermentation and continuous fermentation.

Poster 11-13

Withdrawn

Poster 11-14

Production and toxicity of γ-decalactone and 4-hydroxydecanoic acid from *R. aurantiaca*

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The production of γ-decalactone by the psychrophilic yeast *R. aurantiaca* in a medium containing 2% castor oil was similar to that in medium containing 2% ricinoleic acid. No traces of lactones were detected by using olive oil as substrate. *R. aurantiaca* produced both γ-decalactone and its precursor 4-hydroxydecanoic acid. The production of γ-decalactone and 4-hydroxydecanoic acid was significantly higher when a 20-L bioreactor was used than when a 100-L bioreactor was used. The influence of pH on the extraction of γ-decalactone was also studied. By using 20 g/L of castor oil, 6.5 and 4.5 g/L of γ-decalactone were extracted after acidification at pH 2.0 and distillation at 100°C for 45 min in 20- and 100-L bioreactors, respectively. The effect of γ-decalactone and 4-hydroxydecanoic acid on the growth of *R. aurantiaca* was investigated. Our results show that γ-decalactone must be one of the limiting factors for its production. We confirmed that castor oil, besides being the substrate of bioconversion, acts as an extractant of the lactone. The addition of gum tragacanth to the fermentation medium at concentrations of 3 and 4 g/L seems to be an adequate strategy to enhance γ-decalactone production and to reduce its toxicity towards the cell. We propose a process at industrial scale using a psychrophilic yeast to produce naturally γ-decalactone from castor oil which acts also as a detoxifying agent; moreover the process was improved by adding a natural gum.

Poster 11-15

Development of value added products from hydrolyzed Lignin

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Biochemical conversion of lignocellulosic biomass produces ethanol as a main product and hydrolysed lignin as a major byproduct. A typical corn stover based biorefinery (2000 tons/day) can produce as much as 400 tons/day of lignin and ash residues. Lignin is a complex, cross-linked phenyl propanoid compounds that mainly fills space in the plant cell wall and act as a defense against invading microbes. Although direct combustion of hydrolyzed lignin is normally proposed, several high value products such as binders, resins, polymer composites, phenols, vanillin can be produced from lignin. Recently, there has been significant interest in using lignin as commercial pellet binders and even polymer composites. This paper investigates the binding properties of hydrolyzed lignin to produce high quality biomass (switchgrass) pellets and optimize the amount of lignin required to produce high density and durable biomass pellets. The compaction behavior of hydrolyzed lignin is also studied using uniaxial compression testing method at various preheating temperatures and applied pressures. This paper also reviews the development of other value added low cost bio-products from lignin.

Keywords: hydrolyzed lignin, pellet binder, resins & lignin pellets

Poster 11-16

Growth media optimization for polyhydroxyalkanoate and hydrogen coproduction from Rhodospirillum rubrum cultured on synthesis gas

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The typical biorefinery separates biomass into cellulose, hemicellulose, lignin, and terpenes before processing each component into fuels and chemicals. In a hybrid biorefinery, thermochemical processes first break down recalcitrant components of the biomass into intermediate compounds that can be processed biologically. One such hybrid biorefinery is based on synthesis gas fermentation. This process begins with the gasification of biomass to produce syngas, a flammable gas mixture consisting primarily of carbon monoxide (CO), hydrogen (H_2), and carbon dioxide (CO_2). Microorganisms are then used to ferment the syngas into biofuels and chemicals. *Rhodospirillum rubrum*, a non-sulfur purple bacterium, utilizes the carbon monoxide (CO) in syngas to produce hydrogen, a high fuel value gas, and polyhydroxyalkanoates (PHA), biobased-biodegradable polymers. Information is limited pertaining to the optimization of growth media for *R. rubrum* for its effect on the coproduction of H_2 and PHA. This investigation examines growth medium optimizations considering both cost of media components and the coproduction of H_2 and PHA. Correlations of PHA production to cell density at different growth stages are also examined.

Poster 11-17

Improvement of coenzyme Q_{10} production by *ispB* knockout and *dxs* overexpression in recombinant *Escherichia coli* expressing *Agrobacterium tumefaciens* decaprenyl diphosphate synthase

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Coenzyme Q_{10} (CoQ_{10}) is a lipid-soluble benzoquinone essential for ATP generation and used as an antioxidant in food supplement. Biotechnological production of coenzyme Q_{10} was performed in recombinant *Escherichia coli* expressing decaprenyl diphosphate synthase (Dps) from *Agrobacterium tumefaciens*. From batch fermentations of several *dps* expression systems containing different promoters and replication origins, the modified P_{lac} constitutive promoter and ColE1 *ori* gave the best results of CoQ_{10} production. Even though CoQ_{10} accumulated inside the cells, a major by-product of endogenous CoQ_8 was produced inevitably. To prevent CoQ_8 production and concomitantly increase CoQ_{10} titer, the chromosomal *ispB* gene encoding octaprenyl diphosphate synthase was deleted by homologous recombination. Deletion of the *ispB* gene and expression of the *dps* gene led to the production of CoQ_{10} without CoQ_8 and CoQ_9 accumulation. In addition, Dxs which was already known to boost the pool of a CoQ_{10} intermediate, isopentenyl diphosphate, was coexpressed in recombinant *E. coli* BL21(DE3) strain expressing the *dps* gene and deficient in the chromosomal *ispB* gene. Batch fermentation in LB medium resulted in 1.40 mg/g of specific coenzyme Q_{10}. Fed-batch fermentation of recombinant *E. coli* BL21(DE3)*ΔispB*/pAP1+pDXS in a defined medium with 20 g/L initial glucose was carried out by feeding 800 g/L glucose with the pH-stat strategy. As a result, a final coenzyme Q_{10} concentration of 99.4 mg/L and its volumetric productivity of 3.11 mg/L-hr were obtained in 32 hr of fed-batch fermentation.

Poster 11-18

Effects of overexpression of endogenous *ALD6*, *ACS1* and *Kluyveromyces lactis* GPD1 on xylitol production in recombinant *Saccharomyces cerevisiae*

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Xylitol is a five-carbon sugar alcohol and has been used as a sugar substitute because of its low caloric and anti-cariogenic properties. For the biosynthesis of xylitol from xylose, *Saccharomyces cerevisiae* was engineered to express *Pichia stipitis* xylose reductase (XR) and to modulate some metabolic enzymes catalyzing the regeneration of NAD(P)H cofactor, which are involved in the pentose phosphate pathway. In this study, the effects of NAD(P)H regeneration on xylitol production were investigated by overexpression of *ALD6*, *ACS1* and *GPD1* in recombinant *S. cerevisiae* expressing XR. The *ALD6* and *ACS1* genes encoding aldehyde dehydrogenase and acetyl-CoA synthetase, respectively were PCR-amplified from the genomic DNA of *S. cerevisiae* and the *GPD1* gene coding for $NADP^+$-dependent glyceraldehyde-3-phosphate dehydrogenase was originated from *Kluyveromyces lactis*. Each gene was cloned into plasmid p426GPD, integrated into the chromosome of *S. cerevisiae* BJ3505:δXR and expressed under the control of the constitutive *GPD* promoter. A glucose-limited fed-batch fermentation was carried out in a 3.7 L-bioreactor with YP medium containing 20 g/L glucose and 100 g/L xylose initially. Among three xylitol-producing systems, recombinant *S. cerevisiae* co-expressing XR and ACS1 gave the best results of 94.3 g/L xylitol concentration and 1.62 g/L-hr productivity, corresponding to 1.20- and 1.30-fold increases compared with recombinant *S. cerevisiae* expressing XR only.

Poster 11-19

Biodiesel and H_2 production from CO_2 by sequential use of microorganisms in bioreactors

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Photoconversion of light energy and waste CO_2 into an array of biofuels by sequential use of microorganisms in bioreactors was studied. First the oil production by microalga *Chlorella vulgaris* was investigated in a batch culture and in a photobioreactor using waste CO_2. Both flasks and a photobioreactor were illuminated with fluorescent light continuously. Algal biomass was recovered by centrifugation with subsequent drying under 80°C. Algal oil was extracted with hexane. High oil content (50%) was found in *Chlorella* cells. Algal oil was converted into biodiesel by transesterification. A simple photobioreactor for biodiesel generation from microalgae was made from parallel clear PVC 10 feet tubes (6' diameter) with a small slope (10%). The gas mixture (5% CO_2 and air) flowed up from bottom of PVC tubes to the top as large gas bubbles. Next, glycerol, a by-product of biodiesel production, was used as a substrate for making biohydrogen (H_2) by bacterium *Enterobacter aerogenes* in batch culture and in a bioreactor. Higher H_2 production rates for up to 1600 mL g^{-1}· DW· h^{-1} were observed than bacterial cells grew in the presence of 1% glycerol compared to 10% and 0.1% on a simple medium containing inorganic salts.

Poster 11-20

Effect of biodiesel-derived raw glycerol on 1,3-propanediol production by different microorganisms

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There is a great advantage to use biodiesel-derived raw glycerol for the various chemical productions from economical and environmental standpoints. In this study, the production of 1,3-propanediol (1,3 PD) was investigated with pure glycerol, raw glycerol, and acid-pretreated raw glycerol using different 1,3 PD-producing microorganisms. There was no inhibitory effect of raw glycerol on 1,3 PD production when *Klebsiella pneumoniae* DSM2026 and *K. pneumoniae* DSM4799 were tested. In contrast, the growth and 1,3 PD production of *Clostridium butyricum* DSM15410, *C. butyricum* DSM2477, *C. butyricum* DSM2478, and *C. pasteurianum* DSM525 was inhibited in the presence of raw glycerol. A simple acid treatment of raw glycerol was performed to remove impurities in raw glycerol, and the pre-treated glycerol was tested if it could support cell growth and 1,3 PD production. Using the pretreated raw glycerol, the production of 1,3-PD by *C. butyricum* DSM 2477, *C. butyricum* DSM 2478, and *C. pasteurianum* DSM 525 was similar to that with pure glycerol, whereas there was about 50% inhibition on 1,3 PD production with *C. butyricum* DSM 15410. In conclusion, *K. pneumonia*e DSM2026 and *K. pneumoniae* DSM4799 successfully converted raw glycerol to 1,3 PD without any inhibition effect. Although the tested clostridia strains in this study did not grow well with raw glycerol, a simple acid treatment of raw glycerol was effective to remove impurities and, consequently, support the growth of the clostridia strains. Identification of impurities causing the growth inhibition in raw glycerol has been also investigated in detail for each microorganism.

Poster 11-21

The use of alternative pathways in 1-butanol production from *Escherichia coli*

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Currently ethanol is the most utilised biofuel to supplement conventional gasoline; however recently *n*-butanol has emerged as a potential alternative fuel additive due to advantages over ethanol such as a higher energy content. 1-butanol is synthesised as a fermentation product by solventogenic *Clostridia* spp. and genes encoding the butanol pathway in these microorganisms has previously been expressed in *Escherichia coli*. In order to engineer a novel synthetic butanol pathway in *Escherichia coli* non-clostridial genes encoding enzymes resulting in the overall synthesis of 1-butanol were expressed from a P_{BAD} promoter. Competing metabolic pathways in the host *E. coli* were deleted and the effects of these deletions assessed.

Poster 11-22

Building a Future in Renewable Industrial Chemicals

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The price and price volatility of petrochemical feedstocks has made many renewable feedstock options economically viable. Archer Daniels Midland is perfectly suited to take advantage of these opportunities, being a large, integrated grain processing company with considerable expertise in the commodity grains business, as well as a 30+ year history in the bioethanol business. ADM is significantly expanding its portfolio of industrial chemical products to include commodity monomers/chemicals, specialty green chemicals including isosorbide, propylene glycol, starch-based SAPs, biobased polymers (including PHA, modified starches and proteins) and biomass feedstocks and intermediates. ADM's portfolio of green chemicals and plastics will be described as will marketplace trends that may influence the direction of future efforts. Special emphasis will be given to MirelTM PHA Materials.

Poster 11-23

Thermodynamic models for solid-liquid equilibrium of xylose in water and water-ethanol mixtures

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D-xylose or wood sugar is an intermediate product of human and animal metabolism and of others important metabolic processes. This pentose can be used in the food, chemical and pharmaceutical industries (consumed by diabetics, used as raw material for ethanol, xylitol, penicilim, acetic and lactic acids, resins and brewer's yeast productions). This work reports the study of thermodynamic models for solid-liquid phase equilibrium (SLE) and it was carried out by comparing the performance of different models with experimental data. The experimental data of solubility for the xylose-water and xylose-water-ethanol systems have been measured using a variant of the isothermal method. The experiments were carried out in a 100 mL glass jacketed crystallizer with helix-type agitator. The solution was mixed during 48 h at 450 rpm. A total of 12 experiments were carried by changing the temperature from 0 to 60 °C. Later, the experimental results and other reported in previous publications were fitted using prediction models based on vapor-liquid-equilibrium (VLE) (UNIFAC, GSP); semi-empirical models based on VLE (UNIQUAC, Wilson, NRTL), semi-empirical models based on SLE (Nýlt, λh) and empirical model with fitted parameters (Margules). The results show that the UNIQUAC model with fitted parameters can well describe the SLE with good accuracy (1.3% for binary and 3.4% for ternary systems). The other methods resulted in poor agreement with the system's behavior with systematic deviations from the experimental data. The prediction models must be used only where is not possible to obtain experimental data.

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Poster 11-24

Engineering of *Saccharomyces cerevisiae* metabolism for high-level and energy-independent production of cytosolic acetyl-CoA

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Acetyl-CoA is an important metabolite that participates in many biochemical reactions and is a central precursor for a number of interesting biobased chemicals and biofuels (e.g. n-butanol, industrial chemicals derived from fatty acid biosynthesis, isoprenoids, secondary metabolites). In *S. cerevisiae* large amounts of acetyl-CoA are synthesized from pyruvate by oxidative decarboxylation by the mitochondrial multienzyme complex pyruvate dehydrogenase (PDH). However, in yeast mitochondrial acetyl-CoA cannot be exported to the cytosol. Alternatively, yeast can convert pyruvate to acetyl-CoA in the cytosol via acetaldehyde and acetate. The enzymes involved in this so-called "bypass" pathway are pyruvate decarboxylase, acetaldehyde dehydrogenase and acetyl-CoA synthetase (ACS). However, the last reaction catalysed by ACS is energized by the conversion of ATP to AMP and makes fermentative processes less energy efficient leading to decreased cell and product yields. Moreover, the cytosolic concentrations of acetyl-CoA produced by this pathway are very low and limit the production of follow-up metabolites. In order to increase the cytosolic acetyl-CoA pool we engineered a new energy-independent pyruvate dehydrogenase bypass by overproduction of the CoA-dependent acetaldehyde dehydrogenases MhpF or AdhE from *E. coli*. This invention will be applicable for a broad range of fermentative conversion processes with yeast, especially for the production of butanol and hydrocarbon biofuels.

Poster 11-25

Hydrogen production from COSLIF-treated cellulosic feedstocks

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Clostridium phytofermentans is a recently-discovered anaerobe, previously reported to hydrolyze cellulose and to ferment five and six carbon sugars and starch into ethanol. We developed a chemically-defined medium in which to grow *C. phytofermentans* and observed that after a very long lag time, *C. phytofermentans* would fix its own nitrogen from N_2 gas. The native strain was inoculated into a 5 L chemostat in defined medium with N_2 as the sole source of nitrogen for growth (conditions designed to select for mutants with increased nitrogenase activity). The culture was continuously mutated in a loop run past a UV light under conditions yielding about 90% killing. The growth rate increased dramatically over this time and a variant (cpnit-1), with a 33% increased growth rate under nitrogen fixing conditions. Experiments with cpnit-1 have produced two moles of H_2 per mole of glucose under N_2 fixation conditions. H_2 production has been shown to be inversely proportional to ammonia concentration, consistent with enhanced or deregulated nitrogenase activity as the source of H_2. Experiments with cellulosic feedstocks including *Phragmites australis* show greatly increased H_2 production from Cellulose and Organic Solvent-based Lignocellulose Fractionation (COSLIF) treated feedstock as compared to untreated material.

Poster 11-26

Approach for pentitol production from acid-pretreated rice straw hydrolysate by an adapted *Pichia stipitis*

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Pentitol has been applied widely in the food processing, nutrition and pharmaceutical industries. This study was aimed to develop pentitol production technology from lignocellulosic materials. The capability of production for pentitol such as xylitol and ribitol, was found in the xylose utilization by yeast *Pichia stipitis*, which has been through a prolonged adaptation on the acid-pretreated rice straw hydrolysate. Initial xylose concentration was proposed to greatly influence the pentitol production. In comparison to the case with initial xylose concentration of 2%, the production yields of xylitol and ribitol were individually increased 7.88 and 17.2 fold as initial xylose concentration was reached to 6 %. Furthermore, this adapted *P. stipitis* was also shown the ribitol yield of 5 % (percentage of g product per g consumed sugar) from fermentation of rice straw hydrolysate, which was prepared by twin-screw extruder before acid-catalyzed pretreatment. The mechanism for the production of xylitol and ribitol by adapted *P. stipitis* is needed to be elucidated. Transcriptomic and proteomic profiles analysis of this adapted *P. stipitis* are currently under investigations, and it is hopeful to shed some light on the metabolic pathway of the adapted *P. stipitis*.

Poster 11-27

Withdrawn

Poster 11-28

Influence of nutrients on C_{50}-carotenoids production by *Haloferax mediterranei* ATCC 33500

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This study was to investigate the cultural conditions for the production of C_{50}-carotenoids by *Haloferax mediterranei* ATCC 33500 using a two-stage strategy. At the 1st stage culture, ATCC 1176 medium was used as the basal medium and studied on increasing the growth of *H. mediterranei* ATCC 33500 biomass. The 2nd stage culture was simulated as resting cells culture of the 1st stage harvested cells to conduct the bioconversion of C_{50}-carotenoids and studied on the influence of nutrient factors and cultural conditions for C_{50}-carotenoids production at the stage. The results indicated that the optimum conditions for C_{50}-carotenoids production by *H. mediterranei* ATCC 33500 were to use ATCC 1176 medium contained 1% glucose as the initial medium and incubated the cells to the mid-log phase of growth under 37°C, 150 *rpm* of agitation and 1 *vvm* of aeration at the 1st stage culture. Successively, the 2nd stage was proceed to the bioconversion of C_{50}-carotenoids by means of incubating the 1st stage harvested cells in the synthetic salts medium (briefly contained at 5% NaCl, 0.1% CH_3COONa and 8% $MgSO_4.7H_2O$) under 37°C,120 *rpm* of agitation for 24 hr. The yield of C_{50}-carotenoids was reached 0.604±0.005 Abs/mL broth.

Poster 11-29

Reactor Perspectives in Indoor Cultivation of Phototrophic Algae at Large Scale

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Large scale cultivation of autotrophic and phototrophic oleaginous algae has been proposed as a solution to the twin problems of increasing greenhouse gases in our environment and limiting nature of fossilized sources of energetic materials. Algae undoubtedly offer capacity to sequester large quantities of carbon dioxide while utilizing solar energy to produce biomass. Depending on the type of algae and its cultivation conditions, it is possible to have 20-70% of the biomass in the form of lipids with the remainder mostly as carbohydrates and proteins. Starting with the premise that the technology must be usable throughout the year in all climatic conditions and that it may require co-locating the plants with sources of carbon dioxide, the closed and potentially indoor cultivation facilities need to be considered. A survey of the state-of-the-art of the technology for indoor cultivation of algae, however, suggests several major road blocks which must be overcome before it can be commercially exploited at a scale large enough to make any significant impact on the twin problems mentioned above. This presentation will explore the critical issues facing this technology and outline steps that can be taken to address the challenges.

Poster 11-30

Analysis of hydrocarbons produced by marine microalga, *Scenedesmus* sp. JPCC GA0024

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Microalgae, the largest primary biomass, have been attracting attention as a source of high-lipid material to produce biofuel because photosynthetic conversion is an efficient and alternative process and they do not compete with food crops. Especially, the cultivation of marine microalgae under seawater conditions has several advantages in practical application due to low land costs and free seawaters, and preventing contaminant microorganisms from preferentially growing. Our research group has launched the selection of marine microalgae with high neutral lipid content from marine microalgal culture correction in our laboratories to fulfill the open ocean aquaculture of microalgae. Analysis of hydrocarbons produced by a selected strain, *Scenedesmus* sp. JPCC GA0024 was performed under various culture conditions. Growth of JPCC GA0024 was significantly affected by seawater concentrations, and little growth was observed in 0 % seawater conditions. Lipid accumulation also depended on seawater concentration. The highest lipid accumulation was observed in 100 % seawater conditions. Gas chromatography / mass spectrometry analysis indicates that lipid fraction mainly contained straight-chain hydrocarbons including mainly hexadecane and 1-docosene. The strain JPCC GA0024 will become a promising resource that can grow as dominant species in the open ocean toward production of both liquid and solid biofuels.

Poster 11-31

Maximizing algal growth in batch reactors through sequential change in light intensity

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Algal growth requires optimal irradiance. In photobioreactors, optimal light supply requirements change during the growth cycle because with the increase in culture density, the penetration of light through the algal suspension in the reactor is reduced. This creates zones of dissimilar photon flux density (PFD) inside the reactor, which can cause sub-optimal algal growth. However, it is possible to improve growth through design of mixing patterns that cycle cells between high- and low- PFD zones, and by changing light intensities as culture density increases. In this study a lipid producing algal strain, *Neochloris oleoabundans,* is being grown in bioreactors to test the effects of sequential increase in light intensities on growth rates and yields. Our experiments involve studies at three different light levels - 150, 250, 380 micromoles per square meter per second under fixed illumination as well as temporally changing intensities to ascertain optimum light requirements during batch growth. Preliminary results show that a sequential increase in irradiance levels yields up to a 2-fold increase in culture densities over those obtained with experiments performed with single light levels throughout the growth. We are also examining lipid production during our tests to correlate biofuel potential with irradiance and growth yields.

Poster 11-32

Evaluation of biofuel potential through wastewater treatment using algae

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The Logan City Environmental Department (Utah) operates a facility that consists of 460 acres of fairly shallow lagoons (up to 5'deep) for biological wastewater treatment that meets targets for primary and secondary treatments (solids, BOD and pathogen removal). Significant natural algal growth occurs in these lagoons, which improves BOD removal through oxygenation and also facilitates N removal through volatilization as ammonia at high pH conditions created by algal growth. Phosphorus, however, is non-volatile and stays in water and likely cycles in and out of algal cells as they grow and die in the lagoons. Recently, the regulatory limits on phosphorus released from the Logan wastewater treatment facility have been significantly lowered to counter potential downstream eutrophication. One way to potentially lower phosphorus levels in the wastewater effluent is through management of algal growth in the lagoons. As mentioned above, algae growth naturally occurs in the treatment lagoons and if the algal biomass is harvested when growth yields are highest, the phosphorus contained in the cells could be removed as well. The algal biomass could then be used for production of biodiesel, biomethane, and biohydrogen. We have collected pH, dissolved oxygen, temperature, nutrient, BOD, and suspended solids data from the lagoon system over several years. Analysis of this data as it pertains to algal biomass productivity due to seasonal variation and treatment system operation will be presented. Strategies for management of the wastewater treatment system to improve algal productivity while achieving treatment goals will also be presented.

Poster 12-07

Understanding Natural Paradigms for Plant Cell Wall Deconstruction: Community Dynamics and Structure in Decaying Poplar Wood Pile

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Although the microbial degradation of plant cell wall biomass has been studied extensively in various natural and experimental systems, few studies have comprehensively investigated the structural, biochemical and microbial dynamics involved in the natural degradation of biomass feedstocks by composting. In this study, yellow poplar sawdust, a feedstock representing a fast growing hardwood energy crop, was incubated in rotary microaerobic composters for 27 weeks during which samples were collected at regular intervals. Surface degradation of poplar chips was observable by ordinary microscopy after 6 weeks composting, with much more substantial decay of biomass occurring after 15-week composting. Parallel fluorescence-microscopic experiments on the same series of samples using CBM3-GFP (Carbohydrate Binding Module 3 fused to Green Fluorescence Protein) to label exposed cellulose suggest that more hemicellulose and/or lignin were degraded in early stages of composting and that the cellulose in the biomass was thereby progressively more "unwrapped" and exposed, allowing increased access for the CBM3-GFP to bind to the cellulose. Consistent with this suggestion, we observed that the "cellulase" activities, as measured by assays against fluorogenic model substrates, showed increasing predominance in later stages (24-week) of composting, whereas the measured "hemicellulase" activities were higher in the earlier stages. More direct evidence for shifts in ratios of functional lignocellulolytic enzymes, as well as of microbial populations during the composting, was provided by molecular biological analyses of related gene expression abundances. These data lay a foundation for further genomic and proteomic characterization of the dynamics involved in the natural biomass deconstruction process.

Poster 12-08

Identify Molecular Structural Features of Biomass Recalcitrance Using Advanced Imaging Techniques

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Plant biomass is naturally recalcitrant to be deconstructed to simple sugars that are fermentable to transportation fuels (biofuels). To develop a cost-effective process for biofuels production, fundamental research is required to deeper understand the molecular structure of biomass and its bioconversion processes. We have developed advanced imaging techniques that are suitable for characterizing plant cell wall structure and cellulase enzymes at high spatial and chemical resolutions. These techniques include scanning probe microscopy (SPM), coherent anti-Stoke Raman scattering (CARS) microscopy, and single molecule spectroscopy (SMS). In this presentation, I will summarize our recent findings on nanometer scale imaging of plant cell wall cellulose microfibrils, chemical imaging of lignin distribution in genetically-modified plant cell walls, and single molecule tracking of carbohydrate-binding modules/enzymes bound to cellulose crystals.

Poster 12-09

Chemical Imaging of Lignin in Plant Cell Walls Using CARS Microscopy

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Lignins are considered to be one of the primary contributors in the resistance of plant biomass to be deconstructed to fermentable sugars for biofuels production. To improve biomass conversion processes, deeper understanding of the structural architectures of lignins in plant cell walls is required. We have developed microscopic tools that have *in situ* capability to preferably visualize lignin distribution in cell walls with high spatial resolution. The Coherent Anti-Stokes Raman Scattering (CARS) microscopy selectively images a specific chemical structure via its unique chemical bond vibration. Because the contrast mechanism is based on molecular vibrations, which are intrinsic to the samples, no extra labeling and sample preparation are needed. For lignins, the aromatic ring stretch has a signature Raman mode at 1600 cm-1 that can be used for CARS measurement. We have applied CARS to measure the lignin distribution in transgenic alfalfa plant cell walls, as well as raw and pretreated corn stover. Compared to traditional Raman microscopy of imaging lignin, CARS provides much better signal contrast. Our results have also showed that CARS is an ideal tool to semi-quantitatively measure lignin content *in situ*.

Poster 12-10

Probing structural and chemical properties of cellulose with multi-scale theoretical methods

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A critical roadblock to lignocellulosic biofuel is the efficient degradation of crystalline fibers of cellulose to glucose. It is caused by the unusually high thermal and mechanical stability of cellulose. The redundancy in hydrogen bonding (H-bonding) pattern and the intertwinement of intra- and inter-molecular H-bonds ensure this high stability. We have performed computations both at atomistic and coarse-grained levels to investigate the thermal responses of H-bonding networks of cellulose. We will discuss our use of all-atom simulations to understand the molecular aspects that lead to cellulose adopting its different crystal phases, and coarse-grained modeling to understand the bulk properties of microfibrils composed of those phases. All atom replica exchange molecular dynamics simulations have been used to examine (i) conformational preference of different lengths of single cellulose chains and, (ii) aggregation propensities of multiple cellulose chains. We present results that reveal the flexibility and other thermodynamic and mechanical properties. Conformational biases upon assembly are captured and compared with those of soluble cellulose chains. In the coarse-grained approach, we have constructed a statistical mechanical model at the resolution of explicit H-bonds that takes into account both intra-chain and inter-chain H-bonds in naturally occurring cellulose crystals. This model captures the plasticity of the H-bonding network in cellulose due to frustration and redundancy in available H-bonds. Furthermore, instead of only one stable H-bond pattern, different H-bonding patterns dominate at different temperatures till the disassembly at very high temperature. It provides useful clues on rational procedure for the efficient degradation.

Poster 12-11

Biomass Compositional Analysis Method Errors

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Biomass analysis methods are used to quantify the various components found in lignocellulosic feedstocks. Tracking of the carbohydrates, lignin, and other components is needed to compare different feedstocks, determine feedstock variability, and measure pretreatment yields. The National Renewable Energy Lab has adapted wood compositional analysis methods to herbaceous feedstocks such as corn stover. We will report the method errors associated with the analysis of a corn stover sample by eight analysts running 14 batches of a dozen replicates in two laboratories. We will also present error data of significant steps in the analysis methods and compare to the overall error. Suggestions for method improvement will be offered.

Poster 12-12

Rapid Conversion Analysis of Switchgrass Feedstocks using NIR Spectroscopy

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The ability to obtain an accurate assessment of convertibility of switchgrass feedstocks using rapid and inexpensive methods is a key element in the development of dedicated bioenergy crops with enhanced characteristics for biofuels production, such as high ethanol yields and lower cost of conversion. This poster describes the development and use of rapid analysis methods for conversion characteristics of switchgrass feedstocks. These techniques combine Near Infrared (NIR) spectroscopy and Projection to Latent Structures (PLS) multivariate analysis in methods inexpensive enough to allow the conversion analysis of hundreds of samples per day, while maintaining the precision and accuracy of the wet chemical methods used to calibrate the NIR method. NIR methods for both acidic and basic thermochemical pretreatment methods have been employed. Exploration of the conversion characteristics of switchgrass samples allows Ceres to provide critical information to farmers, enzyme manufacturers, and biomass processors to guide our collective thinking in the development of this new industry.

Poster 12-13

Molecular Mechanics Simulations of Cellulose Microfibrils

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Crystalline cellulose is the most recalcitrant component of fermentable biomass. The structure and shape of microcrystalline cellulose fibrils have been studied with molecular mechanics simulations. These studies provide insight into features of the substrate which may be more accessible to enzymatic hydrolysis, and provide a basis for further studies of cellulase-cellulose interactions. Differences in crystal packing and crystal shapes as they relate to biological samples will be described.

Poster 12-14

Changes in Cellulose Molecular Weight During Biomass Pretreatment

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The molecular weight (or degree of polymerization, DP) of cellulose is considered to be one of the most important properties affecting the enzymatic hydrolysis of cellulose. However, there are few studies available investigating the effects of pretreatment on biomass cellulose DP due to the difficulty in making these measurements. In this study, size exclusion chromatography (SEC) was successfully used to measure the molecular weight distribution of cellulose samples derived from pretreated biomass. It was necessary to prepare tetrahydrofuran-soluble cellulose tricarbanilate derivatives from pretreated corn stover to conduct SEC. We found that in dilute acid and organosolv pretreated corn stover samples the chromatograms of the cellulose carbanilates shifted to longer retention time as pretreatment severity increased, implying that this treatment parameter caused a reduction in apparent molecular weight. These changes in DP should significantly influence enzymatic hydrolysis of the cellulose in the pretreated biomass, because it is expected that β-1,4-exoglucanase activity is dependant on the number of available chain ends.

Poster 12-15

Microscopic evaluation of plant cell wall structure of ensiled corn stover by correlative microscopy

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Ensiling has been proposed as a method of feedstock storage for biorefineries. The traditional use of ensiling is to provide winter forage and to increase forage digestibility for livestock, but the potential effect of ensiling on feedstock bioconversion hasn't been fully examined. As part of a collaboration between INL and NREL to determine the effect of ensiling on biomass conversion, we have used microscopic imaging techniques to analyze the impact ensiling has on cell wall structure. We were able to assess mechanical, chemical, and biological disruption to the structure of ensiled corn stover cell walls. Also, we were able to detect microbial colonization and direct microbial interactions with cell walls. From a microscopic analysis perspective, the cell walls of ensiled corn stover appear to have been altered somewhat by ensiling. While there is not the extensive re-localization of cell wall matrix components as seen in dilute acid pretreated cell walls, the cell walls of ensiled materials do appear loosened compared to senesced, field-dried feedstock. Based on these structural parameters alone, we would anticipate that these materials should be at least as digestible as field-dried material.

Poster 12-16

How Pretreatment Can Overcome the Natural Recalcitrance of Biomass to Cellulase Hydrolysis

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The structure and chemical composition of plant biomass has evolved to make the cellulose recalcitrant to hydrolysis by cellulase enzymes. Contributing factors to the recalcitrance of lignocellulosic biomass include the epidermal tissue of the plant body, the organization and types of cells present in plants, the difficulty of liquid penetration into the plant cell wall, lignification, the diversity of hemicellulose structure, and cellulose structure. Enzymatic conversion of lignocellulosic biomass requires an effective pretreatment to enable efficient cellulase action. Pretreatment conditions cover the entire range from low to high pH, moderate to high temperatures, and minutes to weeks. The main job of pretreatment in a biorefinery is to overcome the natural recalcitrance of biomass, increasing access of enzymes to cellulose, and increasing the ease with which the cellulose can be hydrolyzed. Using a variety of tools, we have developed a better understanding of how pretreatment processes can generate highly digestible cellulosic substrates. Characteristics such as cellulose accessibility, crystallinity, morphology and molecular weight can indicate pretreatment effectiveness. Other factors include substrate porosity and particle size. Microscopic imaging can indicate changes in lignin and xylan distribution in the plant cell wall that can also be important. This paper will summarize our current understanding of how pretreatment can overcome the natural recalcitrance of biomass to cellulase hydrolysis.

Poster 12-17

Compositional Analysis of Lignin in Bioenergy Crops and De-Polymerization via Pretreatment

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Lignin is a complex phenylpropanoid polymer derived from enzyme mediated radical coupling of coniferyl, sinapyl and coumaryl alcohols with main functions to impart strength to plant cell wall, transport water and provide defense against pathogens. Physical, chemical and biological degradation of cellulose and hemicellulose is inversely proportional to the amount of lignification. Lignin therefore confers biomass recalcitrance and necessitates pretreatment steps, which are costly and must be improved if bioenergy from biomass is to be realized. New emerging pretreatment techniques employing ionic liquids have shown great promise and are very effective in breaking inter and intramolecular hydrogen bonding in cellulose microfibrils, thereby making cellulose amorphous and enhancing saccharification in both model cellulose and biomass. However, detailed understanding and insight into the extent of lignin depolymerization during pretreatment processes and the influence of degree of lignification, lignin chemical composition, and chemical association between lignin and hemicellulose are lacking. Towards the goal of gaining molecular level understanding of lignin signatures and their influence on depolymerization via pretreatment, we are utilizing light scattering, spectroscopy, chromatography and modified wet chemistry techniques. We aim to gain knowledge for attaining optimized pretreatment conditions, aiding efforts on altering chemistry in bioenergy crops, and perhaps selective depolymerization of celluloses or lignin. We will present results on our ongoing experiments on potential bioenergy crops with varied dgree of lignification and lignin composition characterized before and after pretreatment. In addition, disruption of lignin-hemicellulose association during pretreatment will be discussed.

Poster 12-18

A Comparative Study of Dilute acid and Ionic Liquid Pretreatment of Biomass and Model Lignocellulosics

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Lignocellulosic biomass has the great potential to serve as the low cost and abundant feedstock for bioconversion into fermentable sugars, which can be further utilized for biofuel production. However, high lignin content, crystalline cellulose structure and the presence of ester linkages between lignin and hemicellulose in the plant cell wall limit the enzymatic accessibility for efficient saccharification. Various physical and chemical pretreatment methods are currently employed to break down the biomass recalcitrant structures, and increase their susceptibility to enzymes. Among these techniques, dilute acid pretreatment has been shown as a leading pretreatment process. However, dilute acid hydrolysis can lead to degradation products that are often inhibitory and significantly lower the overall sugar yields. Glucose and xylose degradation products that result from the pretreatment methods include hydroxymethylfurfural (HMF) and furfural, which produce levulinic and formic acids, respectively, which inhibit the subsequent fermentation of sugars to ethanol. Recently, ionic liquids have demonstrated great promises as efficient solvents for biomass dissolution with easy recovery of cellulose upon anti-solvent addition. However, to date, no comprehensive side-by-side comparative analysis has been conducted in order to evaluate the dilute acid and ionic liquid biomass pretreatment processes. In this study, we are comparing ionic liquid and dilute acid pretreatments acting on switchgrass with numerous analytical techniques to gain a better understanding of both techniques and their saccharification efficiency into fermentable sugars for downstream biofuel production.

Poster 12-19

Molecular dynamics simulations of cellulose-solvent interactions

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Molecular dynamics computer simulations have been performed on model cellulose fibrils immersed in different solvents in order to investigate molecular details of the interatomic interactions between solvent and saccharide units. Different cellulose microcrystalline models comprised of different chain lengths, chain numbers and packing are investigated in the presence of polar solvents, including associative liquids (water and ethanol) and non-associative solvents (acetone and dimethylsulfoxide). Intermolecular structure, solvation structures, chain packing, and the underlying intermolecular interactions are investigated in detail emphasizing the role played by the specific interactions between sugar units and solvent molecules.

Poster 12-20

Application of biological pretreatment to improve thermal and chemical processes releasing sugars and bioproducts from wheat straw

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This study highlights the biological pretreatment process in combination with mild thermochemical treatment to hydrolyze wheat straw releasing fermentable sugars as well as bioproducts. It uses *Phanerochaete chrysosporium* to pretreat the straw up to three weeks at 37 °C. In the sugar analysis 0.1 % Tween 80 treated straw showed a better performance, among Tween 80, lactic acid and H_2O_2, with a total sugar yield of 1.7% of the total dry mass. Decaying of biomass was observed by Scanning Electron Microscopy (SEM). To enhance the hydrolysis, samples were subjected to mild acid (0.2% H_2SO_4) treatment at 121 °C/ 20 mins. The total sugar of ~ 18 gmL^{-1} was detected with the first week sample where the control gave ~ 6 gmL^{-1}. However, the enzymatic hydrolysis gave no significant changes in sugar level comparing to control. Therefore, the experiment suggests that the mild acid treatment can be useful method combining biological pretreatment to extract the hydrolysable sugars. Fourier Transform Infrared (FTIR) spectroscopy showed the distinct absorbance at 1500–1800 cm^{-1} indicating the deposition of C=O stretching compounds, 1497-1530 cm^{-1} the aromatic skeletal and 1383-1360 cm^{-1} for –C-CH_3 & C=O. The result was further verified by fast pyrolysis-GC/MS. A clear increasing trend in ratios of venylguaicyl and syringol with biomass incubation time was noted. Overall, this approach of biomass processing is a new way to utilize the biomass both into fermentable sugars and bioproducts. A further study is warranted to discover the effect of organic compounds after straw biodegradation in ethanogenic fermentation process.

Poster 12-21

Structural changes in lignin and cellulose resulting from the two-step dilute acid pretreatment of Loblolly pine

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Two-step dilute sulfuric acid pretreatment was performed on Loblolly pine to enhance the overall efficiency of the enzymatic conversion of lignocellulosic biomass to monomeric sugars prior to their fermentation to bioethanol. Lignin, cellulose and hemicellulose the major components of lignocellulosic biomass, are closely associated with each other at the plant cell level. This close association, together with the partly crystalline nature of cellulose protects it from enzymatic hydrolysis of native biomass. In the overall conversion of biomass to bioethanol, the structure of lignin is also of importance as it may physically hinder cellulase access to cellulose microfibrils and participate in non-productive binding to enzymes. Detailed structural characterization of cellulose and milled wood lignin isolated from Loblolly pine before and after the two-step dilute sulfuric acid pretreatment elucidates the modifications taking place as a result of this pretreatment. Solid-state ^{13}C NMR spectroscopy coupled with line shape analysis has been used to determine cellulose crystallinity and ultrastucture. The results indicate an increase in the degree of crystallinity and reduced relative proportion of less ordered cellulose allomorphs. These changes may be attributed to a preferential degradation of amorphous cellulose and less ordered crystalline forms during the treatment. Milled wood lignin structural elucidation by quantitative ^{13}C and ^{31}P NMR reveals an increase in the degree of condensation. This is accompanied by a decrease in the number of β-O-4 linkages which are fragmented and subsequently recondensed during high temperature acid-catalyzed reactions. The impact of these changes on pine recalcitrance and enzymatic deconstruction will be reviewed.

Poster 12-22

Enzymes Loading Optimization in the Hydrolysis of Sugarcane Bagasse – A Comparison between Bagasse Pretreatment with Lime and Alkaline Hydrogen Peroxide

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Sugarcane bagasse is one of the potential lignocellulosic feedstocks for bioethanol production. The ethanol yield depends on the efficiency of conversion of glucans and xylans to fermentable sugars without generating byproducts that are toxic to fermentative microorganisms. Thus, the rate and extent of enzymatic hydrolysis of lignocellulosic biomass is very important and highly dependent on enzyme loadings, hydrolysis periods, and structural features resulting from pretreatments. In this work, the optimization of enzymes loading in the hydrolysis of sugarcane bagasse pretreated using two different agents (lime and alkaline hydrogen peroxide) was performed. The effect of enzymes loading in the fermentable sugars yield was studied through analyses using central composite design (response surface) to determine the optimal loadings of cellulase and b-glucosidase. The responses evaluated were glucose and xylose yield released from pretreated bagasse after enzymatic hydrolysis. Experiments were performed using bagasse as it comes from an alcohol/sugar factory, pretreated with lime and alkaline hydrogen peroxide in previously optimized conditions. The higher hydrolysis yield for bagasse pretreated with lime was found using 66.5 FPU/g dry pretreated biomass of cellulase and 25 CBU/g of dry pretreated biomass of beta-glucosidase. Yields were of 78.6% glucose and 100.0% xylose. For the pretreatment with alkaline hydrogen peroxide, the best result was using 12.7 FPU/g dry pretreated biomass and 7.3 CBU/g of dry pretreated biomass for cellulase and beta-glucosidase, respectively, with yields of 100.0% glucose and 52.6% xylose. The influence of pretreatment on hydrolysis yield and on the enzymes loading necessary for optimal performance were discussed.

Poster 12-23

Alkaline hydrogen peroxide pretreatment of sugarcane bagasse for enzymatic hydrolysis: The influence of temperature and pretreatment time on delignification and sugars yield

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The raw material used in this research was sugarcane bagasse, which has great potential to produce ethanol fuel and other products. In this work, pretreatment of sugarcane bagasse with alkaline hydrogen peroxide and the subsequent enzymatic hydrolysis performance were addressed. Starting from an optimization performed for sugarcane bagasse from a different source and with different granulometry from the bagasse used in this work, the best conditions of temperature, pretreatment time and hydrogen peroxide concentration to maximize glucose yield were defined. The hydrolysis yield and the composition of the two different bagasses before and after pretreatment were compared. After the best pretreatment conditions have been defined, kinetic data of the pretreatment were obtained for different temperatures (25, 45 and 65°C) as a function of time, with analysis of the contents of lignin, cellulose and hemicellulose. The content of lignin was lower at 65°C, however, at this temperature, there is loss of cellulose and hemicellulose.

Poster 12-24

Optimization and kinetics of lime pretreatment of sugarcane bagasse to enhance enzymatic hydrolysis

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Renewable energy sources, such as lignocellulosic biomass, are environmentally friendly because they emit less pollution without contributing for net carbon dioxide to atmosphere. Among lignocellulosic biomass, sugarcane bagasse is an economically viable alternative to produce biofuels. The objective of this work is to study the pretreatment of sugarcane bagasse with lime to enhance enzymatic hydrolysis and subsequent ethanol fermentation. The first stage was an evaluation of the influence of solids loading (4-10%) and stirring speed (150-350rpm) during pretreatment on sugars yields after hydrolysis. Afterwards, a 2^3 central composite design was performed to determine the values of temperature, reaction time and lime mass that maximize glucose release after hydrolysis. The maximum glucose yield was 228,45mg/g raw biomass, corresponding to 409.9 mg/g raw biomass of total reducing sugars, with the pretreatment performed at 90ºC, for 90 h and with 0,4 g $Ca(OH)_2$/g dry biomass. The enzymes loading was 5FPU/dry pretreated biomass of cellulase and 1CBU/ dry pretreated biomass of b-glucosidase. After the optimal conditions were determined, kinetic data of the pretreatment were obtained for different temperatures (60, 70, 80 and 90ºC) as a function of time. Bagasse composition (cellulose, hemicellulose and lignin) was measured and the study has shown that 50% of the original material was solubilized, lignin and hemicellulose were selectively removed, but cellulose was not affected by lime pretreatment in mild temperatures (60 – 90ºC). The delignification is highly dependent of temperature and time.

Poster 12-25

Parallel Plate Processing for High Throughput Pretreatment and Enzymatic Saccharification of Lignocellulosic Materials

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Improved understanding on the fundamental nature of biomass recalcitrance is required to advance technologies for the conversion of lignocellulosic materials. This understanding is central to the core research within the DOE-funded BioEnergy Science Center (BESC). Within this center, which includes industrial, academic, and governmental partners, one primary research focus is to identify key factors that contribute to biomass recalcitrance and to apply this knowledge to the development of "improved" (less recalcitrant) plant cell wall materials. Many thousands of plant variants are being screened for increased susceptibility to both pretreatment and enzymatic saccharification, necessitating a high throughput pipeline capable of analyzing thousands of samples per day. In order to meet the screening demands of this effort, a parallel plate processing system was developed for high-throughput analysis of lignocellulosic materials' response to pretreatment and enzymatic saccharification. The system incorporates state-of-the-art robotics systems for both solids and liquids handling, a novel multi-plate 96-well-plate pretreatment reactor system for running up to 1920 simultaneous chemical/thermal reactions, and enzyme-linked oxidation-reduction assays for the detection of the principle sugars released by the combined processes. This screening system will be an effective tool for the BESC Analysis Pipeline in identifying variations in the recalcitrance of lignocellulosic materials. Outliers identified through this system will be further subjected to a more rigorous analysis of how these specific variants react to chemical pretreatments and subsequent enzymatic saccharification.

Poster 12-26

Structural analysis of steam pretreated spruce after enzymatic hydrolysis

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Due to the depleting sources and growing environmental concerns on fossil fuels, there is an increasing need for alternative energy sources. Fuels from renewable resources have a great potential to contribute towards a more sustainable economy. Among the renewables, lignocellulosic raw materials, especially from side steams of agriculture and forest industry are promising alternatives for the production of platform sugars and various chemicals. Their recalcitrance structure, however, still poses a scientific challenge.

The enzymatic hydrolysis is restricted by several factors related either to the physico-chemical structure of the lignocellulosic substrates or the efficiency of the enzymatic system. These potential bottlenecks have to be overcome in order to reduce the overall amount and costs of enzymes. Various chemical treatments and enzymes can be used to enhance the conversion of polysaccharides by hydrolyzing or modifying the residual polymers in the matrix.

To gain a better understanding on the factors structurally limiting the hydrolysis, the conversion of carbohydrate polymers was studied with the well-characterised *Trichoderma reesei* enzyme system. The chemical composition and the structure of the steam pretreated spruce substrate was modified with combinations of purified enzymes and analyzed with various analytical and spectroscopic methods. Especially X-ray microtomography revealed interesting morphological features of the substrate. These results will help to better understand and overcome the bottlenecks in the hydrolysis of recalcitrant plant biomass raw materials.

Poster 12-27

Synergism of a bacterial expansin, BsEXLX1 with the catalytic doamin endo-β-1,4-glucanase in cellulose hydrolysis

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The expansin is the plant cell wall protein which is known to induce the extension of plant cell wall polysaccharides in plant growth. Expansins are receiving much attention due to their application potential since they are also found to have a synergistic effect on enzymatic hydrolysis of cellulose when used in combination with cellulase. However, the expression of plant expansins in organisms other than plants has not been reported yet. Recently, we characterized the structural expansin homologs from bacteria and found that they had synergistic activity for cellulose hydrolysis by cellulase in addition to binding and weakening activities for cellulose (Kim, E. S. et al., J. Biotechnol. 136S: S426, 2008; H. J. Lee et al., J. Biotechnol. 136S: S343, 2008; Kim, E. S. et al., Biotechnol. Bioeng., in press). In this study, important characteristics of BsEXLX1, which was cloned from *Bacillus subtilis* and overexpressed in *E. coli*, were investigated. In the adsorption isotherm study of BsEXLX1 using Avicel, the binding activity was dependent on both pH and temperature, and it also showed binding activity to other polysacchartides such as agarose, starch, and xylan. When a catalytic domain of endo-1,4-β-glucanase was used with BsEXLX1 for cellulose hydrolysis, the hydrolysis by endo-1,4-β-glucanase was promoted by BsEXLX1. This is the first to study of the bacterial expansin for its adsorption isotherm and synergism with cellulase containing a catalytic domain only.

Poster 12-28

Functional analysis of a bacterial expansin from *Hahella chejunsis* for promoting hydrolysis of xylan

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Expansins are plant cell wall proteins that are known to involve in extension of cell wall during plant growth, and it is presumably considered to be induced by disrupting hydrogen bonds within the cell wall polymer matrix. The cell wall-loosening activity gives high potential for effective deconstruction of lignocellulose that is the main component of plant cell wall. Due to the difficulty of expression of plant expansin in hosts other than plant, bacterial expansins are receiving increasing attention (H. J. Lee et al., J. Biotechnol. 136S: S343, 2008; Kim, E. S. et al., Biotechnol. Bioeng., in press). Previously, we identified a bacterial expansin in *Hahella chejunsis* (HcEXLX) based on its amino acid sequence homology with BsEXLX1, a bacterial expansin from *Bacillus subtilis*, where the molecular function of HcEXLX was initially unknown and annotated as a hypothetical protein (H. J. Lee et al., J. Biotechnol. 136S: S343, 2008). HcEXLX was overexpressed in *E. coli* in a soluble form at 30 mg/L, and the bacterial expansin was found to bind various polysaccharides such as Avicel, BMCC, xylan from oats spelt slightly (12%). In addition to the synergistic activity toward cellose hydrolysis with cellulase, HcEXLX showed a synergistic effect (~ 300% compared with the control without HcEXLX) in the hydrolysis of xylan (from oat spelt) by xylanase. This is the first report showing the synergistic effect of a bacterial expansin on hydrolysis of xylan.

Poster 12-29

Using carbohydrate-binding module as molecular probe to map biomass polysaccharides

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Carbohydrate-binding modules (CBMs) are noncatalytic domains found in many carbohydrate-hydrolyzing enzymes, such as the cellulases and hemicellulases. They are thought to function as recognition modules that convey the catalytic modules of these enzymes to the target substrates. We have constructed a library that contains various families of CBMs, each of these CBMs has been tagged with either various fluorescent protein or genetic tag that could be used to conjugate to other fluorophores,such as quantum dots and fluorescence dyes. The fluorescently-labeled CBMs have been applied to map polysacchride distribution of raw and chemically/biologically-pretreated biomass,and further investigeted by semi-quantitative fluorescence microscopy.

Poster 12-30

Rates and Yields of Cellulosic Ethanol from Maize Silage with Effect of *Brown Midrib* Mutations

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The processing characteristics of biofuel feedstocks are strongly affected by the quantity and quality of lignin in the cell wall structure. We present the effect of *brown midrib* mutations on rates and yields of cellulosic ethanol production from maize silage. Both raw silage and silage from commercial sources were pretreated using liquid hot water (160-180°C) and assessed by enzymatic hydrolysis and fermentation using the glucose/xylose fermenting Purdue recombinant *S. cerevisiae* 424A (LNH-ST). At 20% solids concentration (200 g/L), pretreated *bmr* silage achieved higher yields of sugars than non-*bmr* silage pretreated under the same conditions. At the optimal pretreatment conditions, *bmr* silage achieved 62% of theoretical yield of glucose after 24 hours of enzymatic hydrolysis (15 FPU cellulase per gram glucan) compared to 50% yield from non-*bmr* silage. Sugars from both silage varieties fermented to ethanol at high yields using the Purdue recombinant yeast strain.

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FUTURE SIM MEETINGS

2009

SIM Annual Meeting & Exhibition
SIM 60th Anniversary
Westin Harbour Castle Hotel
Toronto, ON Canada
July 26 – 30, 2009

RAFT VIII
Marriott Mission Valley
San Diego, California
November 8-11, 2009

2010

32nd Symposium on Biotechnology for fuels and Chemicals
Hilton Clearwater Beach
Clearwater Beach, Florida
April 19-22, 2010
(*Monday-Thursday*)

SIM 60th Annual Meeting
Hyatt Regency Embarcadero
San Francisco, CA
August 1-5, 2010

2011

33rd Symposium on Biotechnology for fuels and Chemicals
Sheraton Seattle
Seattle, Washington
May 2-5, 2011

SIM Annual Meeting
Sheraton New Orleans
New Orleans, Louisiana
July 24-28, 2011

RAFT IX
Marriott Marco Island
Marco Island, Florida
November 6 – 9, 2011

2012

SIM Annual Meeting
Hilton Washington
Washington, DC
August 12-16, 2012

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