Oral Presentations

Oral Presentation 1-01
Genomic, Transcriptomic, Proteomic and Functional Analysis of Candidate Genes for Bioenergy Feedstock Improvement
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Here we present the discovery and characterization of candidate genes for bioenergy feedstock improvement in rice and switchgrass from four perspectives. First, comparative genome analysis of ten monolignol biosynthesis gene families in rice, Arabidopsis and poplar revealed a surprising lack of coordinative evolution of monolignol biosynthesis genes and helped to identify important conserved genes for lignin modification. Second, cell wall-related genes are studied with transcriptomic and proteomic approaches. Microarray gene profiling among different tissues revealed a coordinative up-regulation of cell wall biosynthesis and expansion genes such as CES, glycosyltransferase, expansins, XTHs in the cotyledon, whilst fewer cell wall-related genes were found to be up-regulated in the adult stem. Surprisingly, although the adult stems contain more lignin, much more lignin biosynthesis genes were up-regulated in the cotyledon. The transcriptomics thus needs to be complemented by the proteomics, where cell wall proteins from the same tissues are being extracted and compared with 2D-DIGE and on-line shotgun LC-MS/MS approaches. Third, according to the ‘omics’ analysis, mutants with down-regulated lignin biosynthesis were analyzed for biomass conversion efficiency. While most of the rice lignin biosynthesis mutants displayed higher saccharification efficiency, the F5H mutant showed a significant 50% decrease, which indicated the importance of both composition and content of lignin for saccharification efficiency in monocot species. Fourth, based on the rice studies, we have cloned and analyzed important lignin biosynthesis genes in switchgrass, and RNAi mutants are being generated. Overall, the ‘omics’ approaches can be highly effective in identifying key genes for monocot bioenergy feedstock improvement.

Oral Presentation 1-02
Maximizing Photosynthetic Yield by Increasing Sink Strength
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One of the main factors that determine the economic viability of biofuel production is the efficiency of photosynthetic CO2 assimilation into storage and structural products by the plant. Improvements in these areas require a thorough understanding of the metabolic fluxes in plant sinks and of the mechanisms that regulate source-sink interactions. For example, little is known about the molecular switches that partition photosynthesize to growth and storage. We also don’t understand the mechanisms that control feedback inhibition of photosynthesis by sink demand. When we understand these regulatory networks it should be possible to engineer current crops to allocate more carbon to cellulose, starch or other carbohydrates. We study source-sink interactions during cereal development. The long-term objective is to obtain a comprehensive map of the metabolic and regulatory events controlling photosynthetic capacity and sink strength. One direction will be to identify the set of transcription factors that govern sucrose utilization in source and sink tissues. Candidate transcription factors include the SUSIBA family, identified by us (Sun et al., 2003, 2005, 2007). References Sun, C., Ahlandsberg, S., Palmqvist, S., Ohlsson, H, Borén, M. and Jansson, C. (2003) Plant Cell 15, 2076-2092. Sun, C., Olsson, H., Höglund, A.-S., Mangelsen, E. and Jansson, C. (2005) Plant J. 44, 128-138. Sun, C., Ridderstråle, K., Larsson, L.-G., Jansson, C. (2007) Plant J., in press.
Oral Presentation 1-03

Developing a Dedicated Energy Crop for Tennessee

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The primary dedicated energy crop that the University of Tennessee is working on is switchgrass. The goals in this project include a demonstration of switchgrass production within the state of Tennessee under a variety of conditions and topography through on-farm demonstrations, establishment of an information and education bio-feedstock program available to producers as they determine whether to adopt a new cropping regime, and an evaluation of agronomic, logistic, energy conversion, and farming system issues associated with commercialization of a biomass energy industry.

The project, initiated in 2003, has 32.5 acres of switchgrass at Milan, TN, and 92 acres of switchgrass planted and managed by 5 producers. The land at Milan contains several experiments. Switchgrass is planted on different soils and landscape positions. Within the fields, randomized replicated experiments are established on each situation to look at varying management for the widely different environments. The management variables include fertility levels and seeding rates along with different varieties of switchgrass. Once the Milan fields were successfully established, individuals in both Extension and research combined to develop a pilot program. Land was bid into the program by farmers and 92 acres were planted to switchgrass in 2004 following guidelines developed by UT researchers and based on the literature and experience in establishing switchgrass the prior year. This paper reports the findings from this project after four years of harvest data, the development of Extension experience, and the likely impact that this project will have on the establishment of a 1/10 scale bio-refinery.

Oral Presentation 1-04

Switchgrass, lignin, enzymes and ethanol

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Switchgrass populations divergently selected for in-vitro dry matter digestibility (IVDMD) have yielded plants with significant differences in total lignin in stems. These plants constitute a resource to understand exploitable diversity in traits that could improve switchgrass as a biofuel crop. In this study we have used a variety of methods to phenotype select plants from these populations. Carbon-isotope discrimination data revealed a tendency of plants with high lignin to discriminate less than plants with low lignin, suggesting subtle changes in the levels and activities of enzymes involved in lignin biosynthesis. Thioacidolysis of stem tissues indicated that differences in the levels S- and H-lignin and variable incorporation of H-G-lignin into cell walls were genotype dependent. The relative contributions of the different lignin-biosynthetic enzymes and isozymes to the observed lignin compositional data appear to be complex. Ethanol yields from SSF of mild pretreated whole biomass did not differ with stem lignin content, suggesting further studies are needed using fractionated stem material. The relationships between lignin, carbon-isotope discrimination ratios, levels and activities of specific lignin-biosynthetic enzymes to ethanol conversion in these plants will be presented.

Oral Presentation 1-05

Development of Low-lignin Biomass Feedstock for Improved Biofuel Production


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The accessibility of plant cell wall polysaccharides to chemical, enzymatic and microbial degradation is limited by many factors, including the presence of lignin. Lignin is a polymer of hydroxylated and methoxylated phenylpropane units linked via oxidative coupling. It accumulates in secondary cell walls during the development of vascular tissues and fibers, and imparts both mechanical strength and hydrophobicity. The composition and structure of lignified cell walls has a dramatic impact on the technological value of raw materials, and has been identified as a key factor limiting effective biomass to biofuel conversion in processes where the sugar components of polysaccharides are released prior to fermentation to ethanol. In addition to physically preventing access of microbial enzymes to cellulose, lignin may also exert a negative impact on bioethanol production through its partial degradation to inhibitors of the microbial fermentation system. A series of transgenic lines of alfalfa (Medicago sativa) were generated in which either one of the two potentially terminal enzymes of the monolignol pathway, cinnamoyl CoA reductase, coniferyl alcohol dehydrogenase and 4-(hydroxy)cinnamoyl CoA ligate, was down-regulated by expression of antisense genes. Transgenic plants with reduced lignin content could yield more fermentable sugar from cell wall polysaccharides as did wild-type plants, a finding with significant implications for the cost-effectiveness of bioethanol. The relationship between lignin and saccharification of plant cell walls for bioethanol production will be discussed. Additional targets for genetic manipulation to improve cell wall deconstruction, and the impacts of lignin modification on plant growth, will also be discussed.

Oral Presentation 1-06

Engineering the Nation’s First Dedicated Biofuel Crop

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Rising above the increasing din of concern about food vs. fuel, a new incarnation of an age old crop, camelina, is offering hope to a thirsty biodiesel industry. This presentation will chronicle how Seattle-based Targeted Growth has optimized camelina for use as a dedicated bioenergy feedstock and the innovative business model surrounding it. Specifically, it will look at both non GMO (Molecular Assisted Marker Breeding) and GMO (yield increasing genes) to create an oil-rich feedstock that reduces requirements for water and fertilizer and can safely grow in rotation with other cereal grains or in marginal farmland. Camelina represents the first in a new breed of dedicated energy crops that will contribute to the success of the biofuels industry. Finally the presentation will discuss the groundbreaking partnership between Targeted Growth and leading biofuel producer Green Earth Fuels to create a vertically-integrated venture to exploit camelina. This closed-loop model could serve as a prototype for an industry that currently is subject to the volatility of the commodities market.
Fermentability of corn stover hydrolysates resulting from different pretreatments: a side-by-side comparison using recombinant ethanologens

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In order to effectively use lignocellulosic materials as a carbon source for ethanol production, several challenges must be overcome include (1) production of a fermentable hydrolysate from lignocellulose with no (or very low-cost) conditioning and (2) complete utilization of hexoses and pentoses by the ethanogenic strains.

Feedstock pretreatments such as Ammonia-Fiber-Expansion (AFEX) and acid pretreatment dictate the fermentability of the resulting hydrolysate. There is a common perception that hydrolysate from lignocellulosic materials is inherently nutrient-deficient. However, hydrolysate generated from AFEX pretreatment exhibits high fermentability without any conditioning and nutrient supplementation.

Additionally, metabolic engineering for effective ethanol production has been extensively pursued in recent decades. Reports on different ethanologens indicate highly promising results. However, comparison across the strains is difficult due to different experimental settings. The performance of these strains on unconditioned hydrolysate cannot be determined because many experiments have involved extensive detoxification and/or nutrient supplementation prior to fermentation.

We have studied the fermentation performance of several recombinant ethanologens side-by-side using AFEX, acid-pretreated and steam-pretreated corn stover. These ethanologens include Escherichia coli KO11 (U. of Florida), Saccharomyces cerevisiae 424A(LNH-ST) (Purdue University) and Zymomonas mobilis AX101 (National Renewable Energy Lab).

From these investigations, we have obtained comparative data for ethanol yield, productivity and growth robustness in cellulosic hydrolysates. As a result, the most promising ethanologen(s) suited for ethanol production from various pretreatment methods were identified. Based on these strains, we have optimized fermentation conditions with industrially-relevant parameters in both Separate-Hydrolysis-and-Fermentation (SHF) and Simultaneous-Saccharification-and-Co-Fermentation (SSCF) using AFEX-pretreated corn stover as the main feedstock.

The Development of TM242: A Novel Thermophilic Bacillus Capable of High Yield Ethanol Production

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The efficient conversion of lignocellulosic biomass to liquid transport fuels such as ethanol has become a major industrial goal in the effort to reduce dependency on fossil fuels and reduce CO2 emissions. Fermentation of biomass hydrolysates has been hampered by the lack of suitable microorganisms that can effectively metabolise all of the hexose and pentose sugars present in these hydrolysates. Strategies to address this have included engineering yeast to metabolise pentose sugars, and the modification of a number of bacterial species to increase ethanol yields. Thermophilic microorganisms have a number of potential advantages for the development of fermentation processes for alcohol as a biofuel. High growth temperature favours high rates of growth and ethanol productivity and also reduces the risk of contamination which continues to be a significant problem in large-scale commercial operations.

The parent strain of TM242, a Geobacillus sp., was chosen because of its ability to metabolise a wide range of biomass derived monomeric and oligomeric sugars. It is a facultative anaerobe capable of growth between 40-70°C and can ferment both hexose and pentose sugars to generate lactate, formate, acetate and relatively low amounts of ethanol as products. Newly developed metabolic engineering tools have been applied to this organism to alter the expression of key enzymes to produce TM242 in which acid production is essentially eliminated and ethanol is efficiently produced at yields approaching the theoretical maximum value. This presentation will outline the metabolic steps involved in the creation of TM242 and illustrate the performance benefits at each step.

The fungal path for D-galacturonic acid catabolism

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D-galacturonic acid is the major component of pectin and consequently an important carbon source for microorganisms living on decaying plant material or for biotechnological processes where cheap raw materials such as sugar beet pulp are used. A bacterial catabolic pathway had been described while a eukaryotic pathway has remained unknown. For E. coli a pathway was described consisting of five enzymes converting D-galacturonic acid to pyruvic acid and D-glyceraldehyde-3-phosphate. The enzymes of this pathway are uronate isomerase, NADH-utilizing D-tagaturonate reductase, altronate! dehydratase, D-erythro-3-deoxy-D-hexulosonate kinase and D-erythro-3-deoxy-D-hexulosonate-6-phosphate aldolase.

We show that a fungal pathway exists that is distinctly different from any previously described pathway. In this pathway D-galacturonic acid is converted to pyruvate and glycerol. The intermediates are L-galactonate, L-threo-3-deoxy-hexulosonate and L-glyceraldehyde. The pathway contains four enzymes, NADPH-utilizing D-galacturonic reductase, L-galactonate dehydratase, L-threo-3-deoxy-hexulosonate aldolase and a glycerol dehydrogenase that converts L-glyceraldehyde to glycerol in the reverse reaction. All the of the enzymes of this pathway have been cloned, expressed in a heterologous host and their kinetic properties determined. We will present potential biotechnological applications of this novel pathway.

Mining the Natural Biorefinery in a Lignocellulose Degrading Insect, Tipula abdominalis

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Microbial fermentation in insect guts is an important component of global carbon cycling and those insects consuming lignocellulose rich diets have developed numerous mechanisms for surviving on a nutrient poor resource. One such lignocellulose degrading insect is Tipula abdominalis, the aquatic cranefly. Larval Tabdominalis deconstruct lignocellulose with the aid of a hindgut microbial consortium. Cellulose degradation can be enhanced by co-culturing cellulolytic anaerobes and non-cellulolytic aerobes; in nature, cellulose degradation occurs through the cooperation of many microorganisms suggesting a synergistic relationship. Both cellulolytic anaerobes and non-cellulolytic aerobes have been isolated from the T. abdominalis larval hindgut. To examine synergistic cellulose degradation by the hindgut microbial community, using filter paper as the sole carbon source, enrichment cultures were established in glass-stoppered bottles, which allowed aeration and cooperative cellulose degradation by aerobes and anaerobes. Cultures became anaerobic, with visible degradation of filter paper. Successive generations of the enrichment culture communities were cultivated, and isolations were performed both aerobically and anaerobically on nutrient-limited media containing cellulose or cellubiose. Denaturing gradient gel electrophoresis (DGGE) analysis of the complex microbiota provided no discernable patterns of community composition. Functional gene libraries and large insert clone libraries are being screened. Understanding mechanisms used in this conversion of a poor nutrient source into energy for the stream ecosystem has relevance to efficient depolymerization of recalcitrant plant cell walls for bioenergy.
Oral Presentation 2-05
De novo biocatalyst design: an alternative strategy for the petroleum-free synthesis of biobutanol
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Butanol production relies on petroleum-based syntheses since the challenges in improving natural biocatalysts hinder the efficient synthesis starting from renewable biomass. An alternative biocatalyst was developed to allow butanol synthesis using Escherichia coli. The engineering of the biocatalyst entails the simultaneous introduction of the multi-gene butanol biosynthetic pathway of solventogenic Clostridium. To improve the functionality of the engineered pathway, the expression of a synthetic gene encoding for a rate-limiting step enzyme was explored. Furthermore, to elicit high productivity, the native metabolism of the butanol-producer strain was reprogrammed to direct carbon flow and cofactor regeneration. The synergistic combination of the de novo pathway constructions enabled the synthesis of butanol. Manipulability of such synthetic biocatalyst can circumvent impediment of the biochemical synthesis of butanol from renewable feedstock.

Oral Presentation 2-06
Metabolic Engineering of Thermoanaerobacterium saccharolyticum for High Yield Ethanol Production
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Effective utilization of biomass as a sustainable source of biofuels and value-added biochemicals is severely hindered by the high processing costs and relative low sugar yields. Switchgrass has gained national attention as a potential indigenous biomass source well suited to many regions in the US. The success of industrial process depends on the trade-off art of compromising processing costs, capital investment, and product revenues. A new cellulose-solvent-based lignocellulose fractionation (CSLF) technology has been developed for separating lignocellulose components based on their different solubility in different solvents (Zhang et al. Biotechnol. Bioeng. 2007: 97:214). We further optimized pretreatment conditions for high sugar release at lower enzyme loading. The optimal pretreatment conditions for switchgrass were determined to be 84% H2PO4 at 50°C for 60 minutes. In order to reduce enzyme use, we further investigated to decrease the enzyme loading from 15 FPU of cellulase per gram of glucan to lower levels without significant reduction in sugar yields in presence of other potential hydrolysis additives such as surfactants. Scanning electron microscopy (SEM) images clearly show that CSLF can completely destruct fibril structure of lignocellulosic biomass.

Oral Presentation 3-01
Optimization of Switchgrass Treatment by Cellulose-Solvent-Based Lignocellulose Fractionation at Lower Cellulase Cellulose Hydrolysis
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Effective utilization of biomass as a sustainable source of biofuels and value-added biochemicals is severely hindered by the high processing costs and relative low sugar yields. Switchgrass has gained national attention as a potential indigenous biomass source well suited to many regions in the US. The success of industrial process depends on the trade-off art of compromising processing costs, capital investment, and product revenues. A new cellulose-solvent-based lignocellulose fractionation (CSLF) technology has been developed for separating lignocellulose components based on their different solubility in different solvents (Zhang et al. Biotechnol. Bioeng. 2007: 97:214). We further optimized pretreatment conditions for high sugar release at lower enzyme loading. The optimal pretreatment conditions for switchgrass were determined to be 84% H2PO4 at 50°C for 60 minutes. In order to reduce enzyme use, we further investigated to decrease the enzyme loading from 15 FPU of cellulase per gram of glucan to lower levels without significant reduction in sugar yields in presence of other potential hydrolysis additives such as surfactants. Scanning electron microscopy (SEM) images clearly show that CSLF can completely destruct fibril structure of lignocellulosic biomass.

Oral Presentation 3-02
Autoxidative delignification of woody biomass in ethanol and enzymatic saccharification of the resulting pulp
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Although the wet oxidation and the solvolysis are representative pretreatment and delignification methods for the production of ethanol from woody biomass, the attempt of combining these two processes has been scarcely made so far probably from the fear of explosion. In reality, ethanol is readily autoxidized under relatively mild conditions, and diethyl acetal, ethyl acetate, methane, carbon monoxide, hydrogen, carbon dioxide and so on are formed, while this oxidation is suppressed to some extent in the presence of antioxidants such as lignin, lignin model compounds, or lignin containing materials, and the antioxidants themselves are preferably oxidized to carbon dioxide. Based on this fact, the selective delignification of pulverized woody biomass is now achieved by its autoxidation in ethanol under pressure of oxygen or air at 130-160°C. When 1g of pulverized spruce (0.25-0.42mm) was treated with either oxygen or air at the initial pressure of 1 or 5MPa respectively in 10mL of ethanol at 150°C for 16h, 0.64-0.65g of pale yellow pulp containing 3.5% of lignin was obtained. Its similar oxidation but in water led to its carbonization, probably because of the much smaller solubility of oxygen in water than in ethanol or the lack of autoxidation itself. Enzymatic saccharification of the resulting pulp with 10FPU of acremonium cellulase at 4% loading for 120h gave 0.46g of monosaccharides: 56% yield of glucose and mannose based on the dry spruce.
Oral Presentation 3-03
The Influence of Solid/Liquid Separation Techniques on the Sugar Yield in Two-Step Dilute-Acid Pretreatment of Softwood for Bioethanol Production
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Bioethanol as one of the most promising alternative fuels contributes to the reduction of environmental impacts generated by fossil fuel consumption through no net-emission of carbon dioxide. Ethanol can be produced from renewable materials through enzymatic hydrolysis and fermentation. However, to reach high yields and high ethanol concentration, cellulosic biomass must be pretreated. Pretreatment is a crucial step for enzymatic digestibility of biomass and has a key role in the ethanol production cost. During pretreatment, lignocellulosic material is broken down to smaller sugar units and thus available for enzymatic attack. To date two-step, dilute-acid pretreatment of biomass has shown to result in the highest sugar and ethanol yields in lab-scale. The hemi-cel lulose is hydrolyzed in the first step then solid material and liquid fraction are separated. The solid material is fed to second reactor while the liquid solution is taken to the fermentation of hemicellulose sugars. In an industrial process filtration and washing of material between two steps are still difficult as it should be performed at high pressure to save energy. However, the applied pressure forms more compact solids which might affect the subsequent steps. Therefore, the right choice of separation-technique together with the number of separations has an important impact on structure of the pretreated material and thus final ethanol yield. In the current study, a number of methods for liquid/solid separation between two pretreatment steps and the influence on the digestibility of solids, as sugar yield in the second step or by enzymatic hydrolysis are under investigation.

Oral Presentation 3-04
Substrate pretreatment: The key to enzymatic hydrolysis by cellulases?
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Although the structure and function of cellulases continue to be the subject of intense research, it is widely acknowledged that the rate and extent of the cellulolytic hydrolysis of lignocellulosic substrates is influenced not only by the effectiveness of the enzymes but also by the chemical and physical characteristics of the heterogeneous lignocellulosic substrates. Although strategies such as site-directed mutagenesis or directed evolution have been successfully employed to improve cellulase properties such as binding affinity, catalytic activity and thermostability, complementary goals that we and other groups have studied have been the determination of which substrate characteristics are responsible for limiting hydrolysis and the development of pretreatment methods that maximize substrate accessibility to the cellulase complex. Over the last few years we have looked at the various lignocellulosic substrate characteristics at the fiber, fibril and microfibril level that have been modified during pretreatment and subsequent hydrolysis. The initial characteristics of the woody biomass and the effect of subsequent pretreatment play a significant role on the development of substrate properties, which in turn govern the efficacy of enzymatic hydrolysis. Focusing particularly on steam pretreatment, this review examines the influence that pretreatment conditions have on substrate characteristics such as lignin and hemicellulose content, crystallinity, degree of polymerization and specific surface, and the resulting implications for effective hydrolysis by cellulases.

Oral Presentation 3-05
Impact of Surfactants on Pretreatment of Corn Stover
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Lignin in pretreated cellulosic biomass can non-productively adsorb cellulase, resulting in a loss of a significant portion of this expensive protein. In addition, lignin interferes with the path for cellulase action, slowing down hydrolysis. Thus, the effectiveness of enzymatic hydrolysis of pretreated lignocellulosic biomass can be significantly enhanced if lignin is removed or modified before adding enzymes. In this project, the enzymatic digestibility of solids resulting from pretreatment of corn stover with various surfactants at 160 to 220°C was evaluated with and without addition of sulfuric acid. Tween-80 gave the best performance as measured by the increase in the hydrolysis yield and enhanced total sugar recovery. In addition, analytical techniques including DSC, XRD and IR were used to investigate the chemical and hydrogen bonding changes resulting from surfactant pretreatment. A mechanism will be presented to explain the observed impact of surfactants on performance.

Oral Presentation 3-06
Effects of Genetic Modified and Mutant Straws on Bio-ethanol Production
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Wheat and barley straws are two of the most abundant agricultural residues in the United States Northwest that are potential sources of feedstock for production of renewable bio-based energy. It has been estimated that a total of 2 million dry tons of straws are produced annually in the Washington State. Being similar to other lignocelluloses, the recalcitrant cell wall structure makes enzymatic hydrolysis of the cellulose and hemicelluloses in the straw a great challenge. It has been elucidated that the presence of lignin in the straw is one of the major barriers to limit enzyme and microbes access to the cellulose polysaccharides. Lignin reduction and fiber structure changes using various genetic and breeding methods could improve biological conversion efficiency of straws, making them attractive resources for energy and chemical production. In this paper, the compositions of several transgenic and mutant wheat and barley straws were analyzed. Statistic analysis indicated that there were no significant changes on lignin content among different treated straw samples. Enzymatic hydrolysis of straws was then conducted in order to explore the effects of transgenic and mutant treatments on hydrolysis performance. The results demonstrated that there were significant differences in terms of glucose conversion between mutant and wild types. For instance, with acid pretreatment, the difference of conversion rates was as high as 30%. Subsequent Simultaneous Saccharification and Fermentation (SSF) demonstrated the impact of treated samples on ethanol production as well.
Oral Presentation 4-01
Catalytic Conversion of Biomass to Hydrocarbons Utilizing Aqueous-Phase Reforming
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Virent Energy Systems, Inc.’s patented Aqueous-Phase Reforming (BioFormingTM) process enables the economical and energy efficient use of plant based sugars to generate the many hydrocarbon products that drive the world economy. Virent’s patented catalytic process provides an unconventional pathway to generate proven liquid fuels such as gasoline, jet fuel, and diesel from renewable biomass-derived feedstocks. This new category of biofuels can be used and distributed like conventional fuels and, based on present feedstock costs, will compete at current price levels. Utilizing a simple reactor system and operating at relatively low temperatures and pressures, the BioForming process reforms carbohydrates to hydrocarbons. By selecting different catalysts and processing conditions, various types of sugars can be reliably converted into hydrocarbon fuels or chemicals. The technology platform is simple and highly thermal efficient. Once it is operating, no additional energy inputs are needed. This thermal efficiency, combined with lower capital costs compared with multi-stage production processes, and its efficient use of plentiful, inexpensive cellulosic feedstocks, make the BioForming platform a truly cost-effective solution. Together, these unique characteristics position the BioForming platform as the key enabling technology for renewable hydrocarbon production.

Oral Presentation 4-02
A Comparison of Rigorous Process and Economic Data for Biochemical and thermochemical Ethanol Production Technologies
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By signing into law the Energy Independence and Security Act of 2007, a renewable fuels standard (RFS) was established for using 36 billion gallons of ethanol, of which over half will be derived from lignocellulosic feedstocks by 2022. Questions remain as to which technologies will enable this change. Multiple technologies exist for converting lignocellulosic biomass into a multitude of biofuels such as ethanol. Biochemical approaches include dilute acid, concentrated acid, and enzymatic hydrolysis. thermochemical approaches include a number of gasifier technologies with multiple fuel synthesis options as well as pyrolysis conversion options. Previous attempts to examine the pros and cons and comparative economics of biochemical and thermochemical biofuels production have been at high levels with inconsistent metrics of comparison. Hence the need for very rigorous and detailed comparisons of biochemical and thermochemical routes to truly ascertain the real advantages and disadvantages of each.

In this paper, the advantages and disadvantages of biochemical and thermochemical biofuels production will be explained. In addition, detailed capital and operating costs are compared for two rigorous designs: a thermochemical approach using gasification and mixed alcohols synthesis, and a biochemical approach using enzymes and mixed sugar fermentation. Comparative analysis of these two rigorous studies has shown that either present viable technological options for cellulosic ethanol production that have unique advantages depending on the locally available feedstock base and other localized factors. There is no clear winner in terms of yield, capital cost, operating costs, etc. Finally, synergies between the two technical routes will be examined.

Oral Presentation 4-03
Membrane contactor for efficient alcohol recovery
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Membrane contactor for efficient alcohol recovery

Biobased feedstocks are displacing oil and gas for fuels, chemicals, and fibers. This displacement requires energy-efficient conversion and recovery processes. As product titers decrease, the energy required for product recovery increases. Previously, we presented the Separative Bioreactor, a membrane technology to recover organic acids and other charged products from fermentation broths. Here we present a membrane-contactor-based technology to recover alcohols and other neutral species from fermentation broths. The membrane contactor uses functionalized membranes and ionic liquid extraction solvents to drive the separation. The process exhibits higher separation factors than observed for either the membrane or solvent separately. To date, technology has been applied to ethanol and butanol. If we are able to scale it up, the membrane contactor/ionic liquid technology will significantly decrease the energy required to recover butanol from fermentation broths. The technology could also improve the energy efficiency for cellulosic ethanol recovery at titers exhibited in syngas fermentation. The following file types may be uploaded:

Oral Presentation 4-04
Ethanol from diverse feedstocks - Coskata’s syngas biotechnology path
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Gasification of lignocellulosic biomass or other carbonaceous feedstocks enables all of the heterogeneous components to be converted to syngas with high efficiency. Coskata’s technology path utilizes its novel strains with novel and efficient bioreactor designs and membrane based separations. This integrated technology can enable a highly efficient and economical process. This paper will briefly discuss the key features of the technology.

Oral Presentation 4-05
Microbial factories for the production of fit-for-purpose hydrocarbon biofuels from sugar feedstocks
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While ethanol and FAME-based biodiesel represent significant steps towards decreasing petroleum dependence, these liquid fuels have been selected not because of their suitability as fuels, but rather because of their ease of production by naturally occurring organisms or processes. The advances in synthetic biology now allow us to design renewable biofuels that more closely resemble petroleum fuels. In this talk, we will explore the impact of novel liquid biofuels produced through the design of microbes that ferment sugar to liquid hydrocarbon fuels. Work at Amyris has centered on identifying the best molecules for use in today’s transportation fuel infrastructure (including diesel, gasoline, and jet fuels) and the subsequent engineering of microbial systems for their production. Because of the potential fungibility of these fuels within the current transportation fuel infrastructure, this technology has the potential to significantly increase biofuel demand, thus offsetting the need for new sources of liquid fuels while also significantly reducing the carbon footprint of those fuels.
Oral Presentations

Oral Presentation 4-06
Biobutanol – Next Generation Advanced Biofuel

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DuPont innovation in support of biofuels has three main components: (1) creation of improved seed and crop protection chemicals, (2) creation of next-generation advanced biofuel products and processes, and (3) creation of economical cellulosic conversion technologies supporting both ethanol and advanced biofuels.

In 2004, DuPont formed a unique partnership with BP to develop a new advanced process to make a next-generation advanced biofuel called biobutanol. At that time, few people had heard of biobutanol and few knew of its fuel properties that make it similar in characteristics to gasoline along with several advantages compared to existing biofuels, including (1) biobutanol can be used in existing pipelines, (2) biobutanol can be blended at a higher concentration into gasoline, (3) biobutanol can enhance ethanol in blends due to its intrinsic lower vapor pressure, and (4) biobutanol has a higher energy density that provides for better fuel economy.

Similar to our Bio-PDO™ efforts, the DuPont scientists on the biobutanol program are the first in the world to have created and successfully demonstrated the technology to enable biobutanol production in micro-organisms where no such ability existed before. This new technology provides a new paradigm for the cost-efficient production of biobutanol by being able to efficiently design the network of biological processes needed to selectively produce biobutanol.

This talk will focus on the success factors needed for development of world-class biofuel bioprocesses and bioproducts along with highlighting some of the technical achievements that DuPont has made with our biobutanol process development efforts.

Oral Presentation 5-01
Follow–Up to the Billion-Ton Resource Assessment Report: Status and Plans

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The Billion-Ton Resource Assessment report, published in 2005, estimated the current and potential availability of biomass feedstocks. The project was projected as approximately 1.3 billion tons and was what might be reasonably available around mid-century when large-scale biorefineries are likely to exist. The report emphasized primary sources of forest- and agriculture-derived biomass such as logging residues, fuel treatment thinnings, crop residues, and perennially grown grasses and woody crops. These primary sources have the greatest potential to supply large, sustainable quantities of biomass. Since publication of the Billion-Ton Resource Assessment, follow-up efforts have focused on updating the results, disaggregating the resource potential to counties and fine spatial scales, examining how the resource potential is affected by environmental sustainability, and answering questions involving what feedstocks will be used, when will they be used, what will be the costs, and what will be the economic impacts. Answers to these latter questions are focused on near-term time periods coincident with implementation of the Energy Independence and Security Act. This presentation reports on some of the preliminary results for primary cropland and forestland resources.

Oral Presentation 5-02
If switchgrass is the silver bullet, how do we get it into the gun?

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Several herbaceous species have been studied for their potential as herbaceous biomass feedstocks. These will be discussed, but the broader focus of this presentation will be on characteristics of an ideal, or ideotype, feedstock. Those desirable traits include: readily bioengineered, perennially productive, minimal anti-quality factors, not weedy/invasive, readily established (using no-till methods preferably), flexible harvests, no or few pests, drought and heat tolerant, low input requirements, et al.

Oral Presentation 5-03
The Case for Corn Stover

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In many ways corn stover is an excellent potential biomass feedstock for cellulosic ethanol. Currently grown on more than 93 million acres in the United States and annually yielding 300 million tons of biomass that could produce 25 billion gallons of cellulosic ethanol, its planting, cultivation and harvesting costs can be shared with a popular cash crop, corn grain. As a domesticated crop, it is easy to grow and accepted by agricultural producers. Numerous other benefits include well-understood capabilities for bioengineering and breeding and the lack of wild relatives with which it is likely to cross-breed. Surging demand for corn grain as an ethanol feedstock, however, has increased scrutiny of potential drawbacks and risks of using corn stover as a biomass feedstock. Removing too large a fraction of the stover from the field, for example, could reduce soil moisture or organic carbon below desired levels, decreasing soil fertility and crop yields over time. Higher water and fertilizer inputs required for increased biomass yields could add to environmental stresses. This presentation will summarize current research results, suggest areas where further research is needed, and recommend the conditions under which corn stover is likely to play a key role as a crop feedstock for cellulosic ethanol.

Oral Presentation 5-04
Biomass yield and quality of Pacific Northwest grown Switchgrass for Biofuel

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Various switchgrass (Panicum virgatum) cultivars have been grown under research conditions for biofuel in the Pacific Northwest (PNW) since 2002. We have successfully grown the full maturity range of switchgrass varieties, ranging from Dacotah to Alamo in the warm, irrigated region of the PNW. To date, only one planting of SWG has died from winterkill, this being seeded in early August and not advancing into dormancy in the fall that is typical with early June plantings. Additional research in the PNW is needed to identify other planting windows without stand loss due to winterkill. Dakotah and Nebraska 28, the earliest maturity cultivars easily produce 2 fully headed, nearly anthesis stage biomass crops per season. Intermediate varieties such as Trailblazer, Forestburg, Cave-in-Rock, Blackwell and Shawnee will produce 2 excellent biomass harvests with one taken near anthesis and the other harvest slightly less mature. The lowland varieties, Kanlow and Alamo respond very differently in our conditions. Kanlow will provide 2 excellent harvests per season but neither will reach full heading. Alamo often provides only a single good cutting per season and is the last variety to transition into fall dormancy in mid-October. Early maturing upland switchgrasses transitioned into fall dormancy in late September. Intermediate upland varieties transition about 2 weeks later than early varieties with the late, lowland varieties transitioning into fall dormancy last. Biomass yields and quality will be compared among a full range of switchgrass variety maturities grown in the PNW.

Oral Presentation 5-05
Characteristics of an Ideal, or Ideotype, Feedstock

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Various switchgrass (Panicum virgatum) cultivars have been grown under research conditions for biofuel in the Pacific Northwest (PNW) since 2002. We have successfully grown the full maturity range of switchgrass varieties, ranging from Dacotah to Alamo in the warm, irrigated region of the PNW. To date, only one planting of SWG has died from winterkill, this being seeded in early August and not advancing into dormancy in the fall that is typical with early June plantings. Additional research in the PNW is needed to identify other planting windows without stand loss due to winterkill. Dakotah and Nebraska 28, the earliest maturity cultivars easily produce 2 fully headed, nearly anthesis stage biomass crops per season. Intermediate varieties such as Trailblazer, Forestburg, Cave-in-Rock, Blackwell and Shawnee will produce 2 excellent biomass harvests with one taken near anthesis and the other harvest slightly less mature. The lowland varieties, Kanlow and Alamo respond very differently in our conditions. Kanlow will provide 2 excellent harvests per season but neither will reach full heading. Alamo often provides only a single good cutting per season and is the last variety to transition into fall dormancy in mid-October. Early maturing upland switchgrasses transitioned into fall dormancy in late September. Intermediate upland varieties transition about 2 weeks later than early varieties with the late, lowland varieties transitioning into fall dormancy last. Biomass yields and quality will be compared among a full range of switchgrass variety maturities grown in the PNW.
Forest residues including logging residues and understory biomass are a potential source of fuel for biofuels production, if they can be economically harvested and transported to the end user. Most forest residues are currently burned or left in the field after timber harvesting. The southeastern United States is rich in forest resources and almost all of the chips and milling residues produced are currently utilized for either energy production or furnish in the pulp and paper industries. At present, forest residues are the only untapped sources of biomass from forestlands and the economics of harvesting and transporting these residues are very important. Several studies have been conducted to assess the sources of residues available from forestlands. However, there is little information available on the actual cost of harvesting and transport of forest residues for bioenergy production. This paper presents a real-time simulation and modeling of forest residue harvesting, collection and transport operations. The model is an extension of the current DOE/ORNL IBSAL model which has successfully been modeled the supply logistics of crop residue. The model uses the latest version of "EXTENDSIM" discrete-event simulation software platform to estimate the cost, energy input and greenhouse gas emissions for the entire operations of forest residue supply system. The developed simulation model can be used as a decision support tool to design a forest-residue based supply logistic system.

**Oral Presentation 5-06**

The impact of biomass availability and processing cost on optimum size for biomass processing

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Biomass such as agricultural or woody residues can be processed to a variety of useful energy forms. For example, straw and corn stover can be used to produce electricity via direct combustion or gasification, and transportation fuel via lignocellulosic ethanol fermentation or Fischer Tropsch synthesis of diesel from biomass derived syngas. As the scale of processing increases, the delivered cost of field sourced biomass increases due to increased transportation distances, but the unit cost of processing the biomass decreases due to economies of scale in both capital and operating cost. Because of the competition between these cost factors, biomass processing plants have an optimum size at which output cost is minimized. In this work we consolidate available data on the above four processing alternatives, adjusting for currency and inflation. We then model biomass processing for a range of biomass availability (tonnes or GJ per gross hectare, where gross hectares includes the entire geographical area in which the biomass plant is situated) to explore optimum size as a function of delivered cost of feedstock as well as processing cost. The sensitivity of optimum plant size to a relaxation in the constraint of minimum cost is also explored as a function of these factors. Both optimum plant size and the sensitivity to a relaxation in minimum cost vary widely over the range of processing cost and feedstock availability of currently proposed biomass projects.

**Oral Presentation 6-01**

How Is Accessibility of Cellulose to Cellulase Affected by Choice of Pretreatment and Substrate?

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The exposure of reactive sites of cellulose to cellulase is considered one of the most important factors for effective saccharification of cellulose. Previously, Yang and Wyman showed that the rate of hydrolysis does not change with conversion for Avicel cellulose. Contrary to this, Hong et al. using a different method claim that accessibility does change with hydrolysis. Thus, it is unclear whether the substrate or the enzyme or both affect hydrolysis and how pretreatment affects their interaction. In this study, the change in accessibility of cellulose over hydrolysis was evaluated for cellulose in poplar solids prepared by leading pretreatments including ammonia fiber expansion, ammonia recycle percolation, controlled pH, dilute acid, lime, and sulfur dioxide. It was observed that accessibility and its change may be influenced by the type of pretreatment and that substrate and its composition may also play a vital role.

**Oral Presentation 6-02**

Effect of Surfactants on Separate Hydrolysis Fermentation and Simultaneous Saccharification Fermentation of Steam Exploded Lodgepole Pine with Inhibitors

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The addition of surfactants has been shown to improve the enzymatic hydrolysis of lignocellulosics. The positive effects can be mainly attributed to the interaction of the surfactants with lignin at the substrate surface, which minimizes non-productive binding of cellulosates to lignin, and enhances the accessibility of cellulose to cellulas. This study investigated the effects of surfactants on separate hydrolysis and fermentation (SHF) of steam exploded lodgepole pine (SELP) and ethanol pretreated lodgepole pine (EPLP). The addition of surfactant, Tween 80, during cellulose hydrolysis of SELP resulted in a significant increase in the cellulose-to-glucose yield. However, Tween 80 had little effect on improving the hydrolysis of EPLP. The addition of surfactant led to a substantial increase in the amount of free enzymes in the 48-hour hydrolysates of both SELP and EPLP. The effect of surfactant addition on final ethanol yield of simultaneous saccharification and fermentation (SSF) was also investigated by using SELP with added furfural and hydroxymethyl furfural (HMF). The results showed that the surfactants did not have any effect on the consumption rates of furfural and HMF during SSF process by Saccharomyces cerevisiae. The presence of furfural and HMF at the experimental concentrations did not affect the final ethanol concentration either. The strategy of applying surfactants in cellulose recycling to reduce enzyme cost is presented.
**Oral Presentation 6-03**
Detrimental Effect of Cellulose Oxidation on Cellulase-catalyzed Hydrolysis

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Production of fuel ethanol and other chemicals from renewable biomass is of strategic importance. To effectively convert the carbohydrates in complex and recalcitrant biomass, mechanical or chemical pretreatments are often required proceeding enzymatic hydrolysis and microbial fermentation. However, pretreatments may negatively affect the enzymatic hydrolysis by releasing by-products inhibitory to cellulases, or modifying cellulose by oxidation or other processes. To study how oxidative modification may impact cellulose’s reactivity toward the hydrolysis by cellulases, we prepared three cellulose substrates by cupric ion and hypochlorite oxidations, and subjected the derived celluloses to the hydrolysis of several Cel7 and Cel6 cellobiohydrolase homologues, two GH61 cellulase enhancing proteins, and one cellulolytic *Trichoderma reesei* extracellular enzyme mixture. We observed a profound decrease of enzymatic hydrolysis on the oxidized celluloses. The effect was attributed to the interference, from oxidized functional groups in cellulose, on its binding/activation in the active pocket/tunnel of cellobiohydrolases. Potential implication of the observed effect from cellulose oxidation on pretreatment optimization was discussed.

**Oral Presentation 6-04**
Developing high performance enzymes for biomass saccharification by de novo cocktail design and DirectEvolution™ enzyme optimization

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Effective enzymatic saccharification of pretreated biomass requires simultaneous synergistic action of multiple exo- and endo-acting cellulases and, in some cases, hemicellulase enzymes. Significant improvement in enzyme performance is needed in order to survive harsh process conditions and lower the cost contribution of enzymes to biomass saccharification applications. Verenium has leveraged its large collection of cellulase and hemicellulase enzymes to develop custom-made de novo cocktails optimized for specific biomass feedstocks, pre-treatment, and other process conditions. Verenium’s DirectEvolution™ technology is uniquely suited to further improve cellulytic performance and process compatibility of individual enzymes. Reduction of enzyme dose while maintaining the efficiency and extent of biomass conversion is a key property that can be improved by DirectEvolution™. In addition, improvements of specific enzyme properties can make enzymes compatible with harsh process conditions, such as high temperature, extreme pH, or high product concentrations. Novel assays have been developed that permit screening of cellulases in the presence of accessory enzymes on desized and pretreated lignocellulosic and cellulosic substrates under relevant process conditions in a high-throughput manner. Using application-derived biomass as substrate for evolution screening allows rapid improvement of the most commercially relevant properties. In this manner, we have successfully evolved a GH family 11 xylanase for superior thermostotolerance by applying GSSM™ technology to identify best individual thermostabilizing mutations, followed by combinatorial blending of single mutants to produce an improved variant with Tm 25 °C higher than the parent enzyme.

**Oral Presentation 6-05**
Thermophilic fungal CBH enzymes for hydrolysis of lignocellulosic materials

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Enzymatic hydrolysis is currently considered as the primary option to produce sugars from biomass for microbial fermentation to various chemicals, including ethanol. Use of thermostable cellulases could improve the overall efficiency of enzymatic hydrolysis of lignocellulosic materials, due to potentially higher specific activities and increased hydrolysis rates. Higher thermal activity can also provide flexibility in selection of process options. Even though a consortium of synergistic enzymes is required for efficient total hydrolysis, the key cellulases required are cellobiohydrolases. In present commercial cellulases, these comprise mainly of fungal family 7 enzymes. In order to find new improved cellulases and optimal enzyme mixtures, comparison of the key components is required, followed by evaluation of these enzymes in hydrolysis experiments using pre-treated substrates. In this work, thermostable Cel7A enzymes were studied in order to develop new superior enzyme products for lignocellulose hydrolysis.

The kinetic data of thermostable fungal Cel7A enzymes were compared and their performance in hydrolysis of pre-treated lignocellulosic raw materials was analysed. The cellobiohydrolases were compared to CBH1 (Cel7A) of *T. reesei*, which is one of the most thoroughly studied fungal cellobiohydrolases. Several thermostable CBH’s were purified, cloned and their enzymatic properties were characterized. Enzyme constructs containing only the catalytic core modules or the entire two-module proteins (composed of the catalytic and the cellulose-binding modules) were also studied. Interesting substrate-specific differences in the hydrolysis performance were detected. Pre-treated lignocellulosic raw materials were efficiently hydrolysed at elevated temperatures by these enzymes, when used in mixtures with other thermostable enzymes.
Expression of Ethanol and Hydrogen Synthesis Pathway Genes during growth on cellulose in *Clostridium thermocellum* ATCC 27405

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The objective of this study was to identify the catabolic pathways and genes utilized by *Clostridium thermocellum* ATCC 27405 during both hydrogen (H2) and ethanol synthesis. Bioinformatic analyses of the *C. thermocellum* genome identified genes encoding key enzymes in pyruvate catabolism pathways, including two putative lactate dehydrogenases, one pyruvate:formate lyase, four pyruvate:formate lyase activating enzymes, and at least three pyruvate:ferredoxin oxidoreductase (POR) or POR-like enzymes. Quantitative polymerase chain reaction was used to confirm the expression of the identified genes throughout growth on α-cellulose. Our data suggests H2 may be generated through the action of either a ferredoxin (Fd)-dependent, membrane-bound NiFe hydrogenase, often referred to as “Energy-converting Hydrogenases”, or via NAD(P)H-dependent Fe-only hydrogenases. Furthermore, our findings show the presence of a gene cluster putatively encoding NADH:Fd oxidoreductase; suggesting a mechanism in which electrons may be transferred between NADH and ferredoxin. Ethanol production appears in part to be catalyzed by an ADH-E enzyme possessing both acetaldehyde and alcohol dehydrogenase activities. The results presented here provide insight into the metabolic pathways utilized in biofuels production. This information is critical to developing strategies for metabolic engineering and optimizing bioprocesses promoting enhanced and H2 gas or ethanol production.

**Oral Presentations**

**Oral Presentation 6-06**

Exploration of *Trichoderma reesei* enzymatic pools and their hydrolysis potential using proteomic and liquid chromatography tools

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*Trichoderma reesei* is a well-known producer of cellulolytic enzymes, both for fundamental research and industrial production. Sequencing of its genome has revealed an unexpectedprofusion of different cellulolytic enzymes. Both regulation and role of these enzymes are not well characterized. Using 2D-electrophoresis coupled with mass spectrometry, we systematically identified major spots (more than 84% and 95% spot volume). Using this map, we determined the composition of the secretomes of two *T. reesei* strains, RutC30 and CL847 (an industrial strain), produced on different carbon sources and inducers. Some of these enzymes were tested for hydrolysis of steam-exploded wheat straw. This strategy revealed information on regulation of cellulases and hemicellulases produced in *T. reesei*, such as variation in inducibility of xylanases XYN1 and XYN4, of so-called minor endoglucanases EG3, EG4 and EG6 and arabinofuranosidasases ABF1, ABF2 and ABF3 or on the role of the carbon source used for *T. reesei* cell growth.

In another approach, we purified the major *T. reesei* enzymes CBH1, CBH2, EG1, EG2 by MPLC and used them for reconstitution of enzymatic pools in miniaturized hydrolysis assays. This convenient tool contributes to clarify the role of each enzyme in substrate degradation. In addition, testing a more extended range of enzymes obtained after fractionation of secretomes will allow determination of the factors lacking for an efficient hydrolysis of biomass. Comparison with secretomes characterized using 2D-electrophoresis will help fine-tuning the best fermentation conditions for production of this “ideal” enzymatic pool.

**Oral Presentation 7-01**

Substrate Selective Uptake to Remove Acetate and Convert Sugar Mixtures

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We report a new approach for selectively removing acetate from sugar mixtures, and then in a subsequent process step, for converting xylose and glucose simultaneously into products by fermentation. The process for converting sugar mixtures uses two “substrate-selective” strains, one unable to consume glucose and one unable to consume xylose. Using *Escherichia coli* as the model organism, the xylose-selective (glucose deficient) strain has mutations in the *glk, ptsG* and *manZ* genes while the glucose-selective (xylose deficient) strain has a mutation in the *xylA* gene. By combining these two strains in a single process, xylose and glucose are consumed more quickly than by a single-organism approach. The process for removing acetate selectively from acetate/xylose/glucose uses a single strain with all four mutations, thereby rendering this third strain unstable to consume xylose or glucose. The ultimate two-step semi-continuous process—first acetate removal and then xylose/glucose conversion—has several advantages over other approaches. In particular, the process adapts to changing concentrations of any of these components, and the strategy results in the transformation of acetate into nutrients available to microbes in the second step. These results are discussed in the context of ethanol production, and the conversion of variable sugar feed streams.

**Oral Presentation 7-02**

Metabolic Engineering of *Saccharomyces cerevisiae* for Succinic Acid Production

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A class of high added-value chemicals being targeted for biotechnology production is C4 organic acids, encompassing fumaric, malic, and succinic acid. Succinic acid is a key building block molecule for further conversion to precursor molecules such as tetrahydrofuran, 1,4-butanediol, and butyrolactone. Succinic acid has the potential to become a commodity chemical, with world-wide annual demand exceeding $2 billion USD and over 160 million kilograms currently produced from petrochemical conversion of maleic anhydride.

There are several biomass platforms, all prokaryotic, for succinic acid production; however, overproduction of succinic acid in *S. cerevisiae* offers distinct process advantages. For example, *S. cerevisiae* has been awarded GRAS status, grows well at low pH significantly minimizing purification costs, and can utilize diverse carbon substrates in chemically defined medium. Furthermore, *Saccharomyces cerevisiae* is a proven, robust, industrial production platform.

*S. cerevisiae* offers the unique advantage of being the most well characterized eukaryotic expression system. Here we describe the use of systems biology tools to drive C4 carbon flux to succinic acid by enhancement of the two native pathways for succinic acid production: the TCA and glyoxylate cycles. *S. cerevisiae* do naturally only accumulate succinic acid in minor amounts; however, through the use of *in silico* metabolic predictions guiding targeted gene deletions and over-expression, mutants that overproduce succinic acid have been engineered and thoroughly characterised and we can show a 70-fold overproduction compared to the natural level of succinic acid produced. Metabolic engineering approaches developed promise to have broad applicability to industrial biotechnology platforms.
Oral Presentation 7-04
Production of substituted aromatics from biomass-derived feedstock by engineered solvent-tolerant Pseudomonas putida S12

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Pseudomonas putida S12 thrives in minimal medium with a second phase of toluene or 1-octanol. This exceptional solvent tolerance makes strain S12 pre-eminently suited for the production of industrially relevant, but toxic chemicals such as substituted aromatics. We have modified P. putida S12 into a versatile whole-cell biocatalyst with regard to substrate utilization and product formation by different engineering approaches, while employing a systems approach for strain optimization.

In demonstrator cases, tyrosine phenol lyase and phenylalanine ammonia lyase were expressed in P. putida S12 for the production of phenol or t-cinnamate, via L-tyr or L-phe, from glucose and glycerol. The initially modest production characteristics were improved by orders of magnitude by random mutagenesis and high-throughput screening. Transcriptomics analysis of the improved mutants provided useful leads for further targeted optimization and indicated that metabolic fluxes to the aromatic amino acids were significantly increased.

In addition to expanding the range of products and optimizing productivity, we are investigating the efficient utilization of biomass-derived feedstock. Wild-type P. putida S12 readily metabolizes the model renewables glucose and glycerol. However, real-life substrates such as lignocellulose hydrolysates also contain considerable amounts of pentoses that P. putida S12 cannot utilize. By introducing xylose utilization genes from Escherichia coli and subsequent laboratory evolution, a P. putida S12 was obtained that efficiently utilizes xylose as the sole C-source. Utilization of arabinose as well as toxic compounds from lignocellulose hydrolysates is currently investigated for improved efficient production of industrially relevant compounds from cheap biomass-derived feedstock.

Oral Presentation 7-05
ZWF1 overexpression in Saccharomyces cerevisiae protects cells from furfural-induced damage to cellular membranes, chromatin, and the actin cytoskeleton

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Bio-ethanol is a leading alternative to fossil fuels due to environmental and economical reasons. To reach bio-ethanol goals, it is essential to develop efficient strategies to use various lignocellulosic substrates for ethanol production (e.g. agricultural and industrial waste products). The pretreatment process used to generate fermentable sugars from lignocellulosic biomass also generates growth inhibitors, such as furfural and 5-hydroxymethylfurfural. Thus a robust strain tolerant to these inhibitors is needed. Previously, the stress protective gene, ZWF1, has been shown to improve tolerance to furfural when overexpressed. Moreover, furfural treatment induces the accumulation of reactive oxygen species (ROS) that cause cellular damage to membrane structures, chromatin, and the actin cytoskeleton. To test whether ZWF1 is linked to this cellular damage, we examined furfural-induced cellular damage using either cells lacking ZWF1 or cells overexpressing ZWF1. In the present study we demonstrate that ZWF1 overexpression in yeast prevents the accumulation of ROS and the damage to membrane structures, chromatin, and the actin cytoskeleton.

Oral Presentation 7-06
Engineering Escherichia coli for the efficient conversion of glycerol to ethanol and co-products

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Glycerol is generated in large quantity as an inevitable byproduct of the biofuels industry. Availability, low prices, and high degree of reduction make this compound an ideal feedstock to produce reduced compounds via anaerobic fermentation. Though glycerol has been shown to be utilized by many enteric bacteria under fermentative condition, it has very recently been shown in our lab that the E. coli can also utilize glycerol by fermentative metabolism. In this work we make use of the knowledge base created by our previous studies to engineer E. coli for the efficient conversion of crude glycerol into ethanol along with the co-products H2 and formate. To improve the rate of glycerol utilization, we overexpressed the enzymes glycerol dehydrogenase (gldA) and dihydroxyacetone kinase (dhaKLM), which are responsible for the conversion of glycerol to glycolytic intermediate dihydroxyacetone phosphate. We further created two independent strains for the co-production of ethanol and formate and ethanol and hydrogen. Strain SY04 (pZSKL MgldA), containing mutations that minimize the synthesis of succinate and acetate and the consumption of formate, produce ethanol and formate at yields exceeding 90% of the theoretical maximum and specific rates higher than 15 mmole/gcell/h. Strain SY03 (pZSKL MgldA), containing mutations that minimize the synthesis of succinate and acetate and cultivated under acidic and microaerobic conditions, produced ethanol and hydrogen at yields also surpassing 90% of the theoretical maximum and specific rates of about 10 mmole/gcell/h. The engineered strains efficiently converted unrefined glycerol, a byproduct of biodiesel production, to ethanol and coproducts hydrogen and formate.
**Oral Presentation 8-01**

**Application of leading pretreatment technologies to poplar wood: Sugar recovery, fermentation performance, and cost estimates**

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Pretreatment is expensive and strongly affects the cost and performance of most of the other operations. To support selection of suitable technologies by industry, the Biomass Refining Consortium for Applied Fundamentals and Innovation (CAFI) developed first-of-a-kind comparative data for application of the promising pretreatment options of ammonia expansion, aqueous ammonia recycle, controlled pH, dilute acid, flowthrough, lime, and sulfur dioxide steam explosion to poplar wood. Common sources of biomass and enzymes were employed, and identical approaches were used to measure compositions and close material balances. Although prior research showed more uniform performance with corn stover, important differences were observed in sugar yields from poplar using just cellulase. Application of more severe conditions generally improved the results for all of the pretreatments, and supplementation with xylanases was particularly effective in improving sugar release from the solids produced by many of them. The solids were characterized to identify fundamental features that could possibly influence performance. Differences were observed in the results when most of these pretreatments were applied to two different sources of poplar wood that had substantial differences in lignin content. The fermentability of the pretreated hydrolyzates and solids with a recombinant yeast strain was also assessed, with some requiring more conditioning than others to realize good ethanol yields. Finally, a minimum ethanol selling price was estimated for each pretreatment based on the performance data developed and was heavily influenced by yield. Overall, these results demonstrate the importance of evaluating pretreatment when integrated into the entire process.

**Oral Presentation 8-02**

**Profiling of cell wall polymers from wheat straw during pretreatment and enzymatic hydrolysis using novel microarrays**

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The efficient release of fermentable sugars from lignocellulosic material for use in biofuel production depends on the combined effects of pretreatment and enzymatic hydrolysis. In both process steps, various carbohydrate monomers and polymers are extracted from cell walls. This degradation and modification of polymers is conventionally monitored by HPLC analysis of carbohydrates in the liquid fraction. However, many of the released carbohydrates are oligosaccharides, particularly during pretreatment, and cannot be directly quantified by HPLC. In addition, it is also valuable to be able to analyse the recalcitrant degradation residue that is left after pretreatment and enzymatic hydrolysis. We have used a recently developed technique, Comprehensive Microarray Polymer Profiling (CoMPP, Moller et al., 2007) to track changes in specific glycans during lignocellulosic biomass degradation. CoMPP is a technique for cell wall analysis that combines the specificity of monoclonal antibodies with the high-throughput capacity of microarrays. CoMPP analysis was performed on wheat straw material before and after hydrothermal pretreatment at four different temperatures (160, 175, 185 and 195°C) at the DONG Energy IBUS pilot plant. In addition, we used CoMPP in combination with HPLC monosaccharide analysis to assess the effects of enzymatic hydrolysis of the pretreated materials using various commercial cellulases and hemicellulases.


**Oral Presentation 8-03**

**Decreasing of recalcitrance of sugarcane bagasse cellulose by steam pretreatment**

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Several technologies to convert biomass into ethanol have been developed in the last years due to recent alert on fossil fuel’s finitude and environmental damages. To obtain ethanol from biomass usually three steps are needed: (i) pretreatment, (ii) hydrolysis of cellulose and (iii) fermentation of sugars. In this work a new technology of pretreatment to sugarcane bagasse was evaluated: a type of steam explosion with slow decompression. In Brazil, this type of pretreatment is used to produce animal feed. The trials were carried out in a 5m³ reactor, kindly furnished by Usina Vale do Rosário (Sugar and Alcohol Company). After pretreatment, the bagasse was alkaline-extracted using 1% (w/v) NaOH for 1h. Enzymatic hydrolysis was took place using two commercial enzymes (Celluclast 1.5 L e Novozym 1888, both from Novozymes Company). Conversion yield of pretreated followed by alkaline extracted bagasse shows that the two previous steps were important for the enzymatic hydrolysis of cellulose. The hydrolyzate has 15 g.L−1 glucose concentration, corresponding to 85% of conversion yield. [Acknowledgments due to FAPESP and CNPq – Brazilian agencies]

**Oral Presentation 8-04**

**Fundamentals on Size Reduction for Biomass Conversion**

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Physical size reduction of biomass through mechanical means is a necessary step in bioconversion of lignocellulosic materials to increase the substrate surface accessible to enzymes to achieve a satisfactory cellulose conversion efficiency. Unfortunately, reducing biomass size to the scale of sub to millimeter requires a significant amount of electric-mechanical energy, especially for woody biomass. It is estimated that the energy consumed in size reduction can be 30-65% of the total energy in the cellulose ethanol produced using current technology (assume 35% conversion efficiency of thermal to electrical energy). Most literature studies reported cellulose conversion efficiency without providing the substrate size and size reduction energy consumption, while other studies provided the substrate size and size reduction efficiency but without the cellulose conversion efficiency. In this study we took an integrated approach to relate size reduction process to cellulose conversion efficiency and size reduction energy consumption. This presentation demonstrates a methodology for correct shape and size characterization of substrate so that a valid comparison of the effectiveness of size reduction, chemical pretreatment, and enzymatic hydrolysis process can be made. Since lignocellulosic substrates have a very large aspect ratio, measurements of geometric length using traditional sieving or screening methods reported in the literature were unable to provide correct information about enzyme accessibility. The correct definition of substrate size is critical to identify the most efficient size reduction, chemical pretreatment and enzyme process for biomass bioconversion. Our results demonstrated that the developed methodology is effective to differentiate the efficiencies of different size reduction processes.
Incorporating ester interunit linkages into plant lignins could enhance the delignification and saccharification of fiber for fermentation into ethanol or other industrial products. In this study, we examined how substitution of coniferyl alcohol (a normal monolignol) with 0 to 60% coniferyl ferulate (an ester conjugate from secondary metabolism) influenced the formation and alkaline solubility of lignin and the enzymatic hydrolysis of artificially lignified maize cell walls. Although extensively copolymerized into lignin, coniferyl ferulate unexpectedly accelerated peroxidase inactivation, interfered with cell-wall ferulate copolymerization into lignin, and reduced lignin concentrations in cell walls from about 200 to 160 mg/g. In addition to lowering lignin content, coniferyl ferulate increased the extractability of cell wall lignin by up to two-fold in aqueous 0.5 M NaOH at 30 or 100 ºC. Thus, the conjugate provides a means for delignifying cell walls under milder conditions at lower cost. Coniferyl ferulate incorporation also increased sugar yields at the onset of enzymatic hydrolysis by up to 45% and the final yield of sugars by up to 15% for lignified cell walls, both before and after a 30 ºC pretreatment with 0.5 M NaOH. Based on our results, bioengineering of plants to incorporate coniferyl ferulate into lignin should substantially reduce the cost for saccharifying fiber for fermentation into fuels or other industrial products.

**Oral Presentation 8-06**

**Thermodynamics and Kinetics of Competing Reactions of Xylose and Xylooligomers during Dilute Acid Pretreatment from Ab initio Calculations**

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Knowledge of the thermodynamic equilibrium and kinetic rate constants of competing reactions of xylooligomers during dilute acid pretreatment are extremely useful for optimizing the biomass conversion process. *Ab initio* molecular dynamics and metadynamics simulations were used to investigate the energetics of several competing pathways encountered during dilute acid pretreatment. The processes studied include xylooligomer hydrolysis, the condensation reactions of xylose monosaccharide to disaccharides, and xylose degradation reactions. The multi-dimensional free energy surfaces (FES) obtained allows accurate determination of both the reaction free energies and barriers of these reaction processes. The thermodynamic equilibrium and the kinetic reaction rate constants were subsequently determined and compared with available experimental data. The effects of solvent water on the free energies and barriers of the hydrolysis, degradation and reversion reactions were investigated. The stabilities of disaccharides were also examined with consideration for their hydrogen bonding structures.

**Oral Presentation ST-01**

**The Energy Biosciences Institute**

C. Sommerville, Stanford University, Stanford, CA

The Energy Biosciences Institute (EBI) is a new research institution—initiated in 2007 as a collaboration among U.C. Berkeley, the Lawrence Berkeley National Laboratory, and the University of Illinois at Urbana-Champaign—whose primary objective is to understand the scientific, technical, and societal challenges associated with large-scale production of biofuels, and with biological aspects of fossil fuel utilization and sequestration. The energy company BP has committed to provide up to $500 M to support the EBI during the next ten years. The EBI research program is largely focused on five research areas: (1) feedstock development; (2) biomass depolymerization; (3) biofuels production; (4) fossil fuel bioprocessing and carbon sequestration; and (5) environmental, social, and economic dimensions of cellulosic biofuels. A summary of each of the 50 research projects and programs supported by the EBI and the names of the investigators involved is posted on the EBI website at www.energybiosciencesinstitute.org.

**Oral Presentation ST-02**

**BioEnergy Science Center - an overview**

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The challenge of converting cellulosic biomass to sugars is the dominant obstacle to cost-effective production of biofuels in sustained quantities capable of impacting U.S. consumption of fossil transportation fuels. The BioEnergy Science Center (BESC), research program will address this challenge with an unprecedented interdisciplinary effort focused on overcoming the recalcitrance of biomass. The BESC team combines national lab, university and industrial researchers and is described at www.bioenergycenter.org.

By combining engineered plant cell walls to reduce recalcitrance with new biocatalysts to improve deconstruction, BESC plans to revolutionize the processing of biomass. These breakthroughs will be achieved with a systems biology approach and new high-throughput analytical and computational technologies to achieve (1) targeted modification of plant cell walls to reduce their recalcitrance (using Populus and switchgrass as high-impact bioenergy feedstocks), thereby decreasing or eliminating the need for costly chemical pretreatment; and (2) consolidated bioprocessing, which involves the use of a single microorganism or microbial consortium to overcome biomass recalcitrance through single-step conversion of biomass to biofuels.

Within five years the Center will remove biomass recalcitrance as a barrier to cost-effective biofuels production by achieving a minimum two-fold reduction in the projected cost of processing for conversion of biomass to ethanol. We will greatly enhance our understanding of cell wall structure during synthesis and conversion. The data generated will be made available through a Web portal to the bioenergy research community. This talk will provide an overview of the BESC start-up activities and some initial results.
Oral Presentation ST-03
The Great Lakes Bioenergy Research Center: Transformational Science and Sustainability

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The Great Lakes Bioenergy Research Center (GLBRC) was established by the U. S. Department of Energy in 2007. The GLBRC is a partnership between several universities and federal laboratories, with the University of Wisconsin as the lead institution and Michigan State University as the other principal academic institution. The GLBRC’s research program is directed toward transformational discoveries in basic plant science, biomass processing, biofuel production and sustainability research. This presentation will outline the research program of the GLBRC, identify key contacts for each of the major research components and describe how collaborations with the Center might be established by interested parties.

Oral Presentation ST-04
The Joint BioEnergy Institute (JBEI): Biomass Conversion to Alternative Transportation Fuels

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The development of cost-effective and energy-efficient processes to transform the cellulosic content of biomass into fuels is hampered by significant roadblocks, including the lack of specifically developed energy crops, the difficulty in separating biomass components, the high costs of enzymatic deconstruction of biomass, and the inhibitory effect of fuels and processing byproducts on organisms producing fuels.

The Joint BioEnergy Institute (JBEI) draws on the expertise and capabilities of three national laboratories [Lawrence Berkeley National Laboratory (LBNL), Sandia National Laboratories (SNL), and Lawrence Livermore National Laboratory (LLNL)], the University of California campuses at Berkeley (UCB) and Davis (UCD), and the Carnegie Institution for Science to provide the scientific and technology underpinnings needed to convert cellulose into transportation fuels. JBEI’s approach is based in three interrelated scientific divisions and a technologies division. The Feedstocks Division will create the knowledge required to develop improved plant energy crops to serve as the raw materials for biofuels. The Deconstruction Division will investigate the conversion of this lignocellulosic plant material to usable forms of sugars and aromatics. The Fuels Synthesis Division will create microbes that can efficiently convert sugar and aromatics into ethanol, butanol and advanced biofuels. JBEI’s cross-cutting Technologies Division will develop and optimize a set of enabling technologies—including high-throughput, chip-based and ‘omics platforms, tools for synthetic biology, multi-scale imaging facilities, and integrated data analysis. (www.jbei.org)

Oral Presentation ST-05
Department of Energy Deployment of Biofuels Technologies

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DOE’s Office of Biomass Program has announced over $650 million dollars in investments since 2006 for research, development, demonstration and deployment to accelerate biofuels production and development of supporting infrastructure. Validation of cost-competitive cellulosic ethanol by 2012 and meeting the ambitious goals of the recent Renewable Fuels Standard are driving forces in the direction of the program. This paper will describe current status of programmatic activities toward meeting these goals.

Oral Presentation 9-01
Production of Ethanol from Lignocellulosic Biomass

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In the U.S., about 95% of the fuel ethanol is made from corn as the primary feedstock. Upon completion of new construction and expansion, total capacity will be 14.5 billions gallons of ethanol (2007 Data). It is estimated that there is an upper limit between 12-15 billion gallons a year using starch-based feedstock in the US. If the goal of displacing 30 percent of transportation fuel with renewable fuels is to be reached by 2030, lignocellulosic biomass has great potential to become a major alternative source of the fermentable sugars.

Utilization of lignocellulosic materials as a renewable carbon source however depends on the development of economically feasible methods for both hydrolyzing cellulose to sugar, as well as converting those sugars to usable fuels.

In this presentation, conversion of lignocellulosic biomass such as pretreated corn stover and sugar cane bagasse to ethanol will be described. The performance of Genencor’s new “whole cellulase” commercial product for biomass conversion, Accellerase™ 1000, will be shown. Different process configurations such as separate saccharification and fermentation, simultaneous saccharification and fermentation (SSF), and hybrid hydrolysis and fermentation (HHF) will be compared. The synergistic effect of enzyme systems on the fermentation process and ethanol yield will be discussed.

Oral Presentation 9-02
Membrane extraction for acetic acid and lignin removal from biomass hydrolysates

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A major obstacle to the large scale industrial use of biobased products and biofuels is the lack of efficient, cost-effective separation methods. Separations operations currently account for 60-80% of the processing costs of most mature chemical processes. Here we focus on the development of membrane extraction as a low cost, robust separation process in future biorefineries. As membrane extraction is non-dispersive it overcomes all of the disadvantages of conventional extraction.

Acetic acid is produced during thermochemical pretreatment of lignocellulosic biomass. It is a weak acid that is strongly inhibitory to microorganisms used for bioconversion of sugars. Removal of acetic acid could be essential for increasing ethanol yields during fermentation.

We have conducted experiments using dilute sulfuric acid pretreated corn stover. Acetic acid, in its protonated form, was extracted into an organic phase consisting of octanol and Alamine 336, a tertiary amine, containing 8-10 carbon aliphatic chains. Importantly, acetic acid removal is most efficient at pH values below 4.8, the pKa of acetic acid, thus no pH adjustment is required after pretreatment. Further as sulfuric acid is co-extracted the pH of the hydrolysate increases during extraction. Our results indicate co-extraction of furfural, hydroxymethylfurfural, acid soluble lignin and other phenolic compounds. Thus addition of membrane extraction to remove acetic acid may simplify and/or eliminate current hydrolysate detoxification technologies such as overliming. Development of a practical membrane extraction process for removal of weak acids such as acetic acid depends on carefully choosing the organic diluent and extractant (octanol and Alamine 336 used here).
Oral Presentation 9-04
Simultaneous lipase and ethyl esters production in expanded bed bioreactor using Metarhizium anisopliae - MTCC 892
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Biodiesel is gaining more importance phase because of its renewable sources of production, biodegradable in nature and non-toxic to the environment with low emissions. Conventional methods of biodiesel require high energy as they operate at higher temperature and pressures and use of harsh chemicals. Lipases are known for hydrolysis, transesterification and methanolysis in the presence of alcohol and can be produced by various microorganisms. However low productivity, cost of fermentation media and subsequent recovery of lipase from fermentation broth is a drawback for the up-scaling and commercialization of the process.

In the present investigations a novel strategy of bioreactor operation was proposed to produce bio-diesel in an expanded bed bioreactor (EBR) using a fungal strain Metarhizium anisopliae - MTCC 892. The culture was immobilized on an inert material and used for lipase production using molasses as a sole production media in an EBR. The EBR was operated in continuous mode and various operating conditions like aeration rate, viscosity of the medium, composition of the molasses and affecting lipase production were studied. Without adopting a purification step for lipase recovery from EBR a strategic addition of ethanol and sunflower oil was done to produce ethyl esters. The rate and ratio of ethanol and sunflower oil addition did not affect the further operation of bioreactor in terms of microbial activity. This strategic operation was resulted in increased volumetric productivity of Biodiesel. The results from the present investigation are encouraging and further studies will result in the transfer of technology on cost efficient manner.

Oral Presentation 9-05
Enrichment of electrogenic consortia in microbial fuel cells for conversion of acetate to electricity
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The ability to produce electricity at high power densities in microbial fuel cells (MFCs) requires optimization of biological as well as engineering parameters. Enrichment of electrogenic biofilm-forming bacteria via selective procedures, such as use of flow-through systems and removal of planktonic cells to improve power density, is demonstrated. Engineering design of the MFC targeting low ohmic and charge transfer resistances, via a three-in-one electrode design, was implemented. The progress of microbial community enrichment in the MFC was tracked via electrochemical impedance spectroscopy. Results show a decrease in the anode side resistance over time. A relationship between improvements in power density obtained over the time and the decrease in resistance was investigated. The changes appear to be mediated by the enrichment of electrode-breathing microbial systems on the anode electrode. The study was conducted using acetate as a model substrate, however, the results are applicable for other substrates including other organic acids, sugars, etc. The removal of acetate from aqueous streams has several applications including biorefineries converting biomass as well as grain to ethanol, wastewater streams from plants producing value-added bioproducts, breweries and food-based industries.

Oral Presentation 9-06
Engineering Challenges for Development of High Intensity Nanostructured Biocatalytic Coatings for Gas Phase and Waste Carbon Conversion to Chemical Intermediates and Fuels
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Concentrating and preserving living microbes in thin (<100μm), adhesive nanoporous coatings may be useful for engineering high intensity biocatalysts for environmental and energy applications. Biocatalytic coatings facilitate engineering microchannel or membrane bioreactors for multi-phase biocatalysis for CO2 sequestration and biological conversion of waste organics to fuels (hydrogen, methanol, ethanol) and chemicals. Several model systems and methods for coating self-assembling polymer particles and living microbes are being studied. The goal is to understand how to engineer polymer-cell interactions and coating microstructure. Coatings may be particularly useful for illumination of concentrated photosynthetic microbes. Anoxic coatings of nitrogen-limited Rhodospseudomonas palustris and sulfur-limited Chlamydomonas reinhardtii are model systems we study for optical and light scattering properties using hydrogen production for reactivity. Single and multi-layer latex coatings are investigated to optimize light adsorption, and understand light scattering in relation to the rate of hydrogen production per illuminated surface area. Algal coatings and coatings of non-photosynthetic anaerobic bacteria could also be useful for gas-phase carbon sequestration. A key characteristic of these systems is preservation of microbial activity and viability simultaneous with formation of nanopores during polymer particle coalescence (film formation). Latex coating emulsions are free of biocides and toxic polymer synthesis monomers. Even with non-toxic latex emulsions, drying rate and conditions can kill some microbes as a result of desiccation and osmotic stress. The response of C. reinhardtii during drying is being investigated using cellular stress responses to engineer polymer emulsion and coating methods for adhesive algal coatings with high viability and reactivity.
Biofuel Production Limited by Renewal of Water and Nitrogen Resources

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Plant growth whether in agricultural, grassland, forest, or natural ecosystems is quantitatively limited by the amount of water and nitrogen that can be recovered from the environment. Water is needed to allow plants to open stomata in the leaf surfaces so that carbon dioxide can diffuse into leaves for photosynthesis. Since the pathway for carbon dioxide to diffuse into leaves is the same as water vapor diffusion from the leaves, every increment of plant growth requires a well defined amount of water uptake by plants from the soil. There is no genetic solution to the physical laws of gas diffusion. In addition, a major factor in the amount of soil water available to plants is the rate of water evaporation from the soil surface. Removal of plant residues for biofuel necessarily results in an increase in the total water requirement for subsequent plant production. Equally important is the nitrogen resource. Nitrogen is required by every living cell as an essential component in nucleic acids and proteins. Virtually all growth processes, including photosynthesis, are dependent on the amount of nitrogen accumulated by the plants. Due to the large amounts of nitrogen needed by plants, productivity is commonly limited by the amount of nitrogen available to and accumulated by plants. Consequently, both water and nitrogen place unavoidable quantitative limits on plant productivity. Without renewed water and nitrogen resources, plant productivity will decrease, and eventually fail, no matter the plant species or their genetic make up.

Life Cycle Consumptive Water Use in Fuel Production

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Feedstock and fuel production requires intensive water input, which is particularly true for biofuel feedstocks, such as agricultural crops. Recently there is a growing public concern on the technology development and implementation especially pertaining to water used by energy and energy used by water. It became more urgent because of sever drought in southern states in US this year. The purpose of this work is to address the water use for energy generation in US by providing a baseline of current consumptive water use in the fuel industries including ethanol, petroleum oil, etc. and examining two major steps of the fuel life cycle – feedstock production and fuel processing/production. This work is an attempt to analyze regional variation, historic trend of consumptive water use in the selected fuel production life cycle, and identify opportunities to reduce water use for fuel production. Results from this study could be used to address water resource sustainability, guide R&D directions in renewable fuel development, and to provide a basis for decision-making in order to meet the overarching goal of energy independence for the nation.

Biochar for environmentally-friendly bioenergy

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Incorporating biochar into soils that was produced during pyrolysis for bioenergy, offers a means of energy production with a net carbon removal from the atmosphere. As well as providing energy and a stable long term carbon sink, biochar is able to improve soils that are at risk of becoming degraded by maximizing biomass off-take. Biochar has properties that sets it apart from returns of uncharred plant litter or composts and manures. Biochar is able to retain nutrients better than other types of organic matter in soil, potentially improving soil productivity and additionally reducing emissions of greenhouse gases other than carbon dioxide from agricultural soils. This presentation will give an introduction to biochar, covering the ancient carbon-rich soils of the Amazon as well as its potential future application within the context of modern bioenergy production.

Life Cycle Assessment of Polyhydroxyalkanoates (PHA)

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We have performed a lifecycle assessment on the polyhydroxyalkanoates (PHA) product system to estimate the environmental performance of PHA derived from corn grain, particularly renewable energy consumption and greenhouse gas emissions. The system boundary includes processes from agricultural production to the PHA fermentation and recovery process. Site-specific process information on the corn wet milling, PHA fermentation and recovery processes was obtained from Metabolix and ADM. Most of energy used in the corn wet milling and PHA fermentation and recovery processes is generated from a cogeneration power plant in which corn stover is burned to generate electricity and steam. Off-site power used in the PHA fermentation and recovery process is purchased from a wind power plant. County level agricultural information is used in estimating the environmental burdens associated with both corn grain and corn stover production. Corn farming counties are specified. Four counties in Iowa (Boone, Cedar, Clinton and Jones counties) supply corn grain to a wet milling plant located in Clinton County. County level soil organic carbon dynamics, nitrate losses due to leaching, and nitrogen oxide and nitrous oxide emissions are predicted by the DAYCENT model. The environmental burdens associated with products in multi-output processes are estimated by the system expansion approach, in which alternative product systems for co-products are introduced. Scenario and sensitivity analyses are done to determine the environmental effects of the following aspects on the overall renewable energy consumption and greenhouse gas emissions: tillage practice, winter cover crop practices and allocation procedures.

Environmental and Economic Analysis of the Fully Integrated Biorefinery

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The biorefinery using Ammonia Fiber Expansion (AFEX) pretreatment has been modeled for the production of fuel ethanol from cellulosic biomass, but work has not yet been done to integrate the biorefinery with cropping (agricultural) and animal production systems. Combining these three subsystems in a single integrated analysis will allow for environmental and economic modeling of biomass production, possible secondary products, fertilizer production, and bioenergy production. Using the Integrated Farm System Model (IFSM), the biorefinery is concurrently simulated with the animal and crop production units in various locations within the United States, under various farm production scenarios. This combined approach allows analysis of economic profitability and directs the development of pathways that also enhance the environment.
Oral Presentation 11-01
MixAlco process: fuels and chemicals from biomass
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The MixAlco process converts any biodegradable biomass (e.g., municipal solid waste, sewage sludge, manure, agricultural residues, energy crops) into fuels (e.g., primary alcohols, secondary alcohols) and chemicals (e.g., ketones, carboxylic acids). To enhance digestibility, the biomass is treated with lime. Then, the lime-treated biomass is fed to a mixed culture of acid-forming microorganisms derived from a marine environment. The acids are neutralized with either calcium carbonate or ammonium bicarbonate, thus forming the corresponding carboxylate salts. Using vapor-compression evaporation, the carboxylate salts are concentrated. Finally, the carboxylate salts are chemically converted to a variety of products.

Via “acid springing,” carboxylate salts are converted to carboxylic acids, which may be hydrogenated to primary alcohols (e.g., ethanol). Alternatively, thermal conversion produces ketones, which may be hydrogenated to secondary alcohols (e.g., isopropanol). If desired, the alcohols can be catalytically dehydrated to hydrocarbons, such as gasoline, diesel, or jet fuel.

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 (secondary alcohols and hydrocarbons)
- Low capital cost
- Low product cost

Recent economic evaluations indicate that primary alcohols can be sold for about $1.13/gal (15% ROI, 10-yr depreciation, 15% working capital, $53/ton biomass cost). The capital cost is estimated to be about $0.82/annual gallon. A demonstration plant is under construction.
Borregaard, Sarpsborg, Norway
building a sustainable business long term.
creation on all components of the feedstock will be essential to
petrochemical fuel substitution and for “green” chemicals, but value
applications.
will give an insight in our approach and some examples of new
but also some of the challenges.
A description of the running operations will be given, to provide an
insight into the practicalities of operating a full scale Biorefinery where
more than 95% of the biomass is converted into bio chemicals, bio
insights.
A description of the running operations will be given, to provide an
performance chemicals, vanillin, yeast extracts and bio ethanol – all
based on lignocellulose from different wood species.
A description of the running operations will be given, to provide an
value creation on the lignin component of the lignocellulose feedstock.
Examples of recent product developments and investigations into new
biomass sources will be given to illustrate the potential in the biomass,
but also some of the challenges.
Product development from the lignocelluloses biomass requires a
broad set of skills both in innovation and R&D, and this presentation
will give an insight in our approach and some examples of new
applications.
Lignocellulosic feedstock offers significant opportunities both for
petrochemical fuel substitution and for “green” chemicals, but value
creation on all components of the feedstock will be essential to
building a sustainable business long term.

Oral Presentation 11-05
Integrated Biorefining at Ghent Bio-Energy Valley
Prof. Wim Soetaert, Faculty of Bioscience Engineering, Ghent
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Ghent Bio-Energy Valley is a Public Private Partnership aiming to
support the development of sustainable bio-based activities in the
region of Ghent, Belgium. Ghent Bio-Energy valley is a joint initiative
of Ghent University, the city of Ghent, the port of Ghent, the Province
of East-Flanders and several industrial companies that are active in the
field of bio-energy generation, distribution, storage and use.
Ghent Bio-Energy Valley aims to promote the development of bio-
based products and bio-energy through collaborative programs, joint
initiatives and synergy between the partners in the fields of Research &
Development, structural measures and policy, industrial development,
logistics and communication towards the general public. Currently,
several biofuel projects are already operational or under construction
in the port of Ghent. The main production site is the integrated
biorefinery at the Rodenhuize docks. It has started production in 2008
and is the largest integrated biofuel production site in Europe. It will
produce both 150,000 m3 bio-ethanol and 250,000 ton biodiesel from
wheat and rapeseed. At the same site, 90 MW green power is also being
produced from biomass and biogas production is planned for the near
future. The strong clustering effect at this production site combines an
integrated production concept and excellent logistical connections,
resulting in a high eco-efficiency of production. Also Research &
Development infrastructure is under construction in the form of the
Pilot Plant for Industrial Biotechnology and Biorefineries (PBB), an open
innovation centre for bio-based activities.
Under the impulse of Ghent Bio-Energy Valley, a plethora of new bio-
based activities are generated that have turned the port of Ghent
into the prime Bio-Port of Europe. As fossil resources are becoming
depleted and increasingly expensive, the transition from the present
fossil-based economy to the bio-based economy is quickly picking up
speed in Ghent.

Oral Presentation 11-06
Status of Iogen’s process for production of ethanol from cellulose
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Iogen Corporation has been operating a demonstration plant for
the conversion of wheat straw to ethanol since 2004. The process
includes a pretreatment with steam and dilute sulfuric acid, followed
by enzymatic hydrolysis of the cellulose, and then fermentation of
the glucose and xylose to ethanol by using recombinant Saccharomyces.
The ethanol is finished as E85 fuel for commercial and fleet use.
The demonstration plant is the final step in scale-up of the process
deploying commercial plants that produce ethanol from cellulose.

Oral Presentation 12-01
Screening for new cellulase enzymes to enhance the synergistic
effect by co-displaying cellulases on the surface of E.coli, LY01
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Simultaneous saccharification and fermentation (SSF) steps have been
proposed in the literature to reduce the cost of bioethanol production
process. A whole-cell biocatalyst system has been developed in our
laboratories to directly produce ethanol from cellulose in a single step.
The results were very successful in converting cellulose to ethanol.
This whole cell biocatalyst was constructed with LY01 (provided by Dr.
L. Ingram, University of Florida), which is one of the most developed
ethanologenic Escherichia coli strains, used as a host cell. Previously, the
cellulase genes, celICA, celCCE and β-glucosidase from the
mesophilic strain Clostridium cellulolyticum, were co-displayed.
In this study, by displaying cDNA library of the C. cellulolyticum on the
surface of the LY01, we screen for new cellulases, which show enhanced
enzymatic synergistic effect. Moreover, cellulose hydrolysis rate is
improved by selectively screening enzymes showing higher activity
after performing directed evolution. The cell surface displayed
enzymes are quantitatively measured using flow cytometric analysis.
We are able to control the expression level of three different enzymes,
which show higher synergistic activities. In this research we will
also study the effects various substrates on the cell-surface display
mechanism.

Oral Presentation 12-02
Performance Data of Novozymes New Cellulase Systems
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The US Department of Energy launched a 3-year program to reduce the
cost of cellulase enzymes for ethanol production from pretreated
lignocellulose substrates back in 2001. The impact of research
performed during this program was quite significant according to the
cost metric specified by NREL and DOE, by which the enzyme cost was
reduced 30-fold. The new cellulase enzymes did, however, not only
result in important cost improvements compared to the old cellulase
systems.
From the results presented here, it is also evident that the performance of
these new enzymes was improved at various process conditions. The
presentation will include performance data from cellulose hydrolysis of
pretreated lignocellulosic substrates at various pH and temperature,
and will furthermore include a discussion of how the activity/efficiency
of cellulase enzymes can be evaluated using process relevant
approaches.
Oral Presentation 12-03
Family GH-6 cellulases do not seem to possess a catalytic base
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The structures of five GH-6 catalytic domains have been determined and the residue, that is best positioned to act as a catalytic base, is an Asp residue, which is conserved in all members of this family. Site directed mutagenesis of this residue in three family members, T. reesei Cel6A, T. fusca Cel6A, and Cel6B, gave results that are not consistent with its role as a catalytic base. However, mutations of the C. fimii residue did drastically reduce activity, as predicted for a catalytic base. We have carried out azide rescue experiments on T. fusca Cel6A and Cel6B, which confirm that none of the conserved acid residues in the active sites of these enzymes function as a catalytic base. It appears that the catalytic base function may be shared by several residues in this family.

Oral Presentation 12-04
The Clostridium thermocellum cellulosome, a molecular machine for cellulose degradation
M. Newcomb and J.H.D. Wu
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The C. thermocellum cellulase system exists as a multi-protein complex called the cellulosome. More than 70 subunits have been found to be associated with the cellulosome. In addition, the bacterium produces free cellulases that are not associated with the cellulosome. However, very little is known about the regulation of gene transcription of these enzymes in C. thermocellum.

We identified glyR3, which is co-transcribed with the cellulase/hemicellulase genes celC and licA, as a cellulase transcription regulator. The gel shift assay (EMSA) revealed that the recombinant GlyR3 bound specifically to the celC promoter region. GlyR3 was also identified from the lysate of the lichenan-grown cells, which bound to the same sequence. DNase I footprinting and competitive EMSA showed the binding site to be an 18 bp palindromic sequence with one mismatch. The DNA-binding activity was specifically inhibited by laminaribiose, a beta-1-3 linked glucose dimer, in a dose-dependent manner. In in vitro transcription analysis, celC expression was repressed by rGlyR3 in a dose-dependent manner. The repression was relieved by laminariobiose, also in a dose-dependent manner. These results indicate that GlyR3 is a negative regulator of the celC operon consisting of celC, glyR3, and licA, and inducible by laminariobiose. Thus the bacterium may modulate the biosynthesis of its enzyme components to optimize its activity on an available biomass substrate. The results further indicate that regulation of the degradative enzymes can be accomplished through soluble sugars generated from the insoluble substrate by the action of the enzymes.

Oral Presentation 12-05
Biomass Ethanol from Clostridium thermocellum – A Systems Biology Analysis
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The anaerobic thermophilic bacterium Clostridium thermocellum ferments cellulose directly to ethanol and other metabolic products using its multi-enzyme cellulosome complex without the need for external cellulase addition. Elimination of cellulase production step consolidates the cellulosic ethanol production process significantly reducing costs. In this study, we used microarray technology to probe the genetic expression of C. thermocellum ATCC 27405 during cellulose and celllobiose fermentation. We also used multidimensional LC-MS/MS technology and 15N-metabolic labeling strategy to quantify changes in cellulosomal proteins in response to various carbon sources (celllobiose, amorphous/crystalline cellulose (avicel) and combinations of avicel, pectin and xylan). Transcriptomic analysis involved a time-course analysis of gene expression to identify gene clusters with similar temporal patterns in expression during cellulose fermentation. Broadly, genes involved in energy production, translation, glycolysis and amino acid, nucleotide and coenzyme metabolism displayed a progressively decreasing trend in gene expression. In comparison, genes involved in cell structure and motility, chemotaxis, signal transduction, transcription and cellulosomal genes showed an increasing trend in gene expression. Proteomic analysis identified over 50 dockerin- and 6 cohesin-module containing components, including 20 new subunits. The list included several proteins of potential interest that specifically respond to the presence of ‘non-avicel’ substrates in the culture medium. Quantitative proteomic results also highlighted the importance of glycoside hydrolase (GH) family 9 enzymes in crystalline cellulose hydrolysis. Overall, the transcriptomic and proteomic results suggest a well-coordinated temporal and substrate-specific regulation of cellulosomal composition in C. thermocellum.

Oral Presentation 12-06
Design and Development of an Enzyme Product for Cellulosic Biomass Conversion
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Many companies have begun process development and scale-up of their cellulosic biomass conversion technologies. It was Genencor’s desire to quickly launch a product to support the needs of these process developers. This paper will discuss many of the steps to launching this first commercial product specifically for this market, including the design of the enzyme system, performance testing and validation, confirming reproducible production quality and delivering on needed production cost reductions. Performance comparisons will be shown between Accellerase™ 1000 and Spezyme® CP, a previous benchmark cellulase product from Genencor.
**Posters**

**Poster 1-07**  
Chemical and physical characterizations of *Liriodendron tulipifera* on growth periods  
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In this study, we investigated various chemical and physical characterizations of 2-, 4- and 6-years yellow poplar (*Liriodendron tulipifera*) to build the fundamental data which are able to enhance the applicable value of *L. tulipifera* as a resource of biomass. Holocellulose content was decreased slightly from 77.37 to 75.28% with increasing growth periods, but sufficient amount (more than 75%) of holocellulose content was confirmed in all samples. 6-years *L. tulipifera* had much acid-insoluble lignin content compared to 2- and 4-years trees. It might be caused by the lignification as it grew. Physical characterizations such as crystallinity and strength on various growth periods were investigated. Crystallinity increased from 32.04 (2-years) to 38.57% (6-years) and bending strength increased largely from 21.29 (4-years) to 74.82% (6-years), too.

The digestibility by enzymatic saccharification and the degradation rate by dilute acid pretreatment were decreased depending on growth periods. The digestibility was decreased from 14.55 (4-years) to 10.18% (6-years) and degradation rate after pretreatment at 200°C for 20min was decreased from 14.68 (2-years) to 8.03% (6-years)

Characterizations of 2-years and 4-years trees were very similar, which 6-years one has shown a difference.

**Poster 1-08**  
Improved Multivariate Calibration Models for Corn Stover Feedstock and Dilute-Acid Pretreated Corn Stover  
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NREL researchers have been actively developing rapid calibration models to predict the composition of a variety of biomass feedstocks by correlating near-infrared spectroscopic data to compositional data produced using traditional wet chemical analysis techniques. The rapid calibration models are developed using multivariate statistical analysis of the spectroscopic and wet chemical data. This work will present an overview of the latest version of these calibration models for corn stover feedstock and dilute acid pretreated corn stover.

Specific topics to be addressed include a comparison of the effect(s) of mathematical pretreatments on the adequacy of the resulting models. We will demonstrate that calibration models developed using a variety of mathematical pretreatments provide essentially equivalent prediction models. Also, we will present a comparison of partial least squares models predicting multiple dependent variables simultaneously (PLS-2) and multiple PLS models, each prediction a single dependent variable (PLS-1) for groups of constituents. Finally, we will present a comparison of different measures of uncertainty among different multivariate analysis packages.

**Poster 1-09**  
Biohydrogen and biodiesel co-production with treatment of high solid food waste  
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A two-step process to produce hydrogen and biodiesel with treatment of high solid food waste is developed. The first step of this process is dark fermentative hydrogen production, in which the fermentative bacteria will use glucose derived from the organic waste carbon to produce hydrogen and volatile fatty acids (VFA) (e.g., acetate or butyrate). One third of the carbon is converted to carbon dioxide in this process while two thirds of the carbon is converted to volatile fatty acids. In the second step, the remaining carbon in the form of VFA is used as a carbon feed to yeast with high lipid content in the biomass, which in turn can be used as feedstock for the biodiesel industry. On the whole, not only the waste is treated in this process, but also it is used as a resource and converted to biomass and biofuel.

**Poster 1-10**  
Structural and Quantitative Determination of Lignin in Biomass by qPyGC-MS  
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Our understanding of the most desirable characteristics for lignin in dedicated energy crops has been limited by our inability to accurately measure both concentration and structure of lignin in herbaceous feedstocks. Some studies on woody biomass suggest that decreasing the lignin content enhances saccharification, but lignin contents of herbaceous materials are naturally much lower than those found in wood and may be near the limits for crop viability. Less is known about the effect of modifying the structure of lignin, specifically, the ratio of phenolic subunits, carbon sequestration, thermochemical pretreatment, and enzymatic saccharification. In herbaceous materials, three types of phenolic subunits are present, p-hydroxyphenol (H) units, guaiacyl (G) units, and syringyl (S) units. This study describes the application of pyrolysis gas chromatography-mass spectrometry (PyGC-MS) to the quantitative measurement of lignin concentration and elucidation of lignin structure. Analysis by this method is fast, inexpensive and accurate over a wide range of biomass materials.
**Poster 1-11**

**Seaweeds as Novel Feedstocks for Bio Alcohol**


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The use of ethanol as an alternative motor fuel steadily increasing around the world. Conventionally, ethanol has been primarily fermented from the mono sugar that makes up the starch in grain. Corn is the source of starch for more than 92% of ethanol production in the United States. In addition, ethanol can be produced from cellulosic biomass, for example, stalks, wheat or rice straw, and wood waste. Switchgrass are also promising cellulosic sources due to their fast-growing characteristic. In spite of their various advantages as biomass for the large-scale production of ethanol, there are several criticisms on the use of food (grain) for the motor fuel regardless of the dying people because of starvation, especially in Africa. In the case of the utilization of cellulosic biomass, the cost is relatively expensive arising from the complicated process due to the presence of lignin which is difficult to remove. Recent advances in the extremely new field of biotechnology for the ethanol production are making it possible to use of macro algal biomass, e.g., 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 CO2, know as a greenhouse gas. In this presentation, we describe in detail the hydrolysis of red algae (seaweed) into mono sugars followed by the fermentation to produce ethanol.

**Poster 1-12**

**Introducing for the first time giant reed (Arundo donax) as ideal feedstock for future biorefinery**

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Giant reed (Arundo donax), a perennial grass, is one of the fastest growing plants in the world. It has an excellent potential to be a major source of bioenergy, biochemicals, and pulp and paper in the future biorefinery. Why? At 15 to 20 oven dry tons per acre, giant reed is approximately twice as productive as poplar and switchgrass with less fertilizer demand and low management costs. Arundo donax efficiently removes carbon dioxide from the atmosphere and “fixes” it into plant tissue, above and below ground. It has been previously proven in our lab to be an excellent source of biomass for pulp and paper production, since it requires less bleaching chemicals and energy to achieve the same level of brightness as wood pulp. In this study for the first time we have shown the technical feasibility of utilizing giant reed for pulp and paper as well as bioethanol production in the future biorefinery. The ethanol yields from 3 stage processing combined of SO2-catalysed steam pretreatment, enzymatic saccharification and yeast fermentation will be presented.

**Poster 1-13**

**Comparison of methods for the quantification of fructose-equivalents in straws**

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Cereal and commercial grass-seed straws are promising feedstocks for biochemical and cellulosic fuel-ethanol production. A low, but significant, fraction of the carbohydrate portion of these feedstocks is often composed of fructose/fructosyl-containing components (“fructose equivalents”); such carbohydrates include sucrose, fructo-oligosaccharides, and fructans. Standard methods used for the quantification of neutral monosaccharide equivalents are not particularly well suited for the quantification of fructose equivalents due to the inherent instability of fructose (> 50% degradation) under the prescribed hydrolysis conditions (oligo/polysaccharide hydrolysis conditions of 4% sulfuric acid, 121oC, 1 hr). The objective of the presented study was thus to determine conditions better suited for fructose-equivalent quantification, focusing on fructan/fructo-oligosaccharide hydrolysis. Time, temperature and acid concentrations were considered, using wheat and tall fescue straws as representative feedstocks. Included in the study were model fructose-containing oligosaccharides/polysaccharides. The instability of fructose, relative to glucose and xylose, at higher acid/temperature combinations was demonstrated, all rates being acid and temperature dependent. Fructans are shown to be effectively hydrolyzed at acid concentrations as low as 0.1%, when at 121°C for 1 hr. Lower temperatures can also be effective, with corresponding adjustments in acid concentration and time. The data indicates that the buffer capacity of the feedstock should be taken into account when using the lower acid concentrations, this being relevant to the appropriate conditions for the determination of degradation “correction factors”. The presented hydrolysis data, which focuses on acid-catalysis, is discussed with reference to the application of appropriate hydrolytic enzymes.

**Poster 1-14**

**Conversion of Kraft Paper Mill Sludges into Ethanol by Simultaneous Saccharification and Fermentation**

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Paper mill sludge is a solid waste material composed of pulp residues coming out of paper machine and inorganic additives associated with paper making. The carbohydrate portion of the sludge has chemical and physical characteristics similar to pulp. Because of high carbohydrate content and well dispersed structure, paper sludges can easily be converted into value-added products without a costly pretreatment process, which is a significant economic benefit. The sludges also contain high level of ash content originated from additives used in paper making. This adversely affects enzyme activity and the bioconversion process. In this study, the sludges were de-ashed and put through Simultaneous Saccharification and Co-Fermentation (SSCF) using cellulase (Spezyme-CP) and recombinant E. coli (KO-11), and also Simultaneous Saccharification and Fermentation (SSF) using Spezyme-CP and Saccharomyces cerevisiae (DSF). The ethanol yield in the SSCF was 70-80% of theoretical maximum on the basis of total carbohydrates and 90-100% on the basis of glucon alone. The ethanol yield in the SSF was 70-80% on the basis of glucon. The SSCF and SSF proceeded well without pH control using the deashed sludges. The ash content, which contains calcium carbonate, clay and titanium dioxide, was partially neutralized by the acids produced from the SSCF and SSF acting as a buffer to stabilize the pH during fermentation. When the SSCF and SSF were operated in fed-batch mode, the ethanol concentration in the broth was increased to 50g/L and 65g/L, respectively.
Kraft paper mill produces chemical pulp treating wood chips with white liquor, a mixture of sodium hydroxide, sodium sulfide, and sodium carbonate at 130 - 180 °C. The long fibers of pulp are used for paper making. The short and fine fibers that are rejected in paper making process and eventually become waste material, e.g., paper mill sludge. Due to its high carbohydrate, low lignin, and low ash content, the pulp can be an attractive substrate for bioconversion into various products, one of which is cellulase. Kraft paper mill thus offers substrates for production of cellulase enzyme as well as cellulosic ethanol. Our laboratory data indicate that only about 10 kg of Kraft pulp is required to produce cellulase enzyme that can saccharify one dry-ton of the sludge. In this study, the Kraft pulp was used as the main carbon source for production of cellulase by Trichoderma Reesei, Rut C-30. Partially de-ashed Kraft paper mill sludges were tested as a substrate for production of cellulosic ethanol through fermentation using the cellulase produced in-house and two different microorganisms - recombinant E. coli KO-11 and Saccharomyces cerevisiae DSA. Fermentation process proceeded without any major problems and demonstrated ethanol yields comparable to those of pretreated corn stover. It is projected that 70 gallons of ethanol can be produced from these processes per ton of dry sludge.

Any valuation of a feedstock for bioprocessing is inherently dependent upon detailed knowledge of its chemical composition. Accepted analytical procedures for compositional analysis of biomass currently 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’. Our group recently reported the first compositional analysis of water-soluble materials in corn stover. Of particular significance was the discovery that fermentable sugars (primarily glucose, fructose, and sucrose) represented 30-46% of the dry weight of extractives (4-12% of the dry weight of corn stover feedstocks). Because constituents belonging to this fraction of biomass are not typically considered in current models of bioconversion, these results have heightened interest in the composition of water-soluble materials.

In continuing work, analytical techniques developed in conjunction with our assessment of corn stover are being applied to assess the composition of water-soluble materials in four representative switchgrass samples. To date, analytical characterization has resulted in mass closures approaching 80%, with several analyses yet to be completed. Chemical constituents currently identified as contributing to mass closure include monomeric and oligomeric sugars, organic acids, inorganic ions, and a distribution of oligomers tentatively identified as being derived from phenolic glycosides. Switchgrass results will be compared with previous analyses of corn stover extracts and presented in the context of their potential impact on biomass processing, feedstock storage, and future analyses of feedstock composition.
**Poster 1-19**  
**Analysis of biomass composition and gene expression patterns in the breeding and selection of shrub willow bioenergy crop varieties**  
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It has been shown that high resolution thermogravimetric analysis (HR-TGA) is a reliable technique to identify differences in stem biomass composition among shrub willow (Salix spp.) varieties selected in the breeding program at the State University of New York College of Environmental Science and Forestry (SUNY-ESF). Rapid determination of biomass composition is critical to the breeding and selection of shrub willow varieties with optimized properties for downstream conversion. In order to improve the process for identifying and selecting shrub willow varieties with distinct biomass compositions, HR-TGA was developed as a rapid, low-cost method for analyzing and screening unique willow genotypes. For validation of the HR-TGA method through regression analysis, a set of 25 shrub willow varieties, characterized for growth features including yield, height, stem diameter, and disease and pest resistance, were analyzed using traditional wet chemistry techniques in addition to HR-TGA. The molecular basis of differences in biomass composition is also being investigated to provide molecular tools that will further improve the shrub willow breeding program. Genes encoding enzymes involved in lignin biosynthesis and selected carbohydrate active enzymes (CAZys) are being cloned from willow and their expression patterns are being analyzed. The long-term goal is to develop molecular markers for the early selection of genotypes with improved biomass properties. Correlating patterns of gene expression with wood composition among selected shrub willow varieties and siblings within a hybrid family is a critical step in this process.

**Poster 1-20**  
**Elephantgrass as a Cellulosic Feedstock for the Southeast**  
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Napiergrass or elephantgrass (Pennisetum purpureum Schumach) is a tall C4 grass that is used throughout the world as a forage crop. Breeding efforts at the University of Florida and with USDA-ARS at Tifton, GA produced high yielding cultivars (Merkeron) and breeding lines during the 1980’s. Yields have been reported as high as 40 Mg ha⁻¹ yr⁻¹ in Florida. Merkeron had significantly higher yields than switchgrass cultivar Alamo over 6 years and at three locations in Georgia. Studies are being conducted to compare napiergrass yield with switchgrass and other tall bunch grasses such as energy cane at Tifton, GA. Yields for the first year were over 34 and 28 Mg ha⁻¹ for the two napiergrass genotypes, 27 Mg ha⁻¹ for energy cane, and 8.5 Mg ha⁻¹ for two advanced switchgrass genotypes. Napiergrass has a good deal of genetic variability that can be exploited via selfing or crossing among parents to enhance yield and quality. Approximately 100 napiergrass plant introductions have been evaluated over the past few years. Genetic variability has been assessed via AFLP analyses, as well as phenotypic traits. Crossing has begun between the highest yielding lines and has been assessed phenotypically and genetically via AFLP technology.

**Poster 1-21**  
**Genetic dissection of sorghum traits to enhance biofuel production**  
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Sorghum offers inherent advantages that make it an attractive dedicated bioenergy crop able to generate sugars, starch and lignocellulosic biomass as feedstocks for ethanol and other green chemicals. What makes sorghum appealing is its tolerance to drought, ability to grow on poor soils, deep root system, and ability to produce a ratoon crop after the initial harvest. There is also great genetic diversity for many useful traits, including high biomass production, high stem-sugar production, and cell wall composition. Sweet-stem sorghums were originally grown for the production of syrup and molasses in primarily Kentucky, Tennessee, Mississippi and Texas. Expansion of sweet sorghums for ethanol production beyond those regions will require the production of regionally adapted cultivars that are resistant to specific diseases and that perform well on the local soil types. Since sugar accumulation in the juice is a multigenic trait, the development of regionally adapted sweet sorghums will be much more expedient if the loci that control sugar accumulation are known. The same applies for genes affecting cell wall composition. Once the genes underlying these traits are known, it is possible to develop molecular markers that can be used for selection of the desired genotypes in a breeding population. We are using a genomics-based approach that combines high-throughput expression profiling, the sorghum genome sequence and two populations of recombinant inbred lines to identify and clone genes that impact bioprocessing characteristics.

**Poster 1-22**  
**Genome-enabled discovery of carbon allocation genes in Populus**  
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*Populus* spp. (Black and Eastern Cottonwood, quaking aspen, balsam poplar) represent fast-growing hardwood tree species native to temperate regions of the world with a high carbon sequestration potential and the ability to provide woody biomass for the production of fuels and green chemical feedstocks. The availability of the poplar genome sequence, the genetic diversity among different poplar species, and the ability to generate transgenics, enable the development of poplar trees that are optimally adapted for carbon sequestration per se, or the production of lignocellulosic biomass for bioenergy production. In order for this to become a reality, it is necessary to identify genes involved in carbon sequestration – the ability of the tree to capture CO₂ and generate photosynthesis – and carbon allocation – the partitioning of the photosynthetic carbon to different parts of the tree and between different cell wall polymers and other metabolites. We are using a pseudo-backcross population of (*P. trichocarpa* x *P. deltoides*) x *P. deltoides* to identify genes that affect root biomass, root architecture and root composition. The population (285 individuals, three ramets each, two nitrogen regimens) is genotyped using SSR and AFLP markers. We are developing a high-throughput compositional analysis for root tissue, based on near infrared spectroscopy and pyrolysis mass spectrometry. The genotypic and phenotypic analyses are followed up with high-throughput gene expression analyses using microarrays. Combined with the poplar genome sequence, this approach is expected to lead to the identification of candidate genes that can be further evaluated through transgenic up- and down-regulation.
**Poster 1-23**  
**High Oil Content Algae Species Grown on Wastewater and Flue Gas**  
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Algae are one of the promising biomass feedstock for renewable energy production owing to their widespread availability, fast growth rate, and high oil and other biomass yields. However, the challenges of the high costs of culturing the algae in large scale have limited the practical use of this feedstock. These limitations can, however, be overcome by coupling algae production with wastewater and flue gas treatment. The objective of our ongoing study is to select high oil content and high growth rate algae species that can flourish on wastewater. The growth characteristics and oil yield of selected microalgae in batch cultures and their relationship to removal of nitrogen and phosphorous and consumption of CO₂ are being investigated. The effects of pH, chemical contaminants, aeration rates, and CO₂ supply on mass culture of these microalgae will be evaluated. Recommendation on algae for biomass production and wastewater treatment will be provided.

**Keywords:** microalgae culture; wastewater; feedstock; high oil content; biodiesel

**Poster 1-24**  
**Cell wall composition of sugarcane and related Saccharum species**  
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Sugarcane (Saccharum spp. hybrids) has great potential to provide feedstock to a biofuel industry in the United States. Sucrose from sugarcane can easily be fermented into ethanol. Sugarcane and related species also yield large amounts of biomass that would be suitable for conversion to biofuel. There is limited information on the composition of the biomass from sugarcane. This study was done to determine the cell wall composition of the residue left after expressing the juice from the stalks of a sample of different Saccharum genotypes. Ninety-six Saccharum genotypes representing commercial cultivars and wild species were grown in cans during the spring and summer of 2006. In August 2006, plants were harvested by cutting at soil level. Plant material was chopped, and juice was expressed from a 1000-g sample. The remaining fiber cake was dried, and then ground through a 1 mm screen. Cell wall composition was determined on duplicate 0.5 g samples by sequential extraction using neutral detergent (NDF), acid detergent (ADF), and 72% H₂SO₄ (ADL). Remaining residue was then ashed. The composition of the fiber cakes ranged from 8 to 22% soluble material, 28 to 35% hemicellulose, 40 to 52% cellulose, and 7 to 14% lignin. This is lower than some earlier reports of lignin content in sugarcane bagasse, possibly due to the ADL method used to determine lignin, which underestimates lignin in grasses.

**Poster 1-25**  
**Evaluating low lignin mutants of forage sorghum for increased conversion efficiency to sugars and ethanol**  
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Reduced lignin near-isogenic lines of Atlas bmr-6, bmr-12, and bmr-6 bmr-12 forage sorghum (Sorghum bicolor (L.)) have been evaluated as sources of biomass for conversion to sugars and ethanol. These mutants have the advantages of reduced lignin contents and high biomass yields. Field replicates of wild-type and multiple reduced lignin mutants were harvested whole and without grain, dried, and ground. Representative biomass samples were evaluated for total carbohydrate, Klason lignin and ash contents. Lignin varied widely and total carbohydrates less so. Samples were next treated with a low-severity dilute-acid pretreatment and the washed solids saccharified with commercial cellulase. The relative amount of glucose released (63-90%) was found to be negatively correlated with lignin content and the correlation was R² = 0.80. Currently, the washed solids are being evaluated for differences in ethanol yields following simultaneous saccharification and fermentation with Saccharomyces cerevisiae.

**Poster 1-26**  
**Mass Culture of Microalgae on Wastewater and Flue Gases for Biomass Feedstock Production**  
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Treatment of wastewater and associated gaseous emission is costly and technically challenging. With increasingly stringent regulations and limits on wastewater discharge and gaseous emission, modification of conventional processes must be made to meet these requirements. These process modifications will require substantial capital investment and would also likely substantially increase operating costs. The present proposed study takes a creative approach in which microalgae is grown on nutrients supplied from wastewater and gaseous emission from wastewater treatment plants, harvested and extracted for oil that is converted to biodiesel fuel, and the remaining algal biomass can be used for ethanol and biooil production. This would create a win-win situation where water and air conditions are improved while renewable energy is generated. The goal of our research is to develop technologies to produce high oil content microalgae in large scale. We studied the growth of selected microalgae species on tap water and wastewater with or without supplemental nutrients and CO₂ inputs. Different batch and continuous bioreactors and harvest techniques were evaluated. The biomass yield, oil yield, and nutrient removal ability of micro-algae in responses to the wastewater at different treatment stages under different lighting conditions were investigated. The experimental results showed that 70% of nitrate and phosphate ions in sewage can be effectively removed after six days of algae culture. Dry biomass production reached at 0.25-0.5g/L/day. The technical and economic implications of our results for wastewater treatment and biomass production will be discussed.

**Keywords:** bioenergy; microalgae culture; wastewater; toxicity; growth inhibition; biomass; biodiesel

**Poster 1-27**  
**Screening and optimizing enzymatic liquefaction of designed household waste for gasification**  
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The amount of municipal household waste is increasing in the developed world of the world. This is an environmental problem but also holds a large potential for energy production. A solid-liquid system for gasification of waste material is to be developed in Denmark based on cross-disciplinary research effort. This system depends on liquefaction of solid household waste for feeding a gasifier. The aim of this project is to develop a technique suitable for liquefying a heterogeneous material by thermal and enzymatic hydrolysis. Different commercial enzymes has been screen for their effect on liquefaction of municipal after thermal treatment of the material at 90 °C for half an hour. The enzymes used include a range of commercial proteases, cellulases, amylases and pectinases. These enzymes are responsible for the hydrolysis of proteins, cellulose, starch and carbohydrates, respectively. Small batches of predefined organic waste were used for the identification of critical enzyme activities and bottlenecks in the enzymatic liquefaction of municipal waste. The reported results are composition of the waste material before and after liquefaction, rheological properties and end-product content.
Poster 1-28
Rapid Compositional Analysis of Switchgrass Feedstocks and Effects of Compositional Variation on Conversion
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The ability to obtain an accurate chemical composition 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 chemical characterization of switchgrass feedstocks. These new techniques combine Near Infrared (NIR) spectroscopy and Projection to Latent Structures (PLS) multivariate analysis in methods inexpensive enough to allow the compositional analysis of hundreds of samples per day, while maintaining the precision and accuracy of the wet chemical methods used to calibrate the NIR method. In the development of improved energy crop varieties, information about the effects of cell wall composition on conversion performance characteristics will be critical. A small laboratory-scale, high-throughput assay has been developed that can be used to assess the conversion efficiency of biomass samples. Both acidic and basic thermochemical pretreatment methods have been employed, and the assay is reproducible at the milligram scale. Variation in enzymatic digestibility of washed, pretreated solids was identified not only in biomass with varying compositions, but also in biomass with similar compositions. Exploration of the composition and conversion characteristics of such samples allows us to provide critical information to farmers, enzyme manufacturers, and biomass processors to guide our collective thinking in the development of this new industry.

Poster 1-29
The effect of drying temperature on the composition of biomass
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The compositional quality of different lignocellulosic feedstocks can influence their performance and potential demand at the biofuel refineries. Many analytical protocols for determining the composition or performance characteristics of biomass involve a drying step, and the drying temperature can vary depending on the specific protocol. To get high quality data, it is important to determine the correct drying temperature to vaporize the water without negatively impacting the compositional quality of the biomass. A comparison of drying temperatures between 45°C and 105°C was performed using wheat straw and corn stover. Near-infrared (NIR) spectra were taken of various samples and compared using principal component analysis (PCA). Carbohydrates were analyzed using quantitative saccharification to determine sugar degradation. Analysis of variance was used to determine if there was a significant difference between drying at different temperatures. PCA showed an obvious separation between each drying temperature. Preliminary data suggest that at a 95% confidence interval that there is no significant difference up to 85°C for wheat straw.

Poster 1-30
Potential for biodiesel synthesis from macaúba (Acrocomia aculeata) pulp oil with a high content of free fatty acids
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The macaúba (Acrocomia aculeata) is a native fruit of the Brazilian cerrado with potential to produce up to 6.5 ton of oil/ha. The macaúba pulp oil presents, few weeks after fruit harvest, high content of free fatty acids (FFA) and is usually used to soap production. In this work, two alternatives routes were compared for producing biodiesel from macaúba pulp oil with 35% of FFA. The selected routes, hydroesterification and biocatalyst, are able to use less expensive feedstocks that cannot be converted to biodiesel by conventional methods. The hydrolysis of the oil was carried out at 300°C, followed by acid-catalyzed methanolysis performed at 190°C with niobic acid (NB-340 CBMM) as catalyst at concentration of 10 wt% relative to FFA. A commercial, (Novozym 435) immobilized lipase, at concentration of 10 wt% relative to oil, was employed as a catalyst for converting the macaúba oil to biodiesel, via alcoholysis. The conversion efficiency to methyl esters were analyzed by colorimetric method. The hydroesterification processes reached 78% and 22% of conversion in a small reaction time (60 minutes) with and without catalyst, respectively. The immobilized lipase has produced a superior conversion (85%) after 72 hours. Although the esterification time using lipase is very high, bioconversion appears as a cleaner technology to biodiesel production. Furthermore, the biodiesel presented lower viscosity and better quality due to very low temperature of enzymatic reaction (35°C). The economic feasibility of enzymatic esterification can be enhanced by recycling the immobilized enzyme.

Keywords: macaúba, biodiesel, lipase, hydroesterification.

Poster 1-31
Cell wall fermentation kinetics impacted more by lignin content and cross-linking than by diverse shifts in lignin composition
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We used a biomimetic model system to ascertain how lignification and diverse shifts in lignin cross-linking and composition influence cell wall fermentation. Primary cell walls from nonlignified maize cell suspensions were artificially lignified with varying ratios of normal monolignols (coniferyl and sinapyl alcohols) and with normal plus unusual monolignols (5-OH coniferyl alcohol, coniferaldehyde, sinapyl acetate, and dihydroconiferyl alcohol) identified in normal, mutant, and transgenic plants. Cell walls with normal or reduced feruloylation were also lignified with varying proportions of sinapyl p-coumarate, a precursor of p-coumarylated grass lignins. Cell wall fermentability was determined by measuring gas production during in vitro ruminal fermentation (a proven surrogate measure of fiber saccharification and fermentation to ethanol). Increasing the lignin content of cell walls from 0.5 to 124 mg/g increased lag time by 37%, decreased fermentation rate by 37%, and decreased fermentation extent by 18% from hemicelluloses. Lignification increased lag time for cellulose by 13-fold without influencing the rate or extent of fermentation. Ferulate cross-linking of xylans to lignin accounted for at least one-half of the inhibitory effects of lignin on cell wall fermentation. Copolymerizing sinapyl p-coumarate with monolignols increased the extent of hemicellulose fermentation by 5% without significantly affecting other kinetic parameters. Other shifts in the lignin composition did not alter the kinetics of fermentation. The results indicate that continued selection or engineering of plants for reduced lignification or ferulate cross-linking will improve fiber fermentability more than perturbing monolignol biosynthesis solely for the purpose of altering lignin composition.
**Poster 1-32**

**Evaluation of Energy Cogeneration System Using Urban Wastes in Brazil**

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Urban solid residues and organic wastes from animals are considered potentially useful for clean energy production. Transformation processes of these lignocellulosic resources and urban solid wastes in useful energy, biofuels and diverse chemical products are world widely investigated. Pyrolysis, for example, can produce electrical energy and products that can be found in the form of solids (vegetal carbon), liquids and gasses. Brazil produces daily an amount of 242,000 tons of wastes, in which 90,000 tons corresponds to municipal solid residues (MSR). Almost 76% of these residues are not being properly treated and processed for energy production. In this context, an energy cogeneration system is proposed as a solution of this problem through the generation of electricity and useful heat using MSR as raw material, and at the same time leading in the reduction of 80% of its accumulated mass as well as undesirable effects on the environment.

This work studied the viability of a cogeneration system by using energy and exergy balances and computational tools to analyze the influence of different mass fluxes of MSR. The combined system was consisted of a gas turbine responsible for natural gas burning as well as a steam cycle responsible for MSR burning. The results showed that for any mass fluxes of MSR this system was viable, where a high amount of electrical energy was produced and also the excess energy could be used by other industries. Therefore, combined cogeneration system presents a promising alternative for energy generation from biomass.

**Acknowledgments:** FAPESP

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**Poster 1-33**

**The enzymatic technology to improved oil extraction from pequi pulp (Caryocar brasiliense Camb.)**

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The pequi is widely distributed in the Brazilian cerrado. Its’ fruits are normally used for food, cosmetics and lubricants production. The pequi pulp is mainly composed of lipids (45 to 50%) and fiber. The integral fruits were collected in the Mato Grosso State, Brazil. The fruits were autoclaved at 121°C for peroxidase inactivation and stored under refrigeration for subsequent use. In this study, an enzymatic extracts with pectinase and CMCase activities was used for hydrolysis of pequi pulp prior to oil extraction. The enzyme was produced in bench-scale reactor by solid state fermentation using a mutant Aspergillus niger 3T5B8. The oil extractions were carried out by centrifugation or by hydraulic pressing, with or without enzymatic incubation. The best oil yield was obtained in the following operational conditions: 0.5% volume of enzyme per weight of the sample, 60°C and 1 hour of incubation time in pre-treatment stage and 340 bars in the pressing stage. The oil content in the pequi pulp (45%) and the physicochemical characteristic of the oil was determined according to standard analytical methods. Free fatty acids, peroxide values, iodine and saponification indices were respectively 1.46 mgKOH/g, 2.98 meq/kg, 49.13 and 189.40. The acidity and peroxide values were lower than the obtained values in commercial oil samples, respectively 2.48 mgKOH/g and 5.22 meq/kg. This combined process promotes, simultaneously, the cellular wall hydrolysis and the viscosity reduction of the pulp, contributing to increased the oil yield by pressing in at least 20%.

**Keywords:** Pequi, oil extraction, enzyme-based process.

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**Poster 2-07**

**Lignocellulose biodogradation by wood-feeding termites: Fundamentals and applications**

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Termites are among the most important and effective lignocellulose-digesting invertebrates on the earth. As the world’s smallest bioreactors, the degradation of wood by termites is very unique and highly efficient, which demonstrates combined actions of the termite and its hindgut microbial symbionts in the utilization of 74-99% of the cellulose and 65-87% of the hemicellulose, as well as 2-83% lignin of the ingested plant material. Two main contributions of the termite to the breakdown of lignocellulose are the provision of small wood particles and the excretion of endogenous cellulases or hemicellulases. The utilized wood is grinded by termite mandibles to particles < 50 μm in size that would increase the surface area and, therefore, the accessibility to hydrolyzing enzymes. In lower termites, wood particles are endocytosed by the archaezoa. The passage of the wood particles through the digestive tract of a wood-feeding termite only takes < 24 h, which is a more efficient bioconversion in degrading lignocellulose than wood-rotten fungi. Cellulose and hemicellulose related enzyme systems are identified either from the termite or from its gut symbionts. The symbiosis between termites and the gut flora can be described as a synergistic interaction of termite and microbial origin enzymes. Sequencing work on termite or its symbionts’ genes associated with cellulolytic activities has already shown a promising future. In this presentation, an outlook is made for the present progress in understanding termite digestive systems and particularly how we can possibly, with research on wood-feeding termites, harness the pathways towards biofuel applications.

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**Poster 2-08**

**Regulation of Pyruvate Metabolism Induced by End Product Loading in Clostridium thermocellum 27405**

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Clostridium thermocellum 27405 is a fermentative thermophile producing hydrogen and ethanol, along with CO2, acetate, formate and lactate from cellulosic biomass. Based on its genomic sequence, pyruvate serves as a common intermediate for end-product formation. The genome sequence also predicts specific genes whose expression may be required. We can verify how the various branches of pyruvate metabolism might be regulated by following changes in growth, end product synthesis, activities of enzymes involved in pyruvate metabolism, and corresponding mRNA levels (using quantitative PCR) of corresponding putative genes under different growth conditions. This is illustrated by describing the effects of additions of higher concentrations of single end products to the medium at the beginning of growth in batch cultures with 1.1g/l cellulose as growth substrate. Such additions had little effect on growth. However, changes in concentrations of other end products were observed: H2 and acetate production increased with ethanol or lactate addition, formate increased with H2 addition, and ethanol increased with acetate addition. Pyruvate:fd oxidoreductase, hydrogenase (MV, NAD+, and NADP+ dependant), lactate dehydrogenase, alcohol dehydrogenase (NADH and NADPH dependant), and aldehyde dehydrogenase activities were observed in extracts of exponentially growing cells. Addition of individual end products did not affect the levels of enzymes activities assayed, except for hydrogenase, which increased in cells grown with added of H2 or lactate. Levels of mRNA for alcohol dehydrogenase adhE (gene 423), NiFe-hydrogenase (gene 3010), Fe-hydrogenase (gene 430) and pyruvate:ferredoxin oxidoreductase (gene 2796), determined by Q-PCR varied depending on the presence of specific added end products.
**Poster 2-09**  
**Miscanthus giganteus cell wall degradation by Neurospora crassa**  
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Neurospora crassa is a filamentous ascomycete fungus which has been a model organism for understanding gene regulation and function for over 80 years. Biochemical and molecular tools are well developed with this organism and include a complete genome sequence (Galanagan et al., Nature 2003), full genome microarrays and ~5,000 single gene deletion strains; ~8,000 mutants will be available by 2009 (http://www.dartmouth.edu/~neurosporagenome/). In nature, N. crassa grows on burnt and composted plant material, including sugarcane. It has been known for over 30 years that N. crassa has the capability of producing lignocellulolytic enzymes, which can degrade both lignin and cellulose. Miscanthus giganteus has been designated as a promising crop for the development of plant-derived biofuels by the Department of Energy. Computational predictions and transcriptional profiling data show that at least 509 genes in the Neurospora genome encode proteins that are predicted to be secreted; this dataset is enriched for lignin degradation enzymes and cell wall deconstruction enzymes such as cellulases. N. crassa can grow on Miscanthus as a sole carbon source. To define the global regulatory mechanisms associated with Miscanthus cell wall deconstruction by N. crassa, we will perform comprehensive time-course of gene expression patterns using transcriptional profiling and full genome microarrays for N. crassa.

**Poster 2-10**  
**Development of efficient Escherichia coli cells with minimal metabolic functionality for ethanol production**  
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For the economic production of biofuels it is important that the available feedstock is converted into the product in the most efficient way. An efficient E. coli strain with minimal metabolic functionality for best ethanol production was rationally designed using elementary mode analysis. The entire solution space of more than 15,000 genetically independent pathways of the E. coli central metabolism identified by elementary mode analysis was reduced to less than ten most efficient ethanol producing pathways by elimination of inefficient pathways with gene knockout mutations. In the remaining efficient pathways a tight coupling of cell growth and ethanol production is enforced. The constructed mutant strain is able to efficiently convert hexoses and pentoses into ethanol at high yields. Moreover, the mutant does not exhibit catabolite repression by glucose and is able to simultaneously utilize hexoses and pentoses at similar rates resulting in favorable production kinetics. In addition, the constructed mutant is also able to efficiently convert at high yield glycerol, an abundant and inexpensive biodiesel side product, into ethanol.

**Poster 2-11**  
**Impact of inoculum production conditions on stress tolerance and fermentation efficiency of natural xylose-fermenting yeasts presented xylose and glucose**  
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Efficient fermentation processes to produce ethanol from low-cost lignocellulosic biomass are sought to support the expansion of the biofuels industry. Stress-tolerant microorganisms are needed that are able to consume both hexose and pentose sugars and also withstand, survive, and function in the presence of stress factors common to fermentations of lignocellulosic hydrolysates, including inhibitors such as furfural and hydroxymethylfurfural (HMF) and high concentrations of mixed sugars and ethanol. Data will be presented showing that nitrogen and carbon source composition and culture physiological state significantly impact the ability of Pichia stipitis to survive and detoxify furan inhibitors and to convert high xylose concentrations efficiently to ethanol. The utility of priming inocula with high xylose concentrations to induce faster fermentation rates in ethanol production fermentors and to eliminate diauxic lag during mixed sugar conversion was observed for P. stipitis NRRL Y-7124 as well as the more acid-tolerant Pachysolen tannophilus Y-2460. Implications of these findings on process-based strategies to produce a tolerant initial population and then to foster and maintain an efficient viable population during subsequent ethanol fermentation are considered.

**Poster 2-12**  
**Withdrawn**

**Poster 2-13**  
**Evaluation of thiamine-synthesis pathway and gluconolactonase deficient mutants of Zymomonas mobilis for overproduction of higher value intermediates**  
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In addition to studies on ethanol production using Z. mobilis, research on this bacterium has also focused on the production of higher value metabolites via the engineering of its central metabolic pathways. As examples, the present study aims to overproduce gluconolactone and pyruvate from Z. mobilis ZM4 using a gluconolactonase deficient mutant (gln-) and a thiamine-synthesis pathway deficient mutant (thiD-) respectively. Studies have shown that wild type ZM4 can grow on synthetic medium in the absence of thiamine, thereby confirming the presence of a thiamine producing pathway in Z. mobilis. These mutant strains were created via homologous recombination with the transfer of cat gene encoding chloramphenicol acetyl transferase into the glnD and thiD sites of its genome using the vectors pGEM::cat and pGEM::thiD::cat respectively. Enzymatic assays on both mutants confirmed the loss of gluconolactonase and hydroxymethylpyrimidine/ phosphomethylpyrimidine kinase activities. The analyses showed that the both gln- and thiD- mutants resulted in no detrimental effect on the cell growth or ethanol production in complex media. Following the fermentation studies, the biomass of gln- was collected and permeabilised to investigate the biotransformation of glucose into gluconolactone. Preliminary results of the biotransformation studies using gln- mutant showed some gluconolactone accumulation within the system. Studies on the thiamine dependent mutant (thiD-) strain showed that the expression of pyruvate decarboxylase, the thiamine-dependent enzyme in the ethanol metabolism, could be regulated with low level concentrations of thiamine supplementation in synthetic media. This presentation will further discuss characteristics of both mutant strains for various biotransformation/fermentation conditions.

**Poster 2-14**  
**Fermentation of biomass derived glucose/xylose mixture to ethanol using several strains of Pichia stipitis**  
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The performance of four different strains of Pichia stipitis, CBS 6054, FPL-061, FPL-DX26 and FPL-SH121, engineered for improved ethanol fermentation from C6 and C5 carbon sugars, were evaluated for production of ethanol from a biomass derived glucose/xylose mixture. Distilled dried grains and solubles (DDGS) were used as a feedstock and pretreated using Ammonia fiber expansion (AFEX). The monomeric sugars were generated in two ways: i) use of an enzyme cocktail containing cellulase and hemicellulase, and ii) hydrolysis with cellulase enzyme followed by mild acid treatment. Both processes generated streams that contained 5-carbon and 6-carbon sugars. Ethanol production by the described Pichia stipitis strains was monitored and compared to fermentations using clean, synthetic sugars. The productivity and ethanol yields seen in these fermentations will be discussed in view of using Pichia for the industrial production of ethanol.
Succinate is produced petrochemically from butane through maleic anhydride to satisfy a specialty chemical market, for uses as surfactant, ion chelator, food additive, and pharmaceutical ingredient. While succinate is produced petrochemically from butane through maleic anhydride to satisfy a specialty chemical market, for uses as surfactant, ion chelator, food additive, and pharmaceutical ingredient. Although lactic acid bacteria usually dominate yeast was instead Dekkera bruxellensis, together with a high number of lactic acid bacteria, almost exclusively Lactobacillus vini. D. bruxellensis is a common contaminant in the wine industry, but is also a production organism in the brewing of Lambic beer and in the generation of sour dough. Although lactic acid bacteria usually are unwanted in ethanol fermentations, they did not seem to affect the process in a negative way. Accordingly, the number of yeast cells was highest when the number of lactic acid bacteria was highest, only low levels of non-desired side-products were detected and the ethanol productivity was in the normal range. D. bruxellensis, together with L. vini, can thus be regarded as ethanol production organisms (patent pending).

**Poster 2-16**

**Dekkera bruxellensis and Lactobacillus vini – a new stable consortium for industrial bioethanol production**

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The yeast and lactic acid bacteria populations in an industrial ethanol fermentation process were investigated using PCR-fingerprinting and rDNA sequencing. This process had originally been inoculated with Saccharomyces cerevisiae. However, in the stably running process, the dominating yeast was instead Dekkera bruxellensis, together with a high number of lactic acid bacteria, almost exclusively Lactobacillus vini. D. bruxellensis is a common contaminant in the wine industry, but is also a production organism in the brewing of Lambic beer and in the generation of sour dough. Although lactic acid bacteria usually are unwanted in ethanol fermentations, they did not seem to affect the process in a negative way. Accordingly, the number of yeast cells was highest when the number of lactic acid bacteria was highest, only low levels of non-desired side-products were detected and the ethanol productivity was in the normal range. D. bruxellensis, together with L. vini, can thus be regarded as ethanol production organisms (patent pending).

**Poster 2-15**

**Engineering Actinobacillus succinogenes for succinate production**

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Succinate is produced petrochemically from butane through maleic anhydride to satisfy a specialty chemical market, for uses as surfactant, ion chelator, food additive, and pharmaceutical ingredient. While this specialty chemical market is relatively small at 15,000 t/yr in the US, the development of bio-based succinate production is targeting a much larger commodity chemical market (i.e., 270,000 t/yr) to produce bulk chemicals such as 1,4-butanediol, tetrahydrofuran, and γ-butyrolactone. Gram-negative Actinobacillus succinogenes produces among the highest succinate levels ever reported (up to 110 g/l for some mutants), making it a natural choice for industrial succinate production. We used metabolic flux analysis studies to identify the main sources of NADPH in the cell and the nodes for flux distribution between succinate and alternative fermentation products in glucose-grown cultures. Using the same approach, we identified the mechanisms by which A. succinogenes maintains a constant growth rate and biomass yield when redox demands are changed in the presence of different NaHCO3 and H2 concentrations. A. succinogenes grows on D-glucose, cellobiose, D-xylene, D-mannose, and L-arabinose, the most abundant sugars present in cellulose and hemicellulose, and it can ferment multiple carbon sources simultaneously. These features suggest that we can develop a lignocellulosic-based succinate production process using A. succinogenes. We are evolving A. succinogenes to grow faster on individual lignocellulosic sugars. Growth parameters and fermentation balances are then compared on the different sugars in evolved and non-evolved strains. Follow-up studies will involve sugar mixtures and corn stover lignocellulosic hydrolysates.

**Poster 2-17**

**Application of Pichia pastoris for the production of biochemicals: production of riboflavin as a test case**

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We present a new application for the well known expression system Pichia pastoris. Up to now P. pastoris has been used extensively and successfully for the expression of heterologous recombinant proteins. With the availability of the P. pastoris genome sequence a huge field of possibilities has arisen. Combining the knowledge about this excellent protein and enzyme producer with the information about the metabolic pathways derived from the genome sequence we have been able to take the first steps towards a whole cell biocatalyst P. pastoris system. For testing this new strategy we chose an already known pathway for the application in white biotech: the riboflavin synthesis pathway.

To overcome the obstacle of the tight regulation of the genes within the riboflavin pathway on the transcription level the native promoter regions were replaced by the strong constitutive promoter of the glyceraldehyde-3-phosphate dehydrogenase gene (GAP). These promoter replacements had to be done stepwise following the pathway from the precursors GTP and ribulose-5-phosphate to the final product riboflavin. Already the first deregulation turned P. pastoris to a so called flavinogenic yeast. The more the pathway got deregulated the more riboflavin accumulated in the cells and also in the culture supernatants. Based on these results, developments are ongoing to establish a P. pastoris system for whole cell biocatalysis.

**Poster 2-18**

**Strain engineering enables the conversion of inexpensive raw materials into a variety of higher-value products via a single uniform fermentation process**

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Surfactants are one of the most widely used categories of chemicals. They are found in nearly every formulation marketed today including food, personal care items, paints, plastics and laundry detergent. Most surfactants are derived from petroleum. However, the observation that certain microorganisms, such as Bacillus subtilis, naturally secrete surfactants creates the potential for production of surfactants from biorenewable resources on an industrial scale via fermentation. We have used gene engineering to produce Bacillus strains that secrete novel surfactants with particular desired properties, such as increase water solubility, demonstrating that strain engineering can be used to produce diverse surfactants tailored to particular end-use applications. Although each engineered strain produces a particular novel surfactant, the strains are otherwise identical. All strains are grown using the same culture conditions and the same growth media, which is derived from inexpensive feedstocks such as crude glycerol, hydrolyzed soy protein and distillers grains. These data demonstrate that strain engineering enables the conversion of inexpensive raw materials into a variety of higher-value products via a single uniform fermentation process.
Figure1. Picture shows a comparison of transformation plates between C. acetobutylicum 824 (pJ-GN), A, and control 824 (pJIR), B. C. acetobutylicum 824 (pJ-GN) secretes high amount of riboflavin into the agar plate to form a dark yellow color while 824 (pJIR) which express ribA, a dual function gene in the riboflavin operon, was placed on the above shuttle vectors to yield pJ-GRA and pJ-GNA. Riboflavin and butanol production differences were examined in the various recombinant strains.

Poster 2-20
Expression analysis during fermentation of xylose and cellubiose by yeasts
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Fermentation is largely a physiological balancing act. During the process one portion of a substrate is oxidized while another portion is reduced. Moreover, fermentation is the result of concerted activities among many different enzymes rather than the product of one or two. Overall metabolic flux is rarely determined by the activity of any one enzyme in a pathway, and activities of multiple enzymes often interact. It should not be surprising, therefore, that the engineering of one or two or even a few enzymes in a metabolic pathway does not approach an optimum. Our laboratory has developed techniques for balancing the activities of multiple enzymes in the pathway for yeast xylose fermentations, for discovering novel activities that contribute to improved fermentation rates and for predicting steps that could be rate limiting. In engineered Saccharomyces cerevisiae and Pichia stipitis, the balance between oxidative and reductive activities can determine the flux of xylose to either xylitol or ethanol. The overall, flux, however, is generally determined by the activities of “gateway” enzymes such as kinases or transporters. The required balance of activities can be predicted from global expression analysis of cells with various genetic backgrounds cultivated under different growth conditions. This presentation will review the transcriptional and physiological responses of native and engineered xylose and cellubiose fermenting yeasts during ethanol production.

Poster 2-21
Fermentation Behaviors of Hypocrea jecorina RUT C-30 for Cellulase Production Using Lactose as Substrate
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Lactose is recognized as an inexpensive, soluble substrate that has reasonably good inducing capability for cellulase production by Hypocrea jecorina. The current knowledge suggests that the fungal species does not directly uptake lactose. Instead, lactose is hydrolyzed by extracellular enzymes, lactase and cellulase components, to glucose and galactose for subsequent microbial ingestion. The fermentation behaviors of H. jecorina grown on lactose have not been mechanistically investigated or modeled to consider this critical extracellular hydrolysis step. In this study, H. jecorina RUT C-30 was grown in both batch and continuous culture systems using lactose, lactose and glycerol mixture, glucose, galactose, as well as glucose and galactose mixtures as the substrate. The concentrations of sugar compounds and the activities of lactase and cellulase enzymes were measured along with the cell concentration. Glucose was found to repress the galactose consumption. The continuous culture results were used to calculate the lactose hydrolysis rate and the specific production rates of cellulase and lactase. The lactose hydrolysis rates were used to evaluate the kinetic contributions of lactase and cellulase to the hydrolysis. The specific production rates of cellulase and lactase were used to establish the effects of dilution rate and different sugar concentrations on the enzyme synthesis. A model was then developed to incorporate these relationships with the cell growth and substrate consumption kinetics to describe the overall fermentation behaviors. The experimental results were used in model fitting to generate a set of best-fit model parameters. The study provided significant conceptual and quantitative insights to the lactose metabolism and cellulase production by H. jecorina.

Poster 2-22
Effects of overexpression of NADPH-regenerating glucose 6-phosphate dehydrogenase on caprolactone production in recombinant Escherichia coli harboring cyclohexanone monooxygenase gene
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Caprolactone is an important intermediate compound for production of medical devices, food packages and bio-degradable plastics. As cyclohexanone monooxygenase (CHMO) converts cyclohexanone to caprolactone using NADPH, NADPH might be a critical factor for enhanced production of caprolactone. Whole-cell conversion of cyclohexanone to caprolactone was attempted by recombinant Escherichia coli BL21 (DE3) expressing cyclohexanone monooxygenase (CHMO) of Acinetobacter calcoaceticus NCIMB 9871. High concentrations of cyclohexanone and caprolactone reduced CHMO-mediated bioconversion of cyclohexanone to caprolactone in the recombinant E. coli cells. The metabolically active cells were employed by adopting a fed-batch culture to improve the production of caprolactone from cyclohexanone. A glucose-limited fed-batch Baeyer Villiger oxidation where a cyclohexanone level was maintained less than 6 g/L resulted in a maximum caprolactone concentration of 11.0 g/L. The maximum caprolactone concentration was improved further to 15.3 g/L by coexpression of glucose-6-phosphate dehydrogenase, an NADPH-generating enzyme encoded by the zwf gene which corresponded to a 99% enhancement in caprolactone concentration compared with the control experiment performed under the same conditions.
**Poster 2-25**

Using systems biology to optimize microbial fuel production

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Microbial production of biofuels often places many different burdens upon the cell, and understanding these burdens is critical to ensure the generation of a robust, industrially useful strain. The past two decades have seen an explosion in the amount of cell-wide data being produced, including microarray, proteomics, and metabolite analysis. Industrially applicable strains such as *Escherichia coli*, *Bacillus subtilis*, and *Clostridium* and *Zymomonas* species have been especially well studied. While few of these studies have actively focused on biofuels production, many experiments have been conducted on related issues that are sure to impact biofuels production, such as the metabolic burden of exogenous genes, environmental stress response and adaptation, stationary phase response, and more. In order to capitalize on research that has already been completed, a review of literature has been conducted. We present many common trends between these different studies, and highlight considerations that should serve as a starting point for any microbial biofuels production platform.

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**Poster 2-26**

Elucidating mechanisms of acetate tolerance in *E. coli* using SCALES

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Creating biofuels from hemicellulosic and cellulosic biomass is an important part in transitioning away from the petroleum-based transportation fuel economy. Acetate is a major toxic side product of the requisite pretreatment steps of the feedstock to the microorganisms producing biofuels. Conferring tolerance of acetate upon the microorganism will increase the viability of the biofuels economy. The SCalar Analysis of Library Enrichments (SCALEs) method, created previously by the Gill lab at the University of Colorado, combines a traditional genomic library selection with DNA microarray technology providing genotypic data for selected clones. The method employs multiple libraries of different, but defined, insert sizes to allow greater resolution of beneficial genomic regions. The objective is to find mechanisms of acetate inhibition on *E. coli* growth. These mechanisms can be elucidated using SCALEs. Sections of the genome that confer acetate tolerance, when over-expressed, will be found in a SCALEs selection. This knowledge shows specifically where in the metabolism acetate inhibiting.

We report here a library selection using an *E. coli* K12 library using a 1.75 g/l acetate culture titrated to a pH of ~7.0 with potassium hydroxide. The culture was monitored over a three day period with samples taken at regular intervals. 75% of selected clones show an increase in growth rate of greater than 5% against control. The greatest increase was over 15%. Regions in the genome most overexpressed after the selection fall into the general categories of genes encoding transport proteins, nucleotide synthesis genes, and shock genes.

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**Poster 2-27**

Butanol Tolerance in a Selection of Microorganisms

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As an alternative liquid fuel, butanol offers distinct advantages because of its high energy content, miscibility with gasoline, octane-improving power, and low volatility. With the increasing price of oil, there is renewed interest in producing butanol biologically. Butanol can be produced from anaerobic bacterial (*Clostridia*) fermentations in a process that also produces acetone and ethanol ("ABE" fermentation) but suffers from low yield and productivity. Due to sensitivity to butanol and extremely complex regulatory pathways involved in switching from acidogenesis to solventogenesis in *Clostridia*, it has been difficult to make significant progress in engineering highly productive strains. An alternative strategy would be to establish the butanol production pathway in an alternative host lacking these complex regulatory pathways. However, an important consideration in selecting a host for butanol production is butanol tolerance. We conducted a screening of a variety of microorganisms known to be easily engineered to sample the level of butanol tolerance presently available in typical laboratory organisms. Currently this information is lacking in the literature but is crucial for the future engineering of highly productive butanol producing microorganisms. We present butanol tolerance data in the form of growth rates for strains of *Escherichia coli*, *Saccharomyces cerevisiae*, *Zymomonas mobilis*, *Lactobacillus sp.*, *Clostridium sp.*, and Non-*Saccharomyces* yeast species. The results show that varying levels of tolerance to butanol exist in wild type organisms.

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**Poster 2-28**

Effect of carbon dioxide on propionic acid productivity from glycerol by *Propionibacterium acidipropionici*

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With the increased production of biodiesels, large amounts of glycerol are produced as a byproduct with limited use, causing a significant environmental problem. It is thus desirable to use this waste glycerol as a renewable feedstock to produce industrial chemicals and fuels to replace fossil fuels and petrochemicals. Our previous study indicates that *Propionibacterium acidipropionici* can convert glycerol to propionic acid at high yield and high purity, but with a very low productivity. In this work, the effect of carbon dioxide on the propionic acid productivity was studied. Based on the metabolic analysis, CO₂ (HCO₃⁻) is required in the Wood-Werkman cycle which determines the propionic acid synthesis. Carbon dioxide, with phosphoenolpyruvate (PEP) is converted to oxalacetate by the enzyme phosphoenolpyruvate carboxylase. Through several sequential reactions, oxalacetate is finally converted to propionic acid. It is found that the productivity of propionic acid with CO₂ (HCO₃⁻) is 2.94 g/l/day, which is much higher than that without CO₂ (HCO₃⁻) (1.56 g/l/day). However, the yield of propionic acid is decreased slightly from 0.77 to 0.67 g/g glycerol due to the higher biomass production. In addition, the yield of productivity of succinate, the main intermediate in Wood-Werkman cycle, is increased by 81% and 280%, respectively. These results imply the increase in the Wood-Werkman cycle rate because of the addition of CO₂ (HCO₃⁻). Thus, the activities of key enzymes (e.g. phosphoenolpyruvate carboxylase, oxaloacetate transcarboxylase, and propionyl CoA transferase) involved in the Wood-Werkman cycle were also studied in this work.

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**Poster 2-29**

Enhanced Efficiency of BioEthanol Production with Novel Yeast

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We studied three naturally occurring yeast strains isolated from poplar trees that have several unique properties beneficial to the bioenergy industry. The poplar yeast strains can utilize both 5-carbon and 6-carbon sugars while the conventional strain cannot. Also, the poplar strain is inhibited by the phytochemicals released in the process. We also found that the poplar yeast strains can grow in medium lacking ammonium and nitrate. Therefore, culturing of these microorganisms will have a lower cost than conventional strains of *S. cerevisiae*. The genome of one of the strains is currently being sequenced by the Joint Genome Institute.
A thermostable alcohol dehydrogenase (ADH-I) isolated from the potential thermophilic ethanologen *Geobacillus thermoglucosidasius* strain M10EXG has been characterized. Inverse PCR showed that the gene (*adhI*) was localised with 3-hexulose-6-phosphate synthase (HPS) and 6-phospho-3-hexuloseisomerase (PHI) on its genome. The deduced peptide sequence of the 1,020-bp M10EXG *adhI*, which corresponds to 340 amino acids, shows 96% and 89% similarity to ADH-Ht and ADH-T from *Geobacillus stearothermophilus* strains LLD-R and NCA 1503, respectively. Over-expression of M10EXG ADH-I in *E. coli* DH5 (pNF303) was confirmed using an ADH activity assay and SDS-PAGE analysis. The specific ADH activity in the extract from this recombinant strain was 9.7 (±0.3) U mg⁻¹ protein, compared to 0.1 (±0.01) U mg⁻¹ protein in the control strain. The recombinant *E. coli* showed enzymatic activity towards ethanol, 1-butanol, 1-pentanol, 1-heptanol, 1-hexanol, 1-octanol and 2-propanol, but not methanol. *In silico* analysis, including phylogenetic reconstruction and protein modelling, confirmed that the thermostable enzyme from *G. thermoglucosidasius* is likely to belong to the NAD-2n-dependent family of alcohol dehydrogenases.

**Poster 2-32**

**Carbon source-dependent lactate formation during biohydrogen production**

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Hydrogen is considered one of the potential candidates as an alternative future energy carrier. Microorganisms offer a sustainable, environmentally friendly route for producing the so-called “biohydrogen”. This is simply formed as a by-product during carbohydrate fermentation by heterotrophic anaerobes. Producing the maximum yield of hydrogen necessitates that minimum or no reduced end products, such as lactate or propionate, are formed due to the accompanying loss of some of the reducing power of pyruvate. In our study, we found that the extent of lactate formation, and consequently hydrogen yield, in certain extreme thermophiles of the genus *Caldicellulosiruptor* is greatly dependent on the nature of the carbon source fermented. In addition, the growth phase of the organism as well as the partial hydrogen pressure (P(H2)) in the bioreactor is strongly involved. Comparative fermentations were carried out in stirred-tank reactors using different carbon sources, including glucose, fructose, xylose and sucrose. Metabolite formation patterns together with the theoretical energy gain of the cells on different sugars. This work aims at a deeper understanding of the physiology behind biohydrogen production that should benefit the application of metabolic engineering in that field.

**Poster 2-33**

**Volatilization of mercury in coal via the mercury reductase operon in Acidithiobacillus ferrooxidans, ATCC 53993**

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The mercury reductase genes in the organism *Acidithiobacillus ferrooxidans*, strain ATCC 53993, were identified using the genome sequence completed in 2006. The strain was found to be capable of reduction of Hg²⁺ to elemental mercury and tolerant to high concentrations of Hg²⁺. The mercury reductase operon was constructed by identifying homologous genes involved in known mercury transport and conversion pathways. The ability of the strain to volatilize mercury was applied to removal of mercury from coal using a single step biological process. The process included dissolution of pyritic minerals from the coal, release of mercuric species from the coal into the aqueous phase, volatilization of the Hg²⁺ to elemental mercury, followed by its removal from the coal slurry by air sparging.

**Poster 2-30**

**Heterologous expression of the alcohol dehydrogenase (adhI) gene from Geobacillus thermoglucosidasius strain M10EXG**

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A thermostable alcohol dehydrogenase (ADH-I) isolated from the potential thermophilic ethanologen *Geobacillus thermoglucosidasius* strain M10EXG has been characterized. Inverse PCR showed that the gene (*adhI*) was localised with 3-hexulose-6-phosphate synthase (HPS) and 6-phospho-3-hexuloisomerase (PHI) on its genome. The deduced peptide sequence of the 1,020-bp M10EXG *adhI*, which corresponds to 340 amino acids, shows 96% and 89% similarity to ADH-Ht and ADH-T from *Geobacillus stearothermophilus* strains LLD-R and NCA 1503, respectively. Over-expression of M10EXG ADH-I in *E. coli* DH5 (pNF303) was confirmed using an ADH activity assay and SDS-PAGE analysis. The specific ADH activity in the extract from this recombinant strain was 9.7 (±0.3) U mg⁻¹ protein, compared to 0.1 (±0.01) U mg⁻¹ protein in the control strain. The recombinant *E. coli* showed enzymatic activity towards ethanol, 1-butanol, 1-pentanol, 1-heptanol, 1-hexanol, 1-octanol and 2-propanol, but not methanol. *In silico* analysis, including phylogenetic reconstruction and protein modelling, confirmed that the thermostable enzyme from *G. thermoglucosidasius* is likely to belong to the NAD-2n-dependent family of alcohol dehydrogenases.

**Poster 2-31**

**Strategies for Increasing Fermentative Hydrogen Yield from Sugars**

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Hydrogen is an attractive clean fuel that can be produced biologically either from water or by fermentation of organic compounds. Although organic acids are completely fermented to H₂ and CO₂ by photosynthetic organisms, need for light in this process makes this uneconomical for commercial production of H₂. Fermentation of sugars to hydrogen is fraught with lower yields that are less than the theoretical maximum of 4 H₂ per glucose. To increase the H₂ yield, we are developing a new pathway that couples NADH oxidation to H₂ production. NADH-ferredoxin oxidoreductase (NFOR) from the hydrogenosome of an anaerobic protozoan was cloned and expressed in Escherichia coli as the first enzyme of this pathway. The recombinant enzyme was purified as a heterodimer (46,600 and 25,200 Da). The holoenzyme had a [2Fe-2S] cluster that was localized in the small subunit. Low temperature EPR spectra of dithionite reduced active holoenzyme had a [2Fe-2S] cluster that was localized in the small subunit. Low temperature EPR spectra of dithionite reduced active holoenzyme had a [2Fe-2S] cluster that was localized in the small subunit. Low temperature EPR spectra of dithionite reduced active holoenzyme had a [2Fe-2S] cluster that was localized in the small subunit.
Nowadays, new research strategies have been considered for the use of banana as adjunct or industrial supplement in different processes. Banana, an important component in the diet of the global population, is one of the fruits most consumed in the world. Besides, banana is very favorable to industrial processes (e.g., alcoholic fermentation) due to its richness in soluble solids and minerals, with low acidity. The main objective of this work was to evaluate the influence of factors as banana mass and extraction time during an aseptic hot extraction process (84ºC) of total soluble solids from banana. Thus, a 2⁵ full-factorial star design was carried out and a model was developed to describe the behavior of the dependent variable (total soluble solids, Y) as a function of the factors (17-35 g banana mass, X₁; and 30-60 min extraction time, X₂). The experiments were performed with 105 mL of water, considering the moisture of the ripe banana (65%). Total sugars concentrations were obtained and the results expressed in Plato (ºP, which is the weight of the extract or the sugar equivalent in 100 g solution, 20ºC), in order to facilitate their use by the beverage industry. The resulting model equation was: Y = 5.58 + 1.25X₁ - 0.26X₁², with which it was possible to obtain the optimized extraction conditions of total soluble solids from banana as being: 38.5 g banana mass and 39.7 min of extraction time.

**Acknowledgements:** CAPES and FAPESP/Brasil; FCT and GRICES/Portugal

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**Poster 2-36**
Applicability of Supercritical Technology for Structural Separate of Sugarcane Bagasse to Microbial Use

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At the moment the demand by renewable sources of energy has fomented the use of vegetal residues of diverse nature due to its large and increasing availability in tropical countries. Within innumerable agro-industrial by-products in Brazil, the sugarcane bagasse is the one that presents greater potential due to the great volumes generated by the industry of sugar and alcohol. The best option for its most potential use is using biotechnology techniques to obtain products with great value, for this a previously division of its structure is necessary, because the microorganisms only use carbon sources from short chain and free lignin. There are different processes to separate its structural compounds, but still there is not one with good results about time, effectiveness and costs together. A possible and potential process is using an extractor with supercritical CO2, due this fluid presents diverses advantages. This preliminary study had as aim to evaluate the potentiality of the use of the supercritical technology like assistant in the division of the structure of the sugarcane bagasse, to later use the sugars from the cellulose and hemicellulose using microorganisms. The results demonstrated that using 50% of supercritical CO₂, 50% of ethanol (75% v/v), like modifier, 220 bar, 150º C and total time of reaction of 40 minutes it was possible to extract 91.6% of the lignin from bagasse. The preliminary results conclude that the great potentiality of the use of the supercritical technology to microbial use the sugars of bagasse.

**Acknowledgements:** University of São Paulo, University of Chile

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**Poster 2-37**
Production of higher alcohols, esters, and other potential biofuel compounds by non-Saccharomyces yeast species

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The yeast species Saccharomyces cerevisiae has been used for millennia to convert sugars to ethanol. Over 1,000 other yeast species are known, though only a limited number are used for production of platform chemicals. We have examined a series of non-Saccharomyces species for production of commercially important compounds including potential biofuels. The Phaff Yeast Culture Collection at the University of California Davis contains thousands of yeast strains isolated from a variety of degrading plant materials. In a preliminary screening study, we have identified and quantified volatile compounds produced by 25 different species. Samples of volatiles in the headspace above duplicate 4-day liquid cultures grown in standard lab media were extracted using Solid Phase Microextraction (SPME) with DVB/ CAR/PDMS (divinylbenzene/carboxen/polydimethylsiloxane) fibers followed by GC-MS analysis. Numerous compounds were identified in each sample by comparison with the retention time and mass spectrum of authentic standards. Selected compounds were quantified, including isoamyl alcohol, isobutanol, isobutyl acetate, phenethyl acetate, and phenethyl alcohol. Each yeast species produced a unique and predictable combination of volatile compounds, including alcohols, esters, aldehydes, and ketones. While isoamyl alcohol was produced by most cultures at trace levels, one yeast species produced over 0.5% (w/v). Isobutanol was also detected in trace quantities in the headspace above all cultures, but was produced at a level of over 0.5% (w/v) by two yeast species. Future plans include screening a large number of yeast species to identify those that produce the highest levels of potential biofuel compounds such as higher alcohols and esters.
Posters

Poster 2-38
In vitro $^{13}$C labeling for simultaneous identification and quantification of central carbon intermediates in genetically modified Saccharomyces yeast capable of glucose/xylose co-fermentation using reverse phase liquid chromatography-mass spectrometry (RP-LC-MS) to perform this task is difficult because metabolite diversity in biological samples prevent accurate RP-LC separation. Furthermore, precise metabolite quantization is very difficult due to the lack of commercially available stable isotope-coded standards. A new MS quantitation method, named Global Isotope-labeled Internal Standard (GILISA) was developed for quantitative analysis. Through the use of anilene-$^{13}$C$_2$-labeled internal standards, accurate MS quantification could be readily achieved. Thirty-two common metabolites from yeast lysate involved in glycolysis, the pentose phosphate pathway, and tricarboxylic acid cycle were unambiguously identified and quantified with relative standard deviations smaller than 10%. This method provides a convenient, robust, and high throughput tool for intracellular metabolite analysis. This paper reports the use of this newly developed GILISA method to indentify metabolic bottlenecks in genetically engineered S. cerevisiae 424A(LNH-ST) yeast capable of effectively co-fermenting glucose and xylose to ethanol.

Poster 2-39
Conversion of aliphatic organic acids into di-acids with recombinant Escherichia coli

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Here we present current results of a whole-cell bio catalytic conversion of aliphatic organic acids into di-acids with a recombinant E. coli strain expressing a monooxygenase from Pseudomonas putida.

It has been shown that alkanes are readily oxidised to carboxylic acids by recombinant E. coli expressing a monooxygenase from P. putida. However, the produced acids are generally toxic for the biocatalyst, limiting the product accumulation significantly. There are hints in the literature that P. putida is able to further convert the monocarboxylic acids into less toxic di-acids under certain circumstances.

We determined the ability of recombinant E. coli to oxidise toxic aliphatic organic acids to less toxic di-acids, allowing for example the production of adipic acid from hexanoic acid.

The conditions for the conversion are optimised, giving special emphasis to the regulation of the recombinant operon.

References:

Poster 2-40
Biomonitoring of biosurfactant production by cultivation of green fluorescent protein-marked Bacillus subtilis W1012

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Biosurfactant production was investigated using two strains of Bacillus subtilis, one being a reference strain (B. subtilis 1012) and the other a recombinant of this (B. subtilis W1012) made able to produce the green fluorescent protein (GFP). The results of batch cultures carried out at different initial levels of glucose in the presence of 10 g/L casein demonstrated that the reference strain was able to release higher levels of biosurfactants in the medium at G = 5.0 g/L ($\beta_{max} = 84-110$ mg/L). The recombinant strain exhibited interesting levels of biosurfactants ($\beta_{max} = 90-104$ mg/L) only at higher glucose concentrations ($G > 20$ g/L). Under these nutritional conditions, the fluorescence intensity linked to the production of GFP was shown to be associated with the cell concentration even after the achievement of the stationary phase. The ability of the genetically-modified strain to simultaneously overproduce biosurfactant and GFP even at low biomass concentration makes it an interesting candidate for use as a biological indicator to monitor cell viability either in bioremediation or oil recovery operations.

Poster 2-41
Screening microorganisms to biodegrade different classes of textile dyes solution and the reutilization of the wastewater

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The reutilization of the wastewater in a textile industry will represent an enormous save in terms of water consume considering that it is used 70-100 liter of water/kg of processed fabric material. If it would be possible to remove totally the dyes from the textile wastewater, this could be reuse in several others textile processes, promoting this way a save and friendly environmental use of water supply. The purpose of this study is screening microorganisms capable to biodegrade different classes of textile dyes solution and the reutilization of the wastewater. Different solutions of textile dyes classes were study in solid and liquid culture media, using Phanerochaete chrysosporium, Pleurotus ostreatus e Trametes versicolor to promote the biodegradation. After 7 days of incubation, the best result was obtained with Trametes versicolor in liquid culture with a 99.9% efficiency of the color biodegradation. The treated solution was used again in the textile dying process, using a 100% cotton fabric material pre-bleached and with 3 different dying solution (blue Procion H-EXL, yellow Procion H-EXL and red Procion Crimson H-EXL), according the manufacture recommendation (Dystar). Even though the culture media color interfered in the results, asking for 20% of dilution, the final DE value was 0,28%. Considering that the average rate of DE from the textile industry dying process can be up to 1,1, the results are very good in terms of color productively and specially excellent in terms of saving 80% of the good water by reusing the treated textile wastewater.
Zymomonas mobilis ZM4 Genome Analysis and Reannotation
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Recent interest in the large-scale production of bioethanol for transportation fuel has intensified the search for efficient fermentative microorganisms that produce ethanol. Zymomonas mobilis ZM4 is a promising candidate due to its productivity, high level of ethanol tolerance and its ability to be genetically manipulated. However, the main drawback to using wild-type Z. mobilis is its narrow substrate utilization range, which is limited to glucose, fructose and sucrose. The efficient conversion of lignocellulosic hydrolysates to ethanol requires high-yield ethanol production from most, if not all, available sugars and resistance to industrially relevant stress and inhibitors. We have proposed to elucidate the molecular basis for important process traits using systems biology tools and the completed genome sequence. In order to optimize these studies, we have updated the Z. mobilis ZM4 genome annotation. The reannotation identifies new genes, corrects gene starts, deletes incorrect genes, detects frame shifts, updates the protein functional annotation and identifies CRISPR (Clustered regularly interspaced short palindromic repeats) sequences, which have been shown to play a role in bacteriophage resistance in other systems. The corrections were identified via a new gene prediction algorithm, Prodigal, developed at Oak Ridge National Laboratory and experimental evidence. Two additional annotation tools, one that identifies and categorizes transporters, and a second that identifies and categorizes transporters, will provide important information not included in other annotation pipelines. The updated annotation will be critical in identifying genome features that can be modified to achieve optimal ethanol production.

Optimization of lactic acid production from cheese whey using Bifidobacterium longum
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Lactic acid is a natural organic acid and has many applications in the pharmaceutical, food, and chemical industries. It is used as an acidulant and preservative, and recently its potential as a substrate for the production of biodegradable plastic has been actively pursued. Lactic acid has been produced by fermentation of sugar-containing substrates including cheese whey using Lactobacillus helveticus, and Lactobacillus casei in most of the previous studies. Immobilization of cells was applied in most of these studies to improve the lactic acid yield. Bifidobacterium longum was used to produce lactic acid from cheese whey in this presentation. High lactic acid yield (0.82g/g) was obtained without immobilization of the cells. The fermentation conditions such as nutrient, pH, and temperature were optimized to obtain maximum lactic acid productivity. The results were also compared to that of other bacteria such as Lactobacillus helveticus.

Optimization of ethanol production using a synthetic equivalent to the liquid of the rind of green coconut
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The liquid of the green coconut shell (GCS), an effluent stream from the industrial processing of green coconut rind, is rich in sugars and is a suitable feedstock for fermentation. The purposes of this study were to optimize the production of ethanol using GCS synthetic as a basis for fermentation and to compare with real GCS. Fermentation was carried out in bench-scale bioreactors using Saccharomyces cerevisiae as inoculum, at a working volume of 5 L and using 0.30% of soy oil as antifoam. During fermentations, the effects of different initial sugars concentrations (10 - 20%), yeast concentrations (5 and 7.5%), temperatures (30 - 50°C) and agitation rates (400 and 500 rpm) on pH/sugars profiles and ethanol production were evaluated. The best conditions for ethanol conversion were (1) media containing 15% of sugar; (2) 7.5% yeast inoculum; (3) temperature set point of 40°C and (4) an agitation rate of 500 rpm, which resulted in an ethanol conversion rate of 98% after 6 hours of process. The conditions optimized with synthetic medium demonstrated to be a good model for work with GCS.

Fermentative hydrogen production by a mixed nitrogen-fixing bacterial culture
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Dark fermentation of carbohydrate-rich biomass by bacterial cultures is one approach for producing renewable H₂. In this work, a mixed bacterial culture capable of fermentative H₂ production from substrates lacking combined nitrogen was developed. Comparison of H₂ production from a glucose medium lacking combined nitrogen with and without N₂ confirmed the presence of nitrogen fixation. Yields of H₂ from this medium were as high as 240 mL/g (1.9 mol/mol) (per unit added glucose). The yield of combined nitrogen from glucose was 4.4 mg/g. Use of a common bioenergy crop, sugarcane, gave a hydrogen yield of 170 mL/g (7.5 mmol/g) (per g added volatile solids). A bioenergetic model for the overall bioconversion process was developed. The process described in this work could reduce or eliminate requirements for combined nitrogen addition, and improve the quality of effluent for subsequent bioconversion stages. For example, the organic acids produced via fermentation could be used to produce methane in a subsequent biomethanation stage.
Poster 2-46
Semi-continuous xylitol production from sugarcane bagasse hemicellulose by Ca-alginate entrapped yeast cells in a bench-scale stirred tank reactor

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In Brazil, much more ethanol would be obtained from the same amount of sugarcane if the sugar-alcohol mills used not only the sugarcane juice but also the sugarcane bagasse as a source of carbohydrates. Together with the conversion of hexose sugars into ethanol, the conversion of pentose sugars into value-added products could benefit the economics of using lignocellulosic sugars as raw materials. For instance, xylitol, a specialty sweetener widely used by food and pharmaceutical industries, could be produced from xylose, the major pentose sugar found in sugarcane bagasse hemicellulose. This sweetener is non and anticariogenic, can be consumed by diabetics and can replace antibiotics in the treatment of otitis, among other applications. In the present study, we report the operational stability of the semi-continuous xylitol production from sugarcane bagasse hemicellulose hydrolysate by Ca-alginate entrapped Candida guilliermondii cells during five successive fermentation batches carried out in a stirred tank reactor. The average volume of the Ca-alginate beads was reduced by about 30 % after the 600 h taken to perform out in a stirred tank reactor. The average volume of the Ca-alginate beads was reduced by about 30 % after the 600 h taken to perform out in a stirred tank reactor. The average volume of the Ca-alginate beads was reduced by about 30 % after the 600 h taken to perform out in a stirred tank reactor. The average volume of the Ca-alginate beads was reduced by about 30 % after the 600 h taken to perform out in a stirred tank reactor. The average volume of the Ca-alginate beads was reduced by about 30 % after the 600 h taken to perform out in a stirred tank reactor. The average volume of the Ca-alginate beads was reduced by about 30 % after the 600 h taken to perform out in a stirred tank reactor. The average volume of the Ca-alginate beads was reduced by about 30 % after the 600 h taken to perform out in a stirred tank reactor. The average volume of the Ca-alginate beads was reduced by about 30 % after the 600 h taken to perform out in a stirred tank reactor. The average volume of the Ca-alginate beads was reduced by about 30 % after the 600 h taken to perform out in a stirred tank reactor. The average volume of the Ca-alginate beads was reduced by about 30 % after the 600 h taken to perform out in a stirred tank reactor. The average volume of

Acknowledgements: Fapesp and CNPq

Poster 2-47
The influence of rice bran extract as nutrient on xylitol production

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The bioconversion of xylose into xylitol using rice bran concentration of 5 and 20 g/L was carried out to verify the influence of this supplement on Candida guilliermondii metabolism for xylitol production. Experiments were performed with synthetic medium and sugarcane bagasse hemicellulose hydrolysate treated with ion-exchange resins and by combining alteration of pH and adsorption with activated charcoal in 125 mL Erlenmeyer flasks, at 30 °C, 200 rpm during 72 h. A batch cultivation of hydrolysate treated with active charcoal was carried out in a 1 L fermentor at 30 °C, pH 5.00, 300 rpm, using oxygen transfer coefficient of 22.86 h⁻¹. The results showed that higher values of xylitol productivity (0.70, 0.71 and 0.62 g/g.L) and yield factor of xylitol (0.71, 0.69 and 0.63 g/g) in synthetic medium, hydrolysate treated with resin and treated with active charcoal, respectively were obtained with 5 g/L of rice bran. The use of lower quantity of rice bran eliminates the stage of fermented broth treatment in the process of xylitol production.

Acknowledgements: The authors acknowledge the financial support of Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), Brazil.

Poster 2-48
Bio-Alcohol production from Syngas

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Alcohol derived from lignocellulose in energy crops or organic wastes is one of many ways to use more solar energy and a durable alternative for car fuels derived from oil. Alcohols can be produced from plant materials or wastes either by enzymatic hydrolysis of (hemi)cellulose and fermentation of sugars by yeast or bacteria, or by gasification of (hemi)cellulose plus lignin and fermentation of the resulting syngas by bacteria. The main advantage of the syngas-route is that it allows conversion of non-biodegradable feedstock into alcohol, which may result in 1.5 times more product in case of wood-like materials. Furthermore, the bio-syngas-route is less sensitive to trace pollutants in the syngas compared to the chemical syngas-route (Fischer Tropsch) and is applicable on large as well as small scale.

The aim of our work is to optimize the process of alcohol production from plant (waste) materials. The bio-syngas-route has three main steps: the feedstock gasification to syngas, the syngas conversion into alcohol and recovery of the alcohol with a separation unit. Gasification and alcohol recovery are mature technologies, but syngas fermentation to alcohol is not. Published conversion efficiencies of syngas into alcohol, alcohol concentrations reached in the process and syngas conversion rates are all low. We study novel bioreactors that increase the gas-to-liquid mass-transfer rate by approximately a factor 10-50 (k_a ~ 1 s⁻¹) compared to previously described systems, while at the same time minimizing the power required for the absorption. The first design calculations and experimental results will be presented.

Poster 2-49
Bioremediation of marine sandy sediments impacted by petroleum

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The aim of this work was to optimize a sand sediment through bioestimulation in the mid-tide zone in the Guanabara Bay-Rio de Janeiro-Brazil, contaminated with petroleum at 14g·Kg⁻¹ sediment concentration. The optimization was performed using a complete 2ⁿ factorial experimental design using these factors: (1) addiction of a commercial biosurfactant JENIEL® IBR425; (2) addiction mineral NPK fertilizer. The response variable used was gravimetric losses of the heavy oil fraction and the statistical analysis of the main factors and their interactions was executed using response surface curves in mode 3D, contour curves, Pareto diagram and ANOVA Table. The screening process indicated that the addition of fertilizer at 100:25:25 C:N:P ratio and the biosurfactant at 2g·Kg⁻¹ sediment concentration result in good levels of biodegradation. Some monitored experiments were carried out yielding in 65,5% of gravimetric losses of heavy oil fraction and 100% of n- alkanes between C₁₅ e C₃₀ during 60 process days. At the same time a natural attenuation test was carried out yielding in 85% and 100% of biodegradation of these n- alkanes between C₁₅ e C₃₀. After 60 days, the sediment from the bioestimulation and natural attenuation were conducted to ecotoxicological essays using Tubifex tubifex. In both experiments the essays were positive indicating that the biodegradation of heavy oil fraction wasn't total, producing organic compounds different than CO₂ and H₂O. From the autochthon culture present in the sediment and responsible for bioremediation were isolated the bacteria Sheewanella putrefaciens and a yeast Candida sp.
**Poster 2-50**
Withdrawn

**Poster 2-51**
**Development of Software Sensors for Real Time Operation: Application for Bioethanol Production**
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One of the main difficulties in biotechnological processes nowadays is the lack of robustness of the operation in the presence of fluctuations in the quality of raw material, variations of dominant microorganisms and deviations in temperature, which cause inherent process variability. This can be avoided by frequent adjustments in the operational conditions and control settings of the process, which requires an efficient monitoring with reliable sensors.

The results obtained in this work have shown that it is possible to infer the key variables of an ethanol fermentation process (concentration of biomass, substrate, and bioethanol) from secondary measurements, such as pH, turbidity, CO₂ flow rate and temperature. Two alternatives were considered for the development of the software sensor, a Multilayer Perceptron Neural Network and a Takagi-Sugeno fuzzy model.

The application of the two software sensors on experimental data provided a reasonable description of the concentration trajectories, providing real time information of the key process variables.

**Poster 2-52**
**Proteomic Analysis of Zymomonas mobilis Responding to Acetate Treatment**
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Zymomonas mobilis is the most efficient ethanol producer among the candidates with the production of 1.5-1.9 mol ethanol from each mol glucose, and the production rate is 3-5 folds higher than that of Saccharomyces cerevisiae. Z. mobilis has been genetically engineered to expand its substrate utilization; however, the original engineered strain displayed an acid sensitive phenotype. Based on our observation, the effects of ampicillin on acid tolerance in Z. mobilis ATCC 31821 were observed. To better understand the effects of ampicillin on the enhancement of acetate tolerance of Z. mobilis ATCC 31821, we utilized the proteomic approach to search for potential regulatory factors of ampicillin-inducing acetate tolerance. Z. mobilis ATCC 31821 grown in the presence of ampicillin was significantly more acetate tolerant than grown in the absence of ampicillin. Analysis by 2-DE indicated that 6 proteins are up-regulated and 12 proteins are down regulated, differentiating the protein expression by 5 folds intensity, in 100 mg/mL ampicillin treated samples as compared with those of non-ampicillin treated. It was also differentiated by 5 folds intensity, 13 proteins are up-regulated and 8 proteins are down-regulated in 0.05 M acetated treated cells after ampicillin-pretreated samples as compared with those of 0.05 M acetated treated but non-ampicillin pretreated. Under ampicillin induction, these differentially expressed proteins could play an important role in the development of acid tolerance response observed in acid culture condition. To our knowledge, there is little report concerning proteomic surveillance of ampicillin-induced acetate tolerant response (ATR) proteins from Z. mobilis.

**Poster 2-53**
**Study on mass transfer of isopropylbenzene and oxygen in a two-phase partitioning bioreactor in the presence of silicone oil**
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A two-phase partitioning bioreactor (TPPB) to treat gas effluents polluted by volatile organic compound (VOC) has been developed. In this work, both the mass transfer of isopropylbenzene (IPB) and oxygen have been considered in relation to their influence on the hydrodynamics of the reactor and the type of silicone oils used as a second phase.

The synergistic effect of silicone oil and stirrer speed on the global oxygen mass transfer coefficient (Kₐ a) and gas-hold-up (up to 12%) have been investigated. The addition of 10% of low viscosity silicone oil (10 centistokes) in the reactor does not significantly affect the oxygen transfer rate. The very high solubility of IPB in the silicone oil leads to an enhancement of driving force term, especially for high fraction of silicone oil. However, it does not seem useful to exceed a volume fraction of 10% since K a a decreases sharply at higher proportions of silicone oil. K a a and K a a evolve in the same way with the proportion of silicone oil. These results confirm the potentialities of our bioreactor to improve both the oxygen and pollutant gas transfer in the field of the treatment of gaseous pollutants, even for highly concentrated effluents.

**Poster 2-54**
**Designing an efficient SSF process for co-fermentation of xylose and glucose**
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To meet desired overall yields during ethanol production from lignocellulosic materials, it is important to use both hexoses and pentoses. Currently, genetically modified *Saccharomyces cerevisiae* strains are becoming available for xylose fermentation. However, simultaneous fermentation of xylose and glucose in genetically modified *Saccharomyces cerevisiae* requires a favorable ratio between these sugars. In simultaneous saccharification and fermentation (SSF) of spruce, which has been shown to be a viable process option for ethanol production, the ratio of xylose to glucose in the material is relatively low, which makes xylose fermentation challenging. However, by co-fermentation of the sugars in spruce, the theoretical ethanol yield can be increased by as much as 7-8%, which is significant in an industrial process. By performing enzymatic hydrolysis and model SSF experiments, studies of enzyme kinetics and consumption rates were carried out in the current work, in order to create a model for sugar release and sugar uptake. The model was then used to improve SSF experiments, and the most feasible SSF mode for co-fermentation of xylose and glucose, was established.
**Poster 2-55**

A plasmid-based system for the use in coupling non-selectable phenotypes to selectable phenotypes

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The production of high value commodity chemicals and bio-fuels by microorganisms relies on the identification of genes in mutants that allow for increased production. Having a reporter system that directly responds to the desired product would enable easier screens and/or selections of said mutants. However, most naturally occurring reporters, such as the Escherichia coli lactose promoter, are often sub-optimal as they may express at low levels, exhibit loose regulation, and respond only in natural conditions or environments. The goal of this work is therefore to develop a plasmid-based approach to couple the non-selectable phenotype of chemical production to a selectable phenotype. Using both computational and experimental techniques, we have developed a reporter system in which the selectable response conferred is dependent upon inducer concentration. Development of such plasmids for the improved production of organic acids is the focus of current activities.

**Poster 2-56**

Metabolic profiling of white-rot fungi, Phanerochaete chrysosporium and Dichomitus squalens cultured with lignocellulosic biomass

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White rot fungi, Phanerochaete chrysosporium and Dichomitus squalens have long been recognized to possess the lignin-degrading activity. In this study, to exploit the capability of the white rot fungi’s lignin degradation for tackling the recalcitrance of the lignocellulosic biomass, the physiological characteristics of the fungi on cellulose or lignocellulose biomass were investigated by the metabolic profiling. The cultivation of the white rot fungi was carried out on synthetic media containing a variety of carbon, nitrogen, trace minerals, and vitamin sources. Both the exometabolome and endometabolome were analyzed by using a quadruple type of GC/MS after appropriate quenching and extraction procedures. The mass spectrometric data were statically analyzed by principal component analysis (PCA) and partial least squares discriminant analysis (PLSDA) to find how the environment conditions (carbon, nitrogen and oxygen supply and so on) affect the profiles of endometabolome and exometabolome. This microbial metabolomics approach is expected to provide the holistic view of the response of the fungal metabolism to the culture environment, especially the presence of lignocellulosic biomass in media.

**Poster 2-57**

Construction of a xylose-utilizing recombinant diploid industrial strain of Saccharomyces cerevisiae and its cofermentation with glucose and xylose

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This work was supported by the National Basic Research Program of China (2003CB716006 G2007CB707803) and the National High Technology Research and Development Program of China (No. 2007AA0Z402).

A xylose-utilizing recombinant diploid industrial strain NAN-127 of Saccharomyces cerevisiae, containing the genes YXL1 and YXL2 from Pichia stipitis encoding xylose reductase(XR) and xylitol dehydrogenase(XDH) respectively and the gene KXS1 encoding xylulokinase(KX) for overexpression from S. cerevisiae, was constructed by integrating vector at the rDNA locus. The recombinant strain NAN-127 was stable for more than 60 generations in nonselective medium and displayed at least 9.3 times higher specific activities of these three enzymes (XR, XDH and XK) compared to the parent strain NAN-27. The results of the cofermentation with 80 g glucose L⁻¹ and 50 g xylose L⁻¹ in 5L fermentor showed that xylose consumption ratio was 65.5% by the recombinant strain NAN-127 at 72h, which was 2.8 folds compared with the parent strain, under the optimal conditions: ventilation rate 0.04 L L⁻¹ min⁻¹, pH 4.5, temperature 30.0°C. Meanwhile, both ethanol and xylitol yield by the recombinant strain NAN-127 (Y g Ethanol / g Total sugar consumed = 0.390 and Y g Xylitol / g Xylose consumed = 0.446 ) were higher than the parent strain.

**Poster 2-58**

Microbial and genetic analysis of a microbial community actively decaying poplar biomass

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As primary decomposers, microbial communities have evolved as competitors and collaborators in deconstructing biomass. To understand and exploit these complex microbial communities and their dynamics for the conversion of recalcitrant plant biomass to useful bioenergy feedstocks, a highly integrated research initiative is required. A prerequisite is to have insight into the composition and metabolic potential of this lignocellulosic biomass degrading community. To achieve this goal several complementary strategies are used to study the microbial community actively decaying poplar woodchips:

Analysis of total community composition: After the isolation of metagenome DNA, 16S rRNA genes were PCR amplified, shotgun cloned and sequenced. In total, 238 clones were sequenced and identified to their closest matching species. The distribution of the species showed that members of the order Clostridiales, many of which are closely related to uncultivatable bacteria, comprise 85% of this community. The presence of the majority of members of this order in the community is expected because of the mesophilic, anaerobic conditions characteristic for the sample.

Isolation and characterization of cultivable microorganisms: the high number of uncultivable microorganisms in this consortium was confirmed by cultivation studies. None of the cultivable bacteria represented the dominant members of the community as determined via 165 RDNase sequencing. Isolated strains are presently screened for their glycosylhydrolase activity.

Metagenome sequencing: in order to obtain a thorough understanding of the diversity, structure, functional interdependence, and metabolic capabilities of this community. This approach should provide unprecedented insights in the diversity of glycosylhydrolases present in plant biomass decomposing microbial communities.
**Poster 2-59**

**Cellulases production by solid-state fermentation in different bioreactors scales**

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Solid-state fermentation (SSF) is an alternative to submerged cultures because it is possible to achieve high specific productivity and to apply low-cost substrates such as corncobs, wheat bran and sugarcane bagasse. Scaling up SSF processes raises several engineering problems due to the build-up of gradients of temperature, pH, oxygen and moisture. The objective of this study was to compare cellulases production under two types of bioreactors and two scales: 0.3 L packed bed columns and a 30 L internally agitated horizontal drum bioreactor. Fermentations were performed by Trichoderma reesei RUT C30, using solely wheat bran and a mix of wheat bran (20%) and sugarcane bagasse (80%) as substrate. Culture conditions were 60% moisture (wet basis), inoculation with 10⁷ spores.g⁻¹, 30°C temperature. Aeration rate was 100 mL.min⁻¹ in packed-bed columns and 20L.min⁻¹ in the bioreactor, employing an agitation pattern of 4 turns every 6 hours. Enzyme extraction was performed with 20 mL of a 1:1 mixture of water and citrate buffer (pH 4.8) per g of fermented medium (wet basis), and FPase and CMCase activities were estimated. The best activity values were observed for wheat bran fermentations at the third day, at 3.8 U/gdm FPA and 9.54 U/gms CMC. The activities obtained in the reactor were smaller, mainly due to the temperature gradients observed with both substrates, impairing the microbial growth and cellulase production. Considering this condition, further studies including different agitation patterns are to be conducted, to obtain successful scale-up criteria.

**Poster 2-60**

**Cellulases production by solid-state fermentation using different agroindustrial residues**

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Solid state fermentation (SSF) is an alternative to submerged cultures, because it is possible to achieve high specific productivity and to apply low-cost substrates such as corncobs, wheat bran and sugarcane bagasse. The objective of this work was to compare the production of cellulases in different substrates: wheat bran, sugarcane bagasse, and mixtures of both supplemented with either corn steep liquor (3% w/v) and yeast extract (1% w/v) or salt solution. Fermentations were performed by Trichoderma reesei RUT C30 in erlenmeyers flasks, and a packed-bed column, 0.3 L capacity, at 30°C. Medium had initial moisture of 60% (wet basis), was inoculated with 10⁷ spores.g⁻¹. Enzyme extraction was performed with 20 mL of a 1:1 mixture of water and citrate buffer (pH 4.8) per g of fermented medium (wet basis). FPase and CMCase activities were estimated (Table 1).

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Treatment</th>
<th>FPase (U/gdm FPA)</th>
<th>CMCase (U/gdm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FT</td>
<td>day 3</td>
<td>3.16</td>
<td>22.95</td>
</tr>
<tr>
<td></td>
<td>day 5</td>
<td>1.16</td>
<td>10.93</td>
</tr>
<tr>
<td></td>
<td>day 7</td>
<td>1.68</td>
<td>10.13</td>
</tr>
<tr>
<td>FTMEL</td>
<td>day 3</td>
<td>2.48</td>
<td>24.89</td>
</tr>
<tr>
<td></td>
<td>day 5</td>
<td>1.20</td>
<td>17.17</td>
</tr>
<tr>
<td></td>
<td>day 7</td>
<td>1.22</td>
<td>17.65</td>
</tr>
<tr>
<td>BF</td>
<td>day 3</td>
<td>1.91</td>
<td>11.61</td>
</tr>
<tr>
<td></td>
<td>day 5</td>
<td>2.22</td>
<td>7.85</td>
</tr>
<tr>
<td></td>
<td>day 7</td>
<td>2.22</td>
<td>7.85</td>
</tr>
<tr>
<td>gr</td>
<td>day 3</td>
<td>2.02</td>
<td>6.60</td>
</tr>
<tr>
<td></td>
<td>day 5</td>
<td>2.33</td>
<td>7.62</td>
</tr>
<tr>
<td></td>
<td>day 7</td>
<td>2.29</td>
<td>7.95</td>
</tr>
<tr>
<td>FT</td>
<td>day 3</td>
<td>3.40</td>
<td>12.38</td>
</tr>
<tr>
<td></td>
<td>day 5</td>
<td>2.26</td>
<td>10.01</td>
</tr>
</tbody>
</table>

The highest activity was observed in the supplemented wheat bran medium (3.4 U/gdm FPA). Different culture conditions have shown that the supplementation of the medium led to a 10% increase in FPase production, in relation to the pure wheat bran culture. Addition of salts retained the FPase rate, but significantly reduced CMCase production.

**Poster 2-61**

**The potential of bioethanol production from citrus fruit processing waste**

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The use of low-cost feedstock for fuel-ethanol production is nowadays one of the main goals to lower the final cost of the product. Waste materials from food industries appear as a promising alternative substrate provided that they are produced in large amounts and the feasibility of the transformation process to ethanol is demonstrated. The production of citrus fruit in Valencia’s region is estimated in 5 million tons/year. About 40% of the production goes towards human food market, 20% is used for juice manufacture and the remainder is nowadays a surplus without market place. In the industry of citrus processing for juice about 60% of the fruit weight is a residue consisting of waste peel, segment membranes and other by-products. The high amount of polysaccharides present in citrus fruit processing residue makes it a potential feedstock for biological conversion to ethanol. It contains soluble and insoluble carbohydrates as its major components. The soluble carbohydrates are simple sugars, glucose, fructose and sucrose whereas insoluble components are hemicelluloses, cellulose and pectin which can be converted into fermentable sugars by a hydrolysis step. In this work, the potential of ethanol production from citrus fruit processing residue was assessed. Laboratory experiments were performed on orange peel waste by a first step of enzymatic hydrolysis with commercial enzymes (pectinase and cellulase) to convert all carbohydrates present in the substrate into fermentable sugars. Then fermentation tests of sugar containing media into ethanol using conventional baker yeast were carried out. Detailed results of this study will be presented.
Commercialization of lignocellulosic ethanol production process requires the use of cheap and robust pretreatment and fermentation strategies for efficient and complete conversion of all available sugars to ethanol. Native S. cerevisiae strains presently used in corn ethanol industry can readily ferment glucose (C6) but not xylose (C5). The pathway of conversion of xylose to ethanol goes via the formation of xylulose, a ketose isomer of xylose. One approach is to genetically engineer microorganisms (E.coli, Z.mobilis or S.cerevisiae) to enable xylose utilization.

Our approach however is to isomerize the xylose to xylulose using the exogenous enzyme Xylose Isomerase (XI) and then ferment the xylulose to ethanol by using native yeast strains in the same vessel. Isomerization, however occurs optimally at a pH of 7-8 while subsequent fermentation step occurs at a pH of 4-5. We have successfully demonstrated that the maintenance of two vastly different pH microenvironments in a single vessel was possible using XI for isomerization co-immobilized with an outer layer of the enzyme urease for pH control. As hydrogen ions diffuse from the fermentation broth into the pellet, they are neutralized by the ammonia produced in the hydrolysis of urea (added to fermentation media). Using our co-immobilized enzyme system with specific additives for driving the isomerization forward, we have obtained xylose conversions that are higher than those possible with the native XI pellets operating under optimal pH. The results of fermentation of pure xylose, mixed sugars and poplar hydrolysate from leading pretreatment technologies will be presented.

Free energy of separation of glucose oligomers in water

A key step in the enzymatic hydrolysis of semi-crystalline cellulose is the separation and removal of glucose oligomer chains from the surface of the crystalline cellulose. In this work, we focus on the energetic aspect of this non-reaction factor that plays an important role in determining the overall rate of the cellulose hydrolysis process. In particular, we use Molecular Dynamics (MD) simulations to investigate the free energy required for the separation of glucose oligomers in presence of water. Simulations are performed on the non-stacked and stacked arrangement of the cellulobiose molecules; these are considered to represent the amorphous and crystalline domains in cellulose. An oscillatory potential of mean force profile was observed in the latter case. The umbrella sampling technique was used to investigate the free energy required for the separation of cellulobiose and cellotetrose pairs in water, as well as that required for the separation of glucose oligomers from a long cellulose chain. Simulation results are used to decipher the dependence of the energetics of the process on the chain length and the interaction between the sugar and water molecules.
Poster 3-10

**Attempting to unravel the substrate factors affecting enzymatic hydrolysis of lignocellulosics: Experiences with selected measurement techniques**

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The "specific activity" of a cellulase complex during reactions with a pretreated lignocellulosic substrate, they experience a hierarchy of structural and chemical obstacles that hamper the enzymes’ ability to hydrolyze the cellulose which is confined in a matrix of hemicellulose and lignin. Over the past few decades, it has been quite difficult to predict the ease of hydrolysis of a pretreated lignocellulosic substrate as factors such as crystallinity, DP, accessibility and chemical composition have all been implicated in the contributing to lignocellulosic recalcitrance. Of the factors that affect the efficiency of lignocellulosic hydrolysis, it seems intuitive that the surface area which a substrate possesses which is capable of accommodating cellulase components would play a significant role. With this in mind, the focus of this presentation will be to explore and assess various measurement techniques to analyze the physical and chemical properties related to “cellulase-accessible” surface area, such as staining techniques, fiber quality analysis, swelling measurements and x-ray photoelectron spectroscopy, by applying them to various lignocellulosic substrates pretreated by the organosolv and steam explosion pretreatment processes. The information should be valuable to elucidating the substrate related aspects that define the “hydrolytic potential” of the substrate when it comes into contact with the cellulase complex.

**Poster 3-11**

**Theoretical Investigation of Cooperative Hydrogen Bonding Networks in Native Celluloses**

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The cellobextrins in native crystalline celluloses are unusually stable compared to other polysaccharides, not easily prone to hydrolysis even with chemical or biological catalysts. The stability of crystalline cellulose is most likely due to its highly cooperative hydrogen-bonding (HB) network. We carried out ab initio calculations to determine the atomic and conformational structures of native crystalline celluloses and for cellulose polymers with degree of polymerization varying from 2 to 7. A highly cooperative hydrogen bonding interaction was found in these cellulose structures. The average hydrogen bonding interaction energy is found to be enhanced by more than 50%. The origin of this cooperativity will be discussed based on the charge transfer and electrostatic interaction models.

**Poster 3-12**

**Cellulose and Lignin Accessibility to Cellulase for Corn Stover and Poplar Solids Prepared by Leading Pretreatment Technologies**

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The digestibility of solids following pretreatments by leading technologies of ammonia fiber expansion, ammonia recycle percolation, controlled pH, dilute acid, lime, and sulfur dioxide were evaluated at different cellulose loadings for two distinct types of cellulosic biomass from controlled sources: an agricultural residue, corn stover, and a woody biomass, poplar. Furthermore, in order to understand the differences in their digestion performance, these solids were characterized hypothesizing that enzyme adsorption on cellulose and its effectiveness are the two primary factors controlling enzymatic saccharification. The accessibility of cellulose and lignin to cellulase (maximum adsorption capacity, σ) was determined, and the effect of selected substrate features on cellulase adsorption on cellulose and its effectiveness were evaluated.

**Poster 3-13**

**Enzymatic hydrolysis of fibre fractions from manure**

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More than 30 million ton of manure with around 6% dry matter is produced each year in Denmark, giving almost two million tons of fibre fractions that could potentially be used for bioethanol production. High dry matter content is an important factor in commercialising the hydrolysis of biomass due to restrictions in water and energy use and thereby also reduction in process costs. Different methods are commercial available for separating manure into fibre and liquid fractions by chemical flocculation or sieving and pressing. In order to investigate the potential role of manure, as a source of biomass for e.g. bioethanol production, enzymatic hydrolysis has been performed on three different fibre fractions. The fibres were pretreated by either chopping, autoclaving at 120°C or bomb treated at 200°C in oil bath. The convertibility of the various fractions after pretreatment was then investigated by enzymatic hydrolysis. Large amounts of cellulose are converted to glucose, in the bomb treated samples, compared to the other pre treatments. The latest results and the potential of bioethanol production from fibre fractions will be presented.

**Poster 3-14**

**Effect of Particle Size on Enzymatic Hydrolysis of Pure Cellulose and Lignocellulosic Biomass**

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The potential benefits of reducing the size of biomass into very fine particles in various pretreatment processes were investigated. Two major points were addressed in this investigation. The first was to verify whether the fine particle size improves the enzymatic digestibility under alkaline pretreatment conditions. The second was to test the feasibility of high temperature-short treatment time neutral treatment of biomass. The conditions explored are in the range of 190-220°C and 5–60 seconds. The extremely small particle size allows quick heating and uniform temperature within the biomass making high temperature-short treatment time feasible. This approach was taken to see if such condition can alter the crystalline structure of the cellulosic component without decomposing the carbohydrates, and if so, how it affects the enzymatic digestibility. Our data taken from switch grass and corn stover to this point indicate that size reduction to very fine particles gives significant improvement in pretreatment. In the alkaline pretreatment, the improvement was due to increased surface area. The benefit of high temperature treatment appears to be related with the alteration of the crystalline structure of cellulose. The digestibility data of treated biomass affected are presented under various alkaline and neutral treatment conditions. The data were further analyzed to verify the isolated effects of size reduction on biomass structure and pretreatment.
Poster 3-15
An investigation of enzyme binding in the enzymatic hydrolysis of biomass

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So far, there is a lot controversy concerning the binding reversibility of cellulases to substrate. While some researchers have noticed exchange of cellulases on the substrate surface in their experiments, which indicates that the binding is somehow reversible, others insist the binding of cellulases to substrate is irreversible from evidence in their binding isomers. Due to the contradiction among these experiments, it is necessary to investigate whether the binding that occurs during the enzymatic hydrolysis process is reversible or irreversible.

According to the experiments here, the enzyme’s reactivity has dropped during the enzymatic hydrolysis of biomass. To investigate if the reasons for the reactivity drop are related to reversible or irreversible binding, cellulases are first incubated with different substrates. It is observed that substrates with higher crystalline content would cause lower reactivity of enzymes. It is thus suspected that the reactivity drop mainly comes from the irreversible binding of exoglucanases, which are very effective in digesting of crystalline cellulose. When enzyme pre-incubates in substrate-free buffer solution, less reactivity drop is noticed compared to enzyme that pre-incubates with substrate. By this, it is inferred that the reactivity drop is likely caused by inactivation of intact enzyme due to its interactions with the substrate. It is concluded here some exoglucanases are irreversibly bound to substrate.

Poster 3-16
Oxidative delignification of lignocellulosic biomass by \( \text{H}_2\text{O}_2 \) under alkaline conditions

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Hydrogen peroxide is a well-known bleaching reagent in pulping industry. Under alkaline conditions, it promotes hydrolysis as well as oxidative degradation of lignin. Degradation of two different biomass feedstocks (Hybrid poplar and Corn stover) was studied under alkaline conditions with and without the presence of \( \text{H}_2\text{O}_2 \). Various strategies of \( \text{H}_2\text{O}_2 \) addition were tested seeking efficient utilization as an oxidative agent. Ammonia and NaOH were used as alkaline reagents. Reaction mechanism in pretreatment process was investigated by characterizing the liquid and lignin separated in the pretreatment. The mode under which lignin and sugar fragments are solubilized appears to affect the accessibility of enzyme molecules to the carbohydrate section of the treated biomass. Enzymatic hydrolysis of treated biomass by cellulase and other auxiliary enzymes (xylanase, pectincase.) were conducted to understand the degradation of biomass during pretreatment. Significant accumulation of xylo-oligosaccharides occurred during enzymatic hydrolysis by cellulase, which indicates that \( \text{H}_2\text{O}_2 \) increases the rate of hydrolysis of glycosidic linkages in hemicellulose.

Poster 3-17
Fermentation of pressurized batch hot water (PBHW) pretreated warm season grasses and inhibitor analysis for determination of value-added coproducts

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Warm-season grasses for livestock feeding are grown across a large portion of the southeastern United States, thus providing a rich and renewable source of biomass. We investigated Tifton 85 bermudagrass (Cynodon spp.), Merkeron napiergrass (Pennisetum purpureum), and GA 993 switchgrass (Panicum virgatum) as possible sources of biomass for conversion to ethanol using an engineered Escherichia coli strain. Grass samples were either left untreated or were pretreated using a pressurized batch hot water (PBHW) reactor. Napiergrass consistently produced the lowest ethanol yield of the three grasses investigated. All three grasses had similar levels of p-coumaric and ferulic acid, known inhibitors of microbial metabolism, indicating that some other compound(s) may be responsible for the differences in fermentability observed. To investigate this phenomenon further and to determine the content of additional phenolic-based value-added coproducts, fermentation samples were analyzed for 42 compounds after pretreatment, after enzymatic digestion, and after 144 hours of fermentation. Of the three grasses, Tifton 85 and GA 993 hold the most potential as possible bioenergy crops for ethanol production based on consistently higher ethanol yields than those obtained using napiergrass. Value-added coproduct removal prior to inoculation is being investigated and may increase ethanol yields further as some coproducts are inhibitory to bacterial growth and metabolism.

Poster 3-18
Kinetics of xylose reversion reactions during acid pretreatment

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Acid pretreatment of cellulosic biomass solubilizes hemicellulose (i.e. xylan), allowing subsequent enzymatic hydrolysis of the cellulose. Conversion of cellulose and xylan into constituent sugars in high yields is essential for the economic viability of bio-ethanol. Thus, acid-catalyzed reactions that result in the loss of sugars are of particular interest. A loss reaction that has received little attention is the reversion reaction of sugars that result in the formation of oligomers, which cannot be fermented. The economics of pretreatment dictate that high biomass loadings are necessary and under these conditions reversion reactions may be important. In this study, the reversion reactions of xylose (and for comparison, glucose) under dilute acid conditions were examined. Experiments were conducted in highly agitated microwave-irradiated reactor vessels. This reactor set-up allowed for rapid heating of the sample and limited the effects of mass transport, permitting the measurement of intrinsic kinetics. At high sugar loadings, xylose reversion reactions were found to result in conversion of up to 10% of the xylose into oligomers that are most likely to be dimeric. Under similar conditions the reversion reactions of glucose produced readily identifiable 1,2-linked disaccharides. Using kinetic modeling of the experimental results we obtained the kinetics for formation and hydrolysis of reversion products, in addition to the equilibrium constants. From these measurements, we were able to calculate activation energies. The kinetic measurements from this study are critical for designing and operating pretreatment processes to optimize xylose yield.
**Poster 3-19**
Evaluation of matrix interference on quantitation of lignocellulosic degradation products in biomass pretreatment samples using LC-ESI-MS/MS

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Qualitative and quantitative understanding of potentially inhibitory degradation products produced upon pretreatment of lignocellulosic biomass is paramount to technical and economic valuations of biomass-to-ethanol conversion. Production and release of degradation products results from highly complex chemistry that is poorly understood at this time. Continuing work in our laboratory is focused on using liquid chromatography in combination with electrospray ionization mass spectrometry to monitor accumulation trends of degradation products in biomass pretreatment samples. The primary analytical advantage of employing mass spectral detection is an unmatched ability to resolve target analytes away from alternative matrix constituents that co-elute chromatographically. However, successful application of the technique in quantitative analyses requires knowledge of the effect of sample matrix on the analytical response for each target compound.

In the present study, experiments were designed to evaluate matrix interference as a function of various feedstock-pretreatment chemistry combinations. In order to assess the effect of differing feedstocks, dilute acid pretreatments of corn stover and poplar wood were conducted at low, medium, and high severity. Similar APEX pretreatments of corn stover were also conducted to assess the effect of varying pretreatment conditions. Each sample was analyzed using recently developed LC-ESI-MS/MS methodology, and quantitative data derived from an internal standard calibration approach that is subject to matrix effects were compared with data derived using the method of standard additions which compensates for matrix interference. Results of the study will be presented along with practical guidance on appropriate application of developed methodology for analysis of biomass pretreatment samples.

**Poster 3-20**
Wheat straw autohydrolysis: process optimization and products characterization

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Autohydrolysis has been demonstrated has an important pre-treatment method to efficiently separate the main constitutive plant biomass components. Among others, it has the advantage to enable a high recovery of hemicelluloses as soluble saccharides, while both cellulose and lignin could be recovered in the solid phase, with minor losses.

Wheat straw is an abundant byproduct from wheat production in EU and USA. Its chemical composition and low-cost make it an attractive feedstock for a bioethanol biofinery.

In this work, wheat straw was subjected to autohydrolysis treatments in order to selectively hydrolyse the hemicellulose fraction. The effects of temperature (150-240°C) and non-isothermal reaction time on the composition of both liquid and solid phase were evaluated and interpreted using the severity factor (log Ro). The operational conditions leading to the maximal recovery of soluble hemicellulose (xylo-oligosaccharides and xylose) were established for log Ro = 3.96 and correspond to 60% of the feedstock xylen. Under these conditions, a solubilization of 83% arabinan and 98% acetyl groups also occurred. Glucan was only slightly solubilized (10%), which enable an enrichment of the solid phase to contain 57% glucan. Delignification was not extensive, being utmost 15% for the tested conditions.

The yields of soluble products, e.g., glucose, arabinose, acetic acid and degradation compounds, such as, furfural, hydroxymethylfurfural and formic acid are also presented and the overall liquid fraction composition is discussed regarding liquor fitness for fermentation purposes.

This work has been funded by CEBio (Adi) and BIOREFINO (FCT) projects.

**Poster 3-21**
Protein extraction and enzymatic hydrolysis of ammonia-treated cassava leaves (Manihot esculenta Crantz)

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Cassava leaves were treated with 0.5 kg ammonia/kg dry matter at 78°C and 30% moisture content in a 2 kg reactor. Protein extraction was carried out with a calcium hydroxide solution (pH 10) for 30 min at several temperatures (30, 45, 60, 75 and 90°C) and solid/liquid ratios (1:10 y 1:15) in a thermostated bath. Soluble protein content of the extracts was determined by the Lowry’s method. Dry substrate concentrations of 5%, 7.5% and 10%, and enzyme doses of 2 IU/g and 5 IU/g dry matter were used for the enzymatic hydrolysis in an orbital incubator at 50°C and 100 rpm. Both cellulase and xylanase were used. Reducing sugars produced were determined with the DNS method. The highest protein extraction yield for the ammonia-treated leaves was 29.10%, which was 50% higher than with the untreated leaves (20%), and was obtained at 90°C and with a 1:10 solid/liquid ratio. The highest sugar yield was 54.72% with respect to theoretical, and was obtained with 5% solids and an enzyme dose of 5 IU/g dry matter. This yield was 3.2 times higher than the yield of the untreated leaves (16.72%).

**Keywords:** ammonia treatment, protein, protein extraction, reducing sugars, enzymatic hydrolysis, cassava.

**Poster 3-22**
Effect of varying feedstock-pretreatment chemistry combinations on the production of potentially inhibitory degradation products in biomass hydrolysates

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A variety of degradation products, many of which are inhibitory in downstream microbial processes, are produced upon pretreatment of lignocellulosic biomass under different thermochemical conditions. Production and release of degradation products is highly affected by the pH and redox potential of pretreatment reactions. In a previous study, samples of poplar wood and corn stover were subjected to eight different chemical conditions, representing several leading pretreatment methods. The resulting hydrolysates were analyzed using recently developed analytical methodology involving the application of liquid chromatography in combination with UV spectroscopy and tandem mass spectrometry. While qualitative aspects of this study were definitive, the influence of co-extracted matrix components on the mass spectral response of a number of analytes precluded sound quantitative conclusions. As a result, the pretreatment study has been repeated, and an improved quantitative protocol that alleviates limitations caused by matrix effects has been applied to determine analytical concentrations of degradation products in hydrolysates. The scope of previous work has also been expanded to include pine wood as a representative softwood. Tested pretreatment conditions included: 0.7% H2SO4, 0.07% H2SO4, liquid hot water, neutral buffer solution, aqueous ammonia, lime, lime with oxygen pressurization, and wet oxidation. All reactions were carried out at 180 degrees for 8 minutes. Accumulation trends for 39 potentially inhibitory degradation products will be presented as a function of tested feedstock-pretreatment chemistry combinations.
Posters

Poster 3-23
Ammonia Phase Equilibrium in Vapor-Water-Biomass Pretreatment Systems

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Pretreatment of lignocellulosic biomass materials with ammonia has been shown to increase yields of fermentable sugars and ethanol in enzymatic hydrolysis and Simultaneous Saccharification and Fermentation (SSF) processes. Yields from many types of biomass, including corn stover, corn husk, corn fiber, bagasse, and switchgrass, have been shown to increase significantly after ammonia treatment. Ammonia pretreatment processes are currently being scaled up to provide industrially-significant quantities of treated biomass for subsequent production of sugars, alcohols, organic acids, and other products. However, engineering calculations for design of large-scale ammonia pretreatment plants are hindered by lack of detailed thermodynamic data on ammonia-water-biomass mixtures. While ammonia speciation and vapor-liquid equilibria in ammonia-water systems have been described in detail, the effect of the presence of biomass on ammonia phase equilibrium in vapor-water systems has not been carefully studied. Ammonia sorption on biomass is a significant ecological phenomenon that impacts nitrogen distributions in natural soils, but the sorbed and aqueous concentrations found in nature are low. Ammonia sorption on biomass has not been quantified at the high concentrations, temperatures, and pressures used in pretreatment processes. In this study, the effect of biomass on equilibrium vapor pressure in closed ammonia-water systems is measured, and the extent of sorption of aqueous ammonia species on biomass solids is estimated. The implications of the observed ammonia sorption for design of large-scale ammonia pretreatment plants are discussed.

Poster 3-24
Process Modeling Results of Various Leading Pretreatment Technologies for Corn Stover and Hybrid Poplar

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The Biomass Refining Consortium for Applied Fundamentals and Innovation (CAFI) was formed in 1999 as a multi-institutional effort to develop comparative information oncellulosic biomass pretreatment options. Initial work focused on a comparative evaluation of corn stover feedstock for five leading pretreatment technologies (dilute acid, hot water, ammonia recycle percolation, ammonia fiber explosion and lime). More recently completed efforts, known as the CAFI 2 project, included both corn stover and hybrid poplar as feedstocks on which performance data has been collected. Also, an additional pretreatment approach (SO2 pretreatment), has been included in the research and process modeling aspects of the CAFI 2 project. Process modeling updates include the use of a more current costing basis for the economic models, the development of a graphical user interface, and the incorporation of a woody biomass feedstock handling system that is suitable for handling the hybrid poplar feedstock. The final pretreatment, enzymatic saccharification, and hydrolyzate fermentation data on corn stover and hybrid poplar for each pretreatment process has been incorporated into the economic models. Model scenarios that project a Minimum Ethanol Selling Price (MESP) for a “current” technology case (based upon available bench-scale data) and a “target” case (based upon reasonable projections of improved, commercial-scale performance) have been developed for each pretreatment process for both corn stover and hybrid poplar.

Poster 3-25
Xylan Redistribution During Dilute Acid Pretreatment of Corn Stover

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Xylan is an important hemicellulosic component of the plant cell wall, serving as a cross linking polymer with lignins. Xylan is also recognized as a barrier to efficient cellulase action on microbriels. A key function of the pretreatment process is the removal of xylan to ensure cost effective production of cell wall sugars for subsequent fermentation. To better understand xylan hydrolysis in corn stover we have studied changes in the distribution of xylan caused by dilute acid pretreatment. Samples of corn stover rind were labeled with a anti-xylan antibody (LM11) that was conjugated with a fluorescent dye (Alexa Fluor488). Changes in the distribution of xylan were then monitored using laser scanning confocal microscopy. We focused on the schlerenchyma cells near the vascular bundles for consistency in imaging. These cells also contain the largest amounts of mass in the stem and are thus of greatest process relevance. We observed an increase in xylan antibody signal in the middle lamella and lumen that was concomitant with the decrease in the signal from the bulk of the primary and secondary cell walls, throughout the course of pretreatment suggesting that some xylan fractions migrate out of the cell wall before hydrolysis. Furthermore, the signal accumulates and is retained at the middle lamella and lumen, suggesting that some fractions of the xylan are protected from dilute acid hydrolysis. These latter fractions may be the cause of the slow phase of xylan hydrolysis kinetics reported historically from dilute acid treatment of many bioenergy plant feedstocks.

Poster 3-26
Spectroscopic Analysis of Biomass Feedstock Fines

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The process of chopping and grinding biomass to enhance surface area prior to entering a biofuel production plant produces a wide range of small particles with potentially significant compositional differences. The differences in these fractions have implications relative to the size reduction process and, possibly, to the economic value of the various size fractions. Samples of wheat and barley straw were chopped to 0.25”-0.5” and then divided into size fractions by passing a 1.0-kg sample through a forage separator, resulting in size fractionation of the parent material based upon diminishing screen size openings of 19.0 mm, 12.7 mm, 6.3 mm, 3.96 mm, 1.17 mm and the “pan”. The material in the “pan” was further segregated using a series of sieves with US mesh sizes #20, #30, #40, #50, #70, #100 and the “final pan”. The fractions collected on this set of sieves were dried, ground in a Thomas-Wiley Mill, passed through a 2 mm screen and subsequently examined with near-infrared (NIR), fluorescence and synchronous luminescence spectroscopies. Replicate spectra were obtained for each sample using each spectroscopic technique and the respective data sets subjected to chemometric analysis. The results of the chemometric analyses demonstrated clear spectroscopic differences for each size fraction by each spectroscopic technique. Further analyses of the data give some indication as to the compositional differences in the fractions that are responsible for the spectroscopic differences. It is believed that this information can be tied to quality control of the chopping/grinding process and to valuing the resulting size fractions.
Factors affecting lignin/protein measurements in dilute sulfuric acid treated herbage feedstocks

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The Klason lignin method is one of the most popular methods for measuring lignin content in biomass materials. We used the Klason method to measure lignin content in herbage materials before and after dilute sulfuric acid pretreatment and measured more lignin in the treated material than could be accounted for in the untreated material. This phenomenon was investigated with using a combination of \(^{13}\)C cross polarization/magic-angle spinning (CP/MAS) solid state NMR spectroscopy and lignin removal using acid chlorite bleaching to identify possible interfering compounds in the treated material’s lignin including residual carbohydrates and protein. \(^{13}\)C CP/MAS spectra of acid insoluble residues (AIR) from pretreated corn stover were compared with those from the residues which remain after additional acid chlorite bleaching treatment of the AIR. Only minimal contamination due to carbohydrate and protein was observed in the acid insoluble residues. We are currently investigating the incorporation of degradation products from sugars present in the extractives using a combination of \(^{13}\)C CP/MAS spectroscopy and \(^{13}\)C-labeled sugars such as fructose, xylose and glucose. Our results indicate that degradation products derived from fructose are present in the insoluble Klason residue and may be intimately associated with the lignin. These results indicate that sugars in extractives may be rapidly degraded and then re-polymerized during pretreatment.

A Rapid Simultaneous Saccharification and Fermentation (SSF) Technique to Determine Ethanol Yields

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A relatively simple simultaneous saccharification and fermentation (SSF) technique was developed to determine ethanol production potential of a given large set of biomass sample. The technique is based on soaking approximately 0.5 grams of a biomass sample in aqueous ammonia at room temperature and atmospheric pressure for 24 hours and then fermenting with \textit{Saccharomyces cerevisiae} for 24 hours using Spezyme CP, for the enzymatic hydrolysis. The technique has been demonstrated on a set of corn stover samples supplied by University of Wisconsin, Madison. The samples were weighed into the modified Ankom filter bags (F57) before soaking to avoid biomass loss during the pretreatment process. Simultaneous saccharification and fermentation of corn stover samples were performed inside 25 mL flasks with a total working volume of 10 mL. Fermentation samples taken at 24 hours were analyzed for ethanol production by HPLC. Theoretical ethanol yields of the samples were calculated (ranging 44.9% – 73%) and significantly different corn stover varieties were determined. It was observed that theoretical ethanol yields were highly correlated (\(r^2 = 0.90\)) with acid detergent lignin values while low correlation was determined between cellulose, hemicellulose and ethanol yields. The results suggest that this technique could successfully and easily be performed on a large set of biomass samples to determine ethanol production potentials and to compare the different biomass samples in terms of theoretical ethanol yields.

**Keywords:** Fermentation Technique, Simultaneous Saccharification and Fermentation, Corn Stover, Aqueous Ammonia Soaking

Structural Analysis of Residues from Enzymatic Hydrolysis

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Fuels from lignocellulosic biomass have a high potential to reduce green house gas emissions, and hence are important means to fulfill the road transport CO\(_2\) emissions targets. An increasingly important aspect is to utilize wastes and raw materials which do not compete with food production. Advanced conversion technologies are, however, needed to produce biofuels from a wider range of resources, including lignocellulosic biomass. The major obstacles in the enzymatic hydrolysis of lignocellulose into sugars are related to the recalcitrance and complex structure of the raw material itself, posing a scientific challenge and opportunity for biotechnological development.

The role of hemicellulose and lignin in the complete hydrolysis of lignocellulosic substrates is still not fully understood. To reduce the overall amount and costs of enzymes, the potential bottlenecks decreasing the enzymatic hydrolysis rate should be identified. Various enzymes can be used to enhance the conversion by hydrolyzing or modifying the residual polymers in the matrix.

To understand the disassembling mechanisms of lignocellulosic components, the limiting factors in the conversion of carbohydrate polymers into sugars were studied by characterization of the substrates. After enzymatic hydrolysis with the well characterized \textit{T. reesei} cellulolytic system, modifications in the chemical composition and structures of the hydrolysis residue were followed. These analyses included various chemical and spectroscopic methods, combined with enzymatic and chemical treatments. The results will help to develop improved enzymes required to overcome the bottlenecks in the hydrolysis of recalcitrant plant biomass.

Identification of phenolic compounds in lignocellulosic hydrolysates and its detoxification by enzyme for the production of butanol by \textit{Clostridia}

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With inevitable depletion of fossil fuel and increase of problem of greenhouse effect, the needs of alternative and non-petroleum-based sources of energy being on the rise. Increased attention has been focused on alcoholic liquid fuels, such as ethanol and butanol, prepared from lignocelluloses as an alternative to fossil fuels due to its net reduction of carbon dioxide emission. Lignocellulosic materials provide abundant and renewable resources and have great potential as a substrate for fermentation. Lignocelluloses contain cellulose and hemicellulose which can liberate sugars by steam and dilute acid hydrolysis. Lignocellulosic hydrolysates, however, contain not only fermentable sugars but also some compounds that inhibit microbial fermentation to desirable products, such as furan, weak acids, and various phenolic compounds [1]. Therefore, detoxification of hydrolysates, i.e., removal of inhibitory compounds is necessary before the fermentation for achieving high yield of products.

Phenols are oxidized by peroxidase to generate phenoxy radicals, which couple with other substrate molecules to form dimeric, oligomeric, and polymeric compounds. This enzymatic polymerization method can be exploited for the treatment of wastewater polluted with phenolic compounds. In the present study, we identified phenolic compounds and investigated the enzymatic detoxification of phenolic compounds found in lignocellulosic hydrolysates. The enzyme reaction was optimized as a function of external variables, such as pH, enzyme dose, and hydrogen peroxide to substrate ratio. The detoxified materials were used as substrate for butanol production from \textit{Clostridium beijerinckii} in order to evaluate the toxicity of reaction product.

**References:**

Biomass is converted to ethanol as the result of several processing steps of which enzymatic hydrolysis (saccharification) is a key rate and cost limiting step. To achieve the most economically feasible conversion of solids to glucose through saccharification of biomass, improvements are needed for this step. It is desirable to begin with a high solids concentration in order to maximize the product concentration in the sugar stream, minimize water and energy use, and minimize reactor volume. However, when processing with concentrated slurries, high viscosity prevents efficient mixing and leads to reduced conversion. Previous results show higher saccharification rates with smaller particle sizes. Therefore, the first aim of this study was to determine if the particle size of biomass can be further reduced using ultra-sonication. Results show that ultrasonic irradiation is effective in reducing the particle size of substrates used in this work in terms of both particle size distribution and average particle size of the substrate. Average particle sizes were reduced to as low as 1 micron under the conditions tested. The effect of reduced particle sizes on saccharification rates and rheological properties (viscosity) of biomass slurries are presented. While saccharification rates increased, the viscosity of slurries with particles in this size range surprisingly did not follow the same trends as observed for bigger sizes ranges due to surface characteristics of the particles are presented here. This work also characterizes changes in physical characteristics such as crystallinity caused by ultrasonic irradiation and examines how those changes affect enzymatic digestibility.

**Poster 3-31**

The Effect of Ultrasonic Irradiation on Physical Characteristics and Saccharification of Concentrated Biomass Slurries

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Switch grass is one of the most promising feedstocks for cellulosic ethanol production. In this study, two alkaline reagents, aqueous ammonia and NaOH, were investigated for pretreatment of switch grass. In the case of ammonia based treatment, two different types of pretreatment modes were applied: ammonia recycle percolation (ARP) and soaking in aqueous ammonia (SAA). The ammonia based processes were proven to be highly effective in delignification, yet the enzymatic digestibility of treated switch grass with cellulase did not meet the expectation. The lignin content of untreated switch grass was high (26.4%). It appears that the residual lignin content is the major hurdle limiting the accessibility of cellulase, thus the enzymatic digestibility even after pretreatment of switch grass with these processes. In order to further increase the delignification, \( \text{H}_2\text{O}_2 \) was added in the SAA and NaOH treatment. Addition of \( \text{H}_2\text{O}_2 \) significantly increased delignification and digestibility of switch grass. Addition of xylanase as a supplementary enzyme was highly effective in improving the digestibility of treated switch grass. Lignin separated from the pretreatment was characterized by TGA, DSC and FTIR. The data from these tests were further analyzed to understand the reaction mechanism.

**Poster 3-32**

Cellulase Production from Paper Mill Sludge by *Trichoderma Reesei* Rut C-30

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Paper mill sludge is a solid byproduct recovered from the wastewater stream of the pulping and papermaking. Because of high glucan content and well-dispersed structure containing large amount of short fibers, sludge is considered as a potential feedstock for bioconversion into value-added products. However it also has a high ash content which is toxic to cell growth. It is desirable to remove ash as much as possible from the sludge, while retaining carbohydrates. In this study, the de-ashed sludge via water washing, acid leaching was used as substrates for cellulase production and ethanol fermentation. The cellulase enzyme produced from de-ashed sludge exhibited cellulase activity as high as 8 FPU/ml. Furthermore, in the SSF test using *Saccharomyces cerevisiae* and the cellulase produced from the sludge, ethanol yields in the vicinity of 70% of theoretical maximum, and 6% ethanol concentration were achieved. These results are comparable to those of the processes using commercial cellulases. The results of this study supports that the pulp mill sludge is a feedstock feasible for cellulase as well as ethanol production.

**Poster 3-33**

Alkaline pretreatment of Switch grass

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Switch grass is one of the most promising feedstocks for cellulosic ethanol production. In this study, two alkaline reagents, aqueous ammonia and NaOH, were investigated for pretreatment of switch grass. In the case of ammonia based treatment, two different types of pretreatment modes were applied: ammonia recycle percolation (ARP) and soaking in aqueous ammonia (SAA). The ammonia based processes were proven to be highly effective in delignification, yet the enzymatic digestibility of treated switch grass with cellulase did not meet the expectation. The lignin content of untreated switch grass was high (26.4%). It appears that the residual lignin content is the major hurdle limiting the accessibility of cellulase, thus the enzymatic digestibility even after pretreatment of switch grass with these processes. In order to further increase the delignification, \( \text{H}_2\text{O}_2 \) was added in the SAA and NaOH treatment. Addition of \( \text{H}_2\text{O}_2 \) significantly increased delignification and digestibility of switch grass. Addition of xylanase as a supplementary enzyme was highly effective in improving the digestibility of treated switch grass. Lignin separated from the pretreatment was characterized by TGA, DSC and FTIR. The data from these tests were further analyzed to understand the reaction mechanism.

**Poster 3-34**

Rapid high performance liquid chromatography analysis of sugars in aqueous extracts and dilute-acid hydrolysates of herbaceous biomass

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An improved high performance liquid chromatography (HPLC) method for determination of biomass sugars in aqueous samples has been developed and validated. The method employed a commercially-available anion-exchange column that was modified with sodium carbonate prior to sample analysis, and the modified column resulted in baseline resolution of sucrose, arabinose, galactose, glucose, xylose, mannos, fructose, and an internal standard in approximately 5 minutes. Eluted compounds were detected using pulsed amperometry, and the observed linear response for each analyte resulted in statistically-derived limits of detection (S/N = 3) and quantitation (S/N = 10) ranging from 0.89-2.51 \( \mu \)g/L and 2.87-7.98 \( \mu \)g/L, respectively. Method precision was evaluated by analyzing an aqueous extract and a dilute-acid hydrolysate, derived from both corn stover and switchgrass materials, over a five-day period. Observed retention times varied by <1% after more than 800 sample injections, and inter- and intra-day precision, measured as the relative standard deviation of individual analyte concentrations, ranged from 2-13% and 4-12%, respectively, independent of sample type. Additionally, analysis of matrix spike samples resulted in analyte recoveries ranging from 79-106%, demonstrating excellent accuracy. These data clearly demonstrate the utility of developed methodology for analysis of biomass samples.
**Poster 3-35**

Comparison of accelerated solvent extraction and soxhlet methods for aqueous extraction of herbaceous biomass

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Water-soluble materials have historically been quantified gravimetrically and collectively identified as “extractives” in compositional analyses of herbaceous biomass. However, a recent report has demonstrated that as much as 12% of corn stover dry weight is represented by water-soluble sugars. As a result, there is increasing impetus to evaluate water-soluble constituents of feedstocks for bioethanol production. The first step in such evaluations involves generation of aqueous extracts, and both Soxhlet and accelerated solvent extraction (ASE) techniques have been employed to this end. However, a study demonstrating that the two methodologies provide comparable data is presently absent in literature.

To address this issue, experiments were performed to compare ASE and Soxhlet methods for aqueous extraction of representative corn stover, switchgrass, and sorghum samples. For each extract, the mass percent of water-soluble materials was determined gravimetrically, and sugar composition was determined via high performance liquid chromatography with pulsed amperometric detection (i.e., HPAE-PAD). Subsequent experiments were performed to evaluate the number of extraction cycles required for “exhaustive extraction” using the ASE approach. Mean data (n ≥ 3) were compared at the 95% confidence limit, using a t test or F test, as appropriate, to assess statistical differences in mean data. No statistical differences in % extractives or sugar content were observed among extracts prepared using either the Soxhlet approach or ASE with 2 or more extraction cycles. This study strongly suggests that use of either extraction approach will result in aqueous extracts of comparable composition for a variety of herbaceous biomass materials.

**Poster 3-36**

What are the consequences of bark and whitewood utilization during bioconversion of yellow poplar to ethanol?

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Most of the research in bioconversion of hardwoods to ethanol has explored the use of whitewood feedstocks, failing to appreciate that the realistic commercial woody biomass will likely contain bark since debarking might further increase the overall bioethanol cost. In this study we have examined the feasibility of producing bioethanol from the realistic biomass, bark + whitewood from yellow poplar. The overall sugar recovery, hydrolysis and ethanol yields will be compared with the control biomass, yellow poplar whitewood. Ultimately we will discuss the impact of bark utilization on pretreatment, hydrolysis and fermentation sub-processes as well as biomass storage during bioethanol production.

**Poster 3-37**

Dilute acid hydrolysis of wheat straw oligosaccharides

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Among the promising biomass pre-treatment options available (e.g. autohydrolysis, steam explosion, alkaline and organosolv treatments), many produce a sugar rich liquid stream derived from selective hemicellulose solubilization. Unfortunately, the sugars present are typically in oligomeric form. The hydrolysis of these hemicellulosic oligosaccharides, is a compulsory requirement for direct fermentation to ethanol, as there are not yet any effective microbial catalyst that can directly metabolize them. The posthydrolysis options can be reduced to acid or enzymatic catalyzed hydrolysis, with acid hydrolysis typically presenting both higher yield and productivity. In this work the dilute acid posthydrolysis of wheat straw hemicellulosic oligosaccharides obtained by autohydrolysis was evaluated. We have used a Doehlert experimental design to study the effect of catalyst concentration (sulfuric acid, 0.1-4% w/w) and reaction time (0-60 min) in order to maximize monosaccharide recovery and minimize byproduct formation. Both the main effects are statistical significant for sugar recovery and interaction effects also play a significant role. Conversely byproducts concentration is mainly affected by acid concentration.

Under the optimized conditions it was possible to obtain a significant increase in monosaccharides content (above 15%) together with a slight decrease in inhibitors content compared to the standard acid hydrolysis treatment. Furthermore this is achieved with a close to 60% less acid spending.

This work has been funded by CEBio (AdI) and BIOREFINO (FCT) projects.

**Poster 3-38**

Evaluation of Acid and Enzymatic Hydrolysis from Hemicellulose Hydrolyzates on Mixed Pulping Wood

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Forest biomass is a promising resource for future biofuels and bioproducts. Biorefining wood into paper and chemicals is not as easy as making a single traditional paper product. Paper is made from the solid cellulose fraction of wood, removing lignin and hemicellulose components through a liquid pre-extraction enhance the quality of the pulp. Pre-extraction of hemicellulose by alkaline (Green Liquor) pretreatment produces a neutral-pH extract containing hemicellulose. One near term option is to carefully pre-extract the hemicellulose before the main pulping step and then ferment it to bioethanol. A significant difference with other lignocellulose biomass conversion processes is that the solid fraction has high value to make pulp and paper products and is thus not converted to liquids or boiler fuel. A secondary hydrolysis step is required after primary pre-extraction to hydrolyze oligomeric sugars into monomeric sugars before fermentation. In this study, we investigate the extent of hemicellulose recovery by pre-extraction using green liquor pretreatment and characterize the hydrolysis of the extract with respect to variable concentration via evaporation and comparing acid and enzymatic hydrolysis.
Effect of Drying Method on AFEX Pretreatment and Enzymatic Hydrolysis of Year-Old Poplar
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Previous studies have shown that drying of forages can have an impact on cell wall composition and ruminant digestibility of these materials. Because of the potential variability in drying options following field harvest of biomass for ethanol production, it is important to determine if the type of drying, or the act of drying has an impact on pretreatment susceptibility and/or sugar yields following enzymatic hydrolysis. Ammonia fiber expansion pretreatment (AFEX) followed by enzymatic hydrolysis was performed on year-old NM6 (P. nigra x P. maximowizii) poplar cuttings which had been dried at a variety of conditions. Three samples were dried following mulching and prior to milling: air-dried, oven-dried at 38°C, and oven-dried at 71°C; and one sample was freeze-dried following milling. An additional sample was left undried as a control. Optimal AFEX pretreatment conditions were determined for each drying condition and sugar yields following enzymatic hydrolysis were compared between samples.

Optimizing the hydrolysis of sugarcane bagasse hemicellulose with dilute H2SO4 through empirical modeling: An experimental approach to increase the yield of xylose recovery
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Dilute-acid hydrolysis has frequently been used to hydrolyze the hemicelluloses of many lignocellulosic materials, leading to xylose-rich hydrolysates that can be used for the production of goods like xylitol and ethanol. In the present study, experiments based in a 2⁰ central composite full factorial design were carried out in 200 mL stainless steel containers in order to improve the yield of xylose recovery from the sugarcane bagasse hemicellulose (Yx). The temperature (A), the sulfuric acid concentration (B) and the residence time (C) were considered as the independent variables, while the Yx was taken as the response variable. Bagasse loading of 15 % (dry-mass basis) was used in all the experiments. According to the results, all the three independent variables influenced the Yx significantly (p < 0.05). A quadratic model, $Y_{x} = 37.09 + 8.89 B + 11.26 C - 7.16 B^2 - 9.89 C^2$, was developed to correlate the response variable with both the acid concentration and the residence time, maintaining the temperature at 150 °C. According to the aforementioned model, an $Y_{x}$ of 54.4 ± 7.1 % of the maximum theoretical value would be obtained by performing the hydrolysis at 2.0 % (w/v) acid concentration and 28 min. Such prediction was further confirmed by an additional hydrolysis at the optimum conditions, an $Y_{x}$ of 57.3 % being obtained. This hydrolysate presented a high xylose concentration (33.2 g/L), which would reduce the necessity to increase the substrate content by vacuum concentration prior to the subsequent fermentation.

Acknowledgements: FAPESP and CNPq (Brazil)

Effects of the extraction with ethanol or cyclohexane/ethanol on the structural composition of sugarcane bagasse
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The effects of submitting the sugarcane bagasse to a previous extraction with ethanol or cyclohexane/ethanol (2:1) in its structural composition were determined in this study. Such extraction not only affects the percentages of the main components determined after a two-step acid hydrolysis, but could also lead to a hemicellulosic hydrolysate with lower amounts of extractives, compounds that can inhibit the microbial activity. After milling, the bagasse was maintained in contact with each one of the solvents in a Soxhlet extractor until the solvent became colorless. After extensive washing with deionized water, the extracted bagasse was dried in an oven at 105 °C and had its composition determined after two-step acid hydrolysis under standard conditions. According to the results, the structural compositions of the non-extracted bagasse, of the bagasse extracted with ethanol and of the bagasse extracted with cyclohexane/ethanol, respectively, were as follows: cellulose (46.9 ± 0.2, 46.1 ± 0.1 and 45.4 ± 0.3), hemicellulose (27.5 ± 0.1, 27.1 ± 0.2 and 26.2 ± 0.1), lignin (26.3 ± 0.7, 23.2 ± 0.4 and 21.5 ± 0.2), ashes (1.6 ± 0.0, 1.6 ± 0.0 and 1.5 ± 0.0) and extractives (0.0, 4.1 ± 0.1 and 6.1 ± 0.0). We observed that the relative percentages of cellulose, hemicellulose and ashes determined in the present study did not differ significantly from those determined previously in our Institution. On the other hand, the amount of extractives determined in this study was higher than those described previously (3.0 %, in average).

Acknowledgements: Fapesp and CNPq (Brazil)

Development of High Throughput Pretreatment Systems for Cellulosic Biomass
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High throughput (HTP) pretreatment systems could facilitate the rapid screening of many biomass feedstocks to identify those with enhanced features for biological conversion. Such HTP systems must accomplish five steps: 1) the loading of milled biomass, water, and acid into the reactor; 2) pretreatment of the biomass; 3) separation of the solids and liquid following pretreatment; 4) washing of the solids; and 5) dispensing the washed solids and liquid into HTP systems for enzymatic hydrolysis and fermentation. This paper will evaluate several approaches that could be employed to deal with the challenges associated with linking these operations at a small scale. For example, the pretreatment reactor can be designed either as a single pretreatment vessel that feeds the enzymatic hydrolysis system or as smaller reactors that each feed separate enzymatic hydrolysis systems. Indirect, direct, or microwave heating can be used with either of these systems. Following pretreatment, solid-liquid separation can be accomplished either by filtration or centrifugation, and washing the solids can be achieved in either a batch or continuous process. Several options are available for automatic and accurate weighing and dispensing of the solids for enzymatic hydrolysis. Advantages and disadvantages of each solution will be also be presented.

Acknowledgements: FAPESP and CNPq (Brazil)
The aim of this study is the search of alternatives for upgrading residues generated by the agro food processing industry. Two consecutive alternatives have been developed in this project: bioactive compounds extraction and bioethanol production.

Bioactive compounds have been extracted from vegetable wastes in the canning industry. Firstly, the characterisation of wastes was carried out, measuring the content of fiber, antioxidant capacity, and different vitamins. Subsequently, the extraction of vitamins was made by supercritical fluid extraction (SFE) from a mixture of waste and water content and particle-size. Extraction conditions were optimised. A yield of extraction of 41.4% (extracted/initial) was obtained working at 280 bar, 50°C and particle-size between 0.3 and 0.5 mm.

In order to optimize bioethanol yields, preliminary pre-treatments were carried out varying temperatures (110°C -120°C) and residence times (5 to 20 min). Assays were performed with dry matter content between 5% and 10% with working volumes that varied from 20 ml to 400 ml, in the absence or presence of sulphuric acid (concentration ranged from 0.5 to 1.0 % (wt/wt)). Enzymatic hydrolysis was carried out at 50°C for 72 h followed by fermentation assayed at 37°C using Saccharomyces cerevisiae.

Characterization of vegetable wastes before and after SFE showed no major differences. Pretreatment process reached a solubilization of 53.6 - 57.7% of the total initial sugar content. Enzymatic hydrolysis increased this yield in between 8.8 to 9.0 %. Fermentation yields obtained varied from 72-92% with respect to theoretical.

Poster 3-44
Pretreatment of Rice Straw with Ammonia Recycled Percolation
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Because of high contents of cellulose (~37 wt%) and hemicellulose (~17%), rice straw seems to be a potential lignocellulosic biomass for production of bioethanol. In this study, Ammonia Recycled Percolation (ARP) pretreatment of rice straw was extensively investigated. In particular, the experimental study included the effects of temperature, reaction time and concentration of ammonia on compositions and enzymatic digestibility of the resulting solid residues; the ranges of pretreatment conditions were, in turn, 150°C-210°C, 10 min ~ 90 min and 0~ 20 wt%. With ARP pretreatment, the lignin content was reduced by as high as ~84% while 20~80 % of the hemicellulose was solubilized. The solid residue resulted from the pretreatment with 15 wt% aqueous ammonia solution at 170°C for 90 min showed as high as ~90% of digestibility with enzyme loading of 15FPU/g of glucan. Addition of the xylanase along with cellulase led to a notable enhancement of digestibility, indicating a speculative inhibitory role of hemicellulose. Simultaneous Saccharification and Fermentation (SSF) and Simultaneous Saccharification and Co-Fermentation (SSCF) were also performed to obtain ethanol productions of 1.38 g/L (corresponding to 81% yield) and 15 g/L (corresponding to 89% yield), respectively.

Poster 3-45
Two complementary methods to determine sugars and organic acids profile of some feedstocks and wastes, using high performance liquid chromatography
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Bioethanol produced by microbial fermentations is an excellent alternative to fossil fuels, particularly when feedstocks used are waste products. A rigorous description of the sugars profile of the feedstocks provides critical information about sugar yields in the subsequent production of bioethanol.

In this work two complementary chromatographic methods are developed to analyze sugar profiles (monosaccharides and disaccharides) and some organic acid that are present in different biomass feedstocks and wastes.

Both methods are based in cation exchange chromatography, first one with hydrogen (H+) and second one with lead (Pb2+) ionic forms, and are operated with a RI detector under isocratic elution conditions.

One of the major drawbacks of the first method is that for certain sugars, a low resolution can be obtained. In fact, mannose, galactose, xylose and fructose elute together in one peak. However, other different sugars can be quantified with a good resolution (cellobiose, glucose, arabinose, ribose) and some organic acid like citric, malic, galacturonic, glucuronic and oxalic.

Nevertheless, the second method allows a good enough quantification of the sugars that can not been well resolved in the first ones: sucrose, glucose, xylose, galactose, arabinose, mannose and fructose.

Finally, we have confirmed the validity of both methods comparing the RSD (Relative Standard Deviation) values of sugars that can be properly quantified with both methods.

In short, with these two complementary methods we have obtained a complete and precise profile of the sugars and certain organic acids that are present in some feedstocks and wastes.

Poster 3-43
Multiple valuation of residues generated by the agro food processing industry
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The aim of this study is the search of alternatives for upgrading residues generated by the agro food processing industry. Two consecutive alternatives have been developed in this project: bioactive compounds extraction and bioethanol production.

Bioactive compounds have been extracted from vegetable wastes generated in the canning industry. Firstly, the characterisation of wastes was carried out, measuring the content of fiber, antioxidant capacity and different vitamins. Subsequently, the extraction of vitamins was made by supercritical fluid extraction (SFE) from a mixture of waste and water content and particle-size. Extraction conditions were optimised. A yield of extraction of 41.4% (extracted/initial) was obtained working at 280 bar, 50°C and particle-size between 0.3 and 0.5 mm.

In order to optimize bioethanol yields, preliminary pre-treatments were carried out varying temperatures (110°C -120°C) and residence times (5 to 20 min). Assays were performed with dry matter content between 5% and 10% with working volumes that varied from 20 ml to 400 ml, in the absence or presence of sulphuric acid (concentration ranged from 0.5 to 1.0 % (wt/wt)). Enzymatic hydrolysis was carried out at 50°C for 72 h followed by fermentation assayed at 37°C using Saccharomyces cerevisiae.

Characterization of vegetable wastes before and after SFE showed no major differences. Pretreatment process reached a solubilization of 53.6 - 57.7% of the total initial sugar content. Enzymatic hydrolysis increased this yield in between 8.8 to 9.0 %. Fermentation yields obtained varied from 72-92% with respect to theoretical.

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Poster 3-45
Two complementary methods to determine sugars and organic acids profile of some feedstocks and wastes, using high performance liquid chromatography
B. Zarranz*, M. Zazpe, I. Alegría, A. Molinero, M. Macaya and I. Echeverría
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In this work two complementary chromatographic methods are developed to analyze sugar profiles (monosaccharides and disaccharides) and some organic acid that are present in different biomass feedstocks and wastes.

Both methods are based in cation exchange chromatography, first one with hydrogen (H+) and second one with lead (Pb2+) ionic forms, and are operated with a RI detector under isocratic elution conditions.

One of the major drawbacks of the first method is that for certain sugars, a low resolution can be obtained. In fact, mannose, galactose, xylose and fructose elute together in one peak. However, other different sugars can be quantified with a good resolution (cellobiose, glucose, arabinose, ribose) and some organic acid like citric, malic, galacturonic, glucuronic and oxalic.

Nevertheless, the second method allows a good enough quantification of the sugars that can not been well resolved in the first ones: sucrose, glucose, xylose, galactose, arabinose, mannose and fructose.

Finally, we have confirmed the validity of both methods comparing the RSD (Relative Standard Deviation) values of sugars that can be properly quantified with both methods.

In short, with these two complementary methods we have obtained a complete and precise profile of the sugars and certain organic acids that are present in some feedstocks and wastes.
**Poster 3-46**  
**Effect of varying AFEX pretreatment severity on corn stover cell wall ultra structure and its related degradation reactions**  
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The severity (time, temperature and ammonia loadings) of AFEX (Ammonia Fiber Expansion) pretreatment is thought to play an important role on modification of the Lignin-Carbohydrate-Complex (LCC) Bridge. The LCC bridge is comprised of hemicellulose side-chains (e.g. acetyl groups, uronic acid and arabino residues) and phenolic aromatic moieties (e.g. ferulic, cinnamic, p-coumaric) that help strongly bind hemicellulose to core lignin. Hence, the severity of AFEX pretreatment would be very closely linked to the digestibility of the treated plant cell wall. The optimum composition and concentration of individual enzyme activities required to achieve higher glucan and xylan conversions is likely dependent on the cell wall ultra structure and ultimately, the severity of pretreatment.

We examined the morphological and ultra structural changes in the corn stover cell wall upon AFEX pretreatment of varying severities, using an environmental scanning electron microscope (eSEM), laser scanning confocal microscope (LSCM) and an atomic force microscope (AFM). The chemistry of AFEX was better understood through identification and quantification of degradation compounds produced during the process. Solvent extraction of the untreated and AFEX treated corn stover was carried out using an accelerated solvent extractor (ASE) for subsequent LC/GC-MS analysis. MALDI-TOF-MS analysis was conducted to determine the degree of polymerization and acetylation of arabino-xylosaccharides produced during AFEX. Quantification of arabino-xylosaccharides was conducted by measuring sugars released during acid hydrolysis of the ASE extract. The overall goal of this project was to study the effect of varying AFEX pretreatment severity on the cell wall ultra structure and its related degradation reactions.

**Poster 3-47**  
**Lime pretreatment of sugarcane bagasse for fuel bioethanol production**  
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In the last years there is a great deal of interest in the utilization of lignocellulosic materials as renewable resources for production of fuel ethanol. Among lignocellulosic materials, the use of agricultural residues has also the benefit of disposal of problematic solid wastes which usually presents few economic alternatives. One of the major lignocellulosic materials to be considered in tropical countries, as Brazil, is sugarcane bagasse.

In this work the pretreatment of sugar cane bagasse with lime (calcium hydroxide) is evaluated. The effect of lime pretreatment on digestibility was studied through analyses using a complete 2^3 factorial design, considering pretreatment time, temperature and lime loading as factors. The responses evaluated were the yield of total reducing sugars (TRS) and glucose released from pretreated bagasse after enzymatic hydrolysis. Experiments were performed using the bagasse, as it comes from an alcohol/sugar factory (non-screened bagasse) and bagasse in the size range from 0.248 to 1.397 mm (screened bagasse) (12-60 mesh). It was observed that the particle size presented weak influence in the release of fermentable sugars after enzymatic hydrolysis using low loading of cellulase and β-glucosidase (3.5 FPU/g dry pretreated biomass and 1.0 IU/g dry pretreated biomass, respectively).

The optimal pretreatment conditions were determined for non-screened bagasse since the preliminary results for pretreatments of non-screened bagasse and for screened bagasse were not very different. As screening is an expensive unit operation, the use of non-screened bagasse was preferred. Analyses were performed using central composite (response surface) to determine the optimal conditions.

**Poster 3-48**  
**Cellulosic ethanol production from sugarcane bagasse using lime**  
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The daily consumption of gasoline in the United States was estimated to be about 400 million gallons, or 146 billion gallons annually in 2004. A goal expressed by the US government is to replace 30% of gasoline consumed in 2004 with ethanol by the year 2030. A Production of ethanol from cellulosic biomass as well as from corn is required to meet this goal. An efficient and economical pretreatment of biomass is essential for cellulosic ethanol production. Sugarcane bagasse was pretreated with lime in a process of producing simple sugars for fermentation to ethanol. After dewatering and pH adjustment with acid, the fibrous material was rapidly solubilized by cellulases, at solids loading ranging from 10 to 30 % (w/w). Composition analysis showed that the lime pretreatment process did not damage either the cellulose or hemicellulose and removed about 28% (w/w) of lignin. Lime treated lignocellulosic material did not produce the inhibitory effects on cellulase activity or fermentation and compared favorably with pure cellulose control. The process was demonstrated at a pilot scale. Production of cellulosic ethanol from sugarcane bagasse was demonstrated.

**Poster 3-49**  
**Comparing AFEX Pretreatment Conditions for Corn Stover Versus Poplar**  
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There is a growing need to find alternatives to crude oil as the primary feedstock for the chemicals and fuels industry. Ethanol has many desirable features as a petroleum substitute and could help smooth the transition from a petroleum-based to a bio-based economy. Among the various feedstocks for producing ethanol, corn stover and hybrid poplar (Populus nigra x Populus maximowiczii) are interesting due to their wide availability in the US. Corn stover could be pretreated effectively using milder AFEX pretreatment conditions, while on the other hand poplar needs harsher AFEX conditions to obtain high sugar yields after enzymatic hydrolysis. Details of the pretreatment conditions and sugar yields with varying enzyme loadings will be discussed. We address subtle ultrastructure differences between corn stover and poplar and also reaction products generated during the AFEX process for both feedstocks. This research was performed within the Consortium for Applied Fundamentals and Innovation (CAFI2).
Poster 3-50

Nutritional evaluation of vapor-phase diethyloxalate (DEO) pretreated corn stover hemicellulosic hydrolysate to improve xylitol production by a Pichia stipitis D-xylulokinase mutant

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Corn stover, contains 70% cellulose + hemicellulose and 15-20% lignin. The hemicellulose can be converted to monomeric and oligomeric sugars by hydrolysis, to form a xylose-enriched liquid hydrolysate fraction that can be used for xylitol production. Xylitol has many interesting applications in the food, pharmaceutical, and odontological industries. *Pichia stipitis* FPL-YS30 (xy3) is a D-xylulokinase mutant that converts xylose mainly into xylitol. This yeast was cultivated in corn stover hemicellulosic hydrolysate, which was obtained by vapor phase diethyloxalate (DEO) pretreatment. This work evaluated supplementation of corn stover hemicellulosic hydrolysate (CSHH) obtained by DEO pretreatment to improve xylitol production by *P. stipitis* FPL-YS30. The hydrolysate pH was increased to 5.5 with Ca(OH)₂ before sterilization by membrane filtration. Different media used urea, yeast extract, phosphates and magnesium salts. Experiments were carried out in 125 mL Erlenmeyer flasks containing 50 mL of medium and inoculum (1.0 g/L) on a rotary shaker at 200 rpm, 30°C. Addition of phosphates and magnesium salts favored the specific cell growth, xylose consumption and xylitol production rates by *P. stipitis* FPL-YS30. The maximum xylitol yield (0.766 g/gxylose) was obtained in medium containing urea, yeast extract, phosphates and magnesium salts. The L-arabinose was totally or partially (17%) consumed depending on the presence or absence of salts, respectively. In general, the acetic acid was consumed concomitantly with the xylose. The untreated CSHH obtained by DEO pretreatment contains low acetic acid and phenolic compounds. Its supplementation by adding phosphates and magnesium salts improved xylitol production by *P. stipitis* FPL-YS30.

Poster 3-51

Optimization of an aqueous ammonia-soaking process for the enhanced enzymatic hydrolysis of rice straw by cellulase

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Rice straw, which is the major lignocellulosic biomass in South Korea, was pretreated by using aqueous ammonia at moderate temperatures for the enhancement of enzymatic hydrolysis to achieve maximum amount of fermentable sugar. In this study, the effects of various operating variables including temperature, time, concentration of ammonia, and solid-to-liquid ratio (S/L ratio) on the degree of delignification and the enzymatic digestibility were investigated and optimized by using a response surface methodology (RSM). The ranges of the batch reaction conditions tested were a temperature of 50-70°C, a reaction time of 4-12 h, and an ammonia concentration of 12-28 wt% at a fixed S/L ratio in 1:6. In the present study, the digestibility of the aqueous ammonia-pretreated rice straw by commercial cellulase was significantly increased in comparison with the control which was not pretreated with ammonia. The experimental and RSM results showed that the effects of reaction time and temperature were significant on the degree of lignin removal and enzymatic digestibility of the rice straw. The ammonia concentrations in the range tested in this study had little impact on the delignification and enzymatic digestibility of the rice straw. The optimum reaction conditions for achieving the maximum delignification and enzyme digestibility, which were determined to be out of experimental range by the canonical analysis, was further found by using the ridge analysis.

Poster 3-52

Effect of wet disk milling-pretreatment without sulfuric acid for enzymatic hydrolysis of rice straw

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Recently, rice straw which can be a potential source for ethanol production has been attracted a lot of interest in Japan. We investigated a wet disk milling-pretreatment without sulfuric acid of rice straw, and compared with the conventional methods such as a ball milling, and a hydrothermal treatment in this study. The rice straw cut out to less than 2 mm by the cutter mill was pretreated by the wet disk milling (10 cycles in continuous treatment), the ball milling (60 min) and the hydrothermal treatment (160 °C or 180 °C, 30 min), respectively. The pretreated materials were hydrolyzed by the mixture of the Acremonium cellulase (4-40 FPU/g dry rice straw) and the commercial hemicellulase Optimash BG. The glucose yields obtained from the wet disk milling, the ball milling and the hydrothermal treatment were 290-350 mg/g dry sample, 300-370 mg/g and 270-320 mg/g, respectively. The xylose yield obtained from these pretreatment were 90 mg/g, 90-120 mg/g and 50-110 mg/g, respectively. These results indicate that the wet disk milling was effective for enzymatic hydrolysis as well as other conventional pretreatments. Moreover, the wet disk milling is not able to treatment continuously but also can save energy as compared with ball milling. The wet disk milling can be an economical pretreatment for enzymatic hydrolysis of rice straw. This work was supported by the Resiionl Biomass Energy Project, Ministry of Agriculture, Forestry and Fisheries, Japan.

Poster 3-53

Olive tree biomass as a raw material for ethanol production. Comparison of pretreatments

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Olive tree biomass obtained by pruning constitutes one of the main agricultural residues in Mediterranean countries. Olive tree culture practices include pruning, an essential annual operation that eliminates unproductive branches and prepares trees for the next crop. Pruning generates a huge amount of a cheap, renewable lignocellulosic residue, lacking of alternative uses, which must be eliminated to prevent propagation of vegetal diseases. To date, pruning residues are either burnt or grinded and scattered in fields, causing environmental concerns and disposal costs. The use of olive tree biomass as a raw material for fuel ethanol production has been proposed. Ethanol production from lignocellulose residues requires, as a first step, a residue pretreatment. In this work, several pretreatment methods including steam explosion (with or without water or acid preimpregnation), liquid hot water, and dilute acid prehydrolysis are compared on olive tree pruning biomass. The pretreatment performance was evaluated based on both the improvement in cellulose digestibility (pretreated solid residue) and the hemicellulose sugar recovery (liquid fractions).

Results show that the highest ethanol yields from the cellulose residue are obtained when applying hydrothermal pretreatments, but the hemicellulosic sugar recoveries are quite low. On the other hand, dilute acid pretreatment produced higher sugar yields taking into account both glucose from enzymatic hydrolysis of the pretreated solid and soluble and hemicellulosic derived sugars in the liquid fractions issued from pretreatment.
Posters

Poster 3-54
Characterization and mapping of lignin during bioethanol process: Comparison between thermochemical and oxidative pretreatment processes

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The objective of the study was to characterize and map changes in lignin during pre-treatment/hydrolysis/fermentation of biomass to ethanol. Chemical composition with regard to cellulose, hemicellulose, lignin, extractives and ash along with the changes in lignin molecular weight distribution and structure throughout different steps of two different bioethanol processes were compared. The two processes included are thermochemical pre-treatment from the IBUS process (www.bioethanol.info) and steam explosion combined with wet oxidation from the BioGasol company (www.biogasol.dk). Both processes include subsequent enzymatic hydrolysis and fermentation. The results reported cover changes in overall composition, lignin molecular weights and location as well as AFM and confocal microscopy of the processed biomass.

Poster 3-55
Withdrawn

Poster 3-56
Preparation and characterization of modified cellulose substrates for cellulase digestion studies

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There has been an increasing interest in the conversion of lignocellulosic biomass to ethanol; however, a major barrier to commercialization of this technology is the high cost of the enzymes needed to saccharify cellulose. A significant effort is underway in laboratories throughout the world to improve our understanding of the action of cellulosylotic enzymes on the cellulose in pretreated plant cell walls. In this study, we will demonstrate a methodology for preparing modified cellulose substrates for advanced cellulase action studies. Cellulose substrates with different degrees of polymerization were prepared and analyzed using high performance size exclusion chromatography (HPSEC) and liquid NMR. The method chosen for this study, carbonilation using phenyl isocyanate, minimizes sugar degradation and the formation of byproducts. Amorphous cellulose substrates were also produced by several different methods and their properties were characterized by x-ray diffraction, NMR, differential scanning calorimetry (DSC), and thermogravimetric analysis (TGA). Microcrystalline celluloses and cellulosics from pretreated biomass have been analyzed. These modified cellulose substrates will be further studied with purified cellulases.

Poster 3-57
Simultaneous Saccharification and Ethanol Production from Pretreated Waste Oak Wood by Aqueous Ammonia

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Oak wood and waste oak wood were pretreated with aqueous NH, in a flow-through column reactor, ammonia recycled percolation (ARP), for ethanol production through simultaneously saccharification and fermentation (SSF). This pretreatment method is highly effective in delignification and in hemicellulose solubilization of lignocellulosic biomass. We have previously investigated on various pretreatment processes using a flow-through (percolation) reactor system in our laboratory. The primary purpose of this investigation is to assess the effectiveness of the ARP treatment as a pretreatment process specifically for oak wood. We were interested in verifying the changes in chemical composition and physical characteristics of biomass brought about by the pretreatment and how those factors affect the enzymatic digestibility. Most of the lignin removal occurred within the first 20 minutes of the reaction. The ARP process solubilizes 40-50% of the hemicellulose in liquor but leaves the cellulose content intact in solid. The solubilized carbohydrate exists in oligomeric form. Decomposition of carbohydrates during the pretreatment is insignificant. The digestibility of the treated waste oak wood is substantially higher than that of a-cellulose. The enzymatic digestibility is correlated with the extent of lignin removal and hemicellulose removal perhaps due to increased surface area and porosity. Conversion of cellulose biomass to ethanol involves SSF.

Poster 3-58
Pretreatment of Rigida Pine Wood by Acid and Ammonia Percolation

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A rigida pine wood collected from a Korean forest has been tested for acid and ammonia percolation pretreatment. The rigida pine wood has different physical characteristics from that of pine wood. However, there is little difference in the chemical compositions between the wood. Due to the difference in physical characteristics, the effects of the pretreatment conditions were also quite different. While the optimum temperature was determined to be 150°C for rigida pine wood, the optimum pretreatment was possible at 170°C with pine wood for ammonia pretreatment. Presoaking for 12 hours with ammonia solution before pretreatment was helpful to increase the delignification efficiency. The acid percolation was conducted for conversion of this biomass into fermentable sugars. A kinetic study was first conducted using batch reactors wherein the maximum yield was found to be less than 50%. In order to improve the yield, a percolation reactor was employed and its performance against rigida pine wood was assessed. The performance data of the percolation reactor over the range of 180-200°C, 0.5-5% acid and other process related factors are reported. On the basis of the data of batch and the percolation reactor, the kinetics and the mechanism of the hydrolysis reaction are discussed.
Steam pretreatment and enzymatic hydrolysis of sweet sorghum bagasse

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Sweet sorghum is an attractive feedstock for ethanol production. The juice extracted from the fresh stem is mainly comprised of sucrose, glucose and fructose and can therefore be readily fermented to ethanol. The solid fraction left behind, the so-called bagasse, is a lignocellulosic residue, which needs to be pretreated before processing to ethanol.

One of the most efficient methods investigated so far for the pretreatment lignocellulosics is steam pretreatment. The process employs saturated high-pressure steam to the material to be treated, which is thereafter decompressed into atmospheric pressure. The method is often combined with acidic or alkaline catalysis.

The objective of this work was to optimize pretreatment of sweet sorghum bagasse by steam pretreatment using varying temperatures (180°C, 190°C, 200°C) and residential times (5 minutes and 10 minutes) after impregnating the raw material with 2% SO2. Pretreatment experiments were performed at Lund University, Department of Chemical Engineering. Efficiency of treatments were evaluated via the enzymatic digestibility of the whole slurry obtained after steam pretreatment using a substrate concentration of 2% and an enzyme (Celluclast 1.5L) loading of 20 FPU/g glucose.

The increasing severity enhanced the enzymatic digestibility. Two of the studied conditions (190°C, 10 min; 200°C, 5 min) were found to be efficient enough to reach a conversion of 90-95%. Efficiency of pretreatments was also checked using separated and washed fibers in the hydrolysis experiments carried out under similar conditions to those applied during the hydrolysis of the whole slurry. Liquid fractions were evaluated upon their sugar and inhibitor content.

Biotechnological Production of Xylitol: Optimization of Monosaccharide Recovery by Post-Hydrolysis of Hemicellulosic Hydrolysate after Acid Hydrolysis of Sugarcane Bagasse

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Sugarcane bagasse hemicellulosic hydrolysate was obtained with an initial xylose concentration of 18.4 g/L. The main product was xylose. The acid hydrolysis represented a conversion efficiency (hemicellulose to xylose) of 73%, considering that the maximum theoretical xylose concentration found in the hemicellulosic hydrolysate is 25 g/L. In order to increase the xylose concentration found in the obtained hemicellulosic hydrolysate, a post hydrolysis stage was carried out. At the end of the post-hydrolysis stage, almost a complete conversion of xylose-oligosaccharides to xylose-monosaccharides took place, resulting in a 20% increase in the total xylose concentration, from 18.4 g/L in the hemicellulosic hydrolysate to 23.5 g/L in the post-hydrolysate. The post-hydrolysis stage consisted in heating the obtained hemicellulosic hydrolysate at 121°C for 10 min. Such heating will prevent the re-polymerization of the produced monosaccharides and at the same time will degrade the formed oligosaccharides, thus leading to an optimum xylose extraction from the hemicellulosic fraction of sugarcane bagasse. Fermentation process for xylitol production using post-hydrolysate showed better xylene-xylitol conversion efficiency of 76% (0.7 g xylitol/g xylose) and volumetric productivity of 0.68 g xylitol/Lh than the original hemicellulosic hydrolysate, which resulted in a conversion efficiency of 71% (0.65 g xylitol/g xylose) and volumetric productivity of 0.61 g xylitol/Lh. Such results were expected since the inhibitors concentrations were higher for the original hydrolysate than that for post-hydrolysate, thus more toxic substances were found in the original hydrolysate that influenced negatively in the fermentation process yield and productivity.

Acknowledgments: FAPESP, CNPq
Poster 3-63
Genencor Pretreatment Survey
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For many years Genencor has been a leading developer of cellulases and supplemental enzymes for the conversion of biomass to fermentable sugars and other subsequent products such as fuel ethanol. As part of a larger commitment to the developing industry, we have greatly expanded Genencor's capability in the biomass applications area. It is understood that there is a need for better and cheaper enzymes; but a greater comprehension of enzyme-substrate interactions is equally important. Enzymatic hydrolysis can have a strong impact on upstream and downstream processes.

A wide range of biomass substrates and pretreatments have been evaluated for enzymatic hydrolysis performance. Presented here are approximately 80 material samples analyzed with Genencor's first commercially available biomass cellulase, Accellerase™ 1000. These are represented by pretreatments such as dilute acid, steam expansion, autocatalysis, and two-stage acid/alkali on substrates such as corn stover, sugar cane bagasse, wood pulp, rice straw, and corn fiber. Saccharifications were performed in 100mL reaction volumes in shake-flasks. Each substrate has been surveyed at a loading of 1%, 7%, and, when possible, 13% cellulose. Enzyme loadings were fixed at 20 and 80mg total protein/g cellulose. Substrates were then ranked on "ease of saccharification" based on a benchmark criterion of what dose of Accellerase™ 1000 would be required to reach 80% glucan conversion in 3 days at a 7% cellulose load. Substrate attributes and pretreatment conditions were correlated with hydrolysis performance such as to give further insight into what makes biomass most amenable to enzymatic hydrolysis.

Poster 3-64
Microscopic investigation of enzymatic hydrolysis of pretreated wheat straw
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Recalcitrance of lignocellulosics to enzymatic hydrolysis is a well-described challenge in the production of bioethanol. A better understanding of parts of the cell wall are exposed to hydrolysis and whether the recalcitrance is possibly due to inefficiencies of the enzymes used is important in overcoming this challenge. We have used atomic force microscopy (AFM) and scanning electron microscopy (SEM) to investigate the effect of various enzyme preparations in the partial hydrolysis of pretreated wheat straw with respect to sugar production and physical changes. When subjected to partial hydrolysis, no apparent opening of the cell wall structures was seen. When hydrolysed more thoroughly, some disruptions and openings could be identified but large areas still seemed untouched.

Poster 3-65
Exploring Advanced Characterization Tools to Study the Structure of the Plant Cell Wall at the Submicron Scale
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Biomass has long been recognized as a potential sustainable source of mixed sugars for fermentation to fuels and other bio-based products. However, biofuels produced by today's technology are not cost competitive, due to the inefficiency of the chemical and enzymatic conversion techniques used in the processes. The improvement of these processes undoubtedly relies on further understanding of the fundamental structure of the plant biomass. The plant cell walls are a complex material on the nanometer scale. Advanced characterization techniques are required to allow us to investigate the plant cell wall structure with high structural and chemical resolution. To accomplish this task, we are developing and applying microscopic and spectroscopic tools, consisting of both nonlinear optical and spectroscopic microscopy, to enable investigating of the plant cell wall constituents and their changes during conversion processes. Preliminary results have demonstrated that it is feasible to map the structure and chemistry of plant cell walls at the submicron scale using an integrated imaging system.

This work has been authored by an employee or employees of the Midwest Research Institute under Contract No. DE-AC36-99GO10337 with the U.S. Department of Energy. The United States Government retains and the publisher, by accepting the article for publication, acknowledges that the United States Government retains a non-exclusive, paid-up, irrevocable, worldwide license to publish or reproduce the published form of this work, or allow others to do so, for United States Government purposes.

Poster 3-66
Pretreatment and Fractionation of Corn Stover by S.E.A.A. (Soaking In Ethanol and Aqueous Ammonia)
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Soaking in Aqueous Ammonia (SAA) has been proven to be an effective pretreatment method for lignocellulosic biomass. SAA is only one of the few methods for pretreatment of biomass in which almost 100% glucan and greater than 80% xylan are retained whereas a significant percentage of lignin is removed. Despite this high efficiency in carbohydrate preservation still about 20% xylan is solubilized together with lignin and hence is not available for conversion to ethanol in the subsequent fermentation step.

We have developed a modification of the SAA method, a Soaking in Ethanol and Aqueous Ammonia method (SEAA), to reduce the loss of xylan due to solubilization. In this method ethanol is added to the aqueous ammonia solution. The presence of ethanol causes re-precipitation of the previously solubilized xylan onto the solid matrix. Thus more xylan is available for conversion to ethanol. The ethanol used in the pretreatment process can be recovered and recycled. In addition to increased availability of the carbohydrates for ethanol production, the solubilized lignin theoretically can be recovered in relatively pure form and converted to high-value products. Re-precipitation of the solubilized xylan does not affect the bioconversion efficiency of the pretreated biomass. The results of pretreatment and fractionation of corn stover will be discussed.
A key element in the utilization of lignocellulosic biomass for the production of ethanol is the complete conversion of cellulose and hemicelluloses into simple sugars by cellulases. In general, pretreatments are designed to enhance the accessibility of cellulose microfibrils to enzymes allowing for more efficient conversion. Effective pretreatments loosen, often by selective removal of components, the plant cell wall matrix enabling better penetration and digestion of the wall. It is thought, however, that excessive removal of xylan and lignin may cause the remaining cellulose to collapse into an even more recalcitrant state. In this study, we have monitored how well the major cellulases in a commercial enzyme preparation (Spezyme CP) penetrated the cell wall matrix following dilute acid pretreatments at 100°C, 120°C, and 150°C. Antibodies to the enzymes cel7A (CBHI), cel6A (CBHII), and cel7B (EGI), as well as antibodies to the cell wall matrix components xylan and lignin were detected using immuno-electron microscopy. Dilute acid pretreatment for 20 minutes at 100°C enabled <1% of the thickness of the cell wall to be penetrated by enzyme, pretreatment at 120°C allowed the enzymes to penetrate ~20% of the cell wall, and pretreatment at 150°C allowed 100% penetration of even thickest cell walls. When correlated with cellulose conversion data, these data allow visualization of the dramatic effect pretreatment has on altering the condensed ultrastructure of biomass cell walls. High-resolution localization of enzyme penetration into pretreated biomass is providing insight into the mechanism of action of cellulolytic enzymes on whole, intact biomass cell walls.
**Poster 3-71**  
**Ethanol Production from Sugarcane Bagasse by a Dilute Ammonia Process**  
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The Audubon Sugar Institute (ASI), a world leader in sugar processing research, is working diligently to enhance the productivity and profitability of the Louisiana sugar and sugar process-related industries by developing integrated technologies to convert sugarcane bagasse, cane leaf matter and molasses into high value products including ethanol, specialty chemicals, biomaterials and animal feeds. The key biomass components in sugarcane bagasse are cellulose (37-43%), hemicellulose (18-25%) and lignin (22-27%). Ethanol is produced from glucose and xylose after the breakdown of cellulose and hemicellulose, respectively. An ethanol process, utilizing dilute ammonia-treated sugarcane bagasse and molasses as feedstock materials, has successfully been developed at ASI. Our process yields 85% enzyme conversion and 9% (w/w) ethanol at 10% (dry weight) solids loading. Organic acids and glycerol concentrations were determined.

**Poster 4-07**  
**Chemical characterization of fast pyrolysis bio-oils produced from pretreatments of pine wood feedstock**  
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It is well known that the pretreatment of biomass prior to fast pyrolysis process can alter the structure and chemical composition of biomass feedstocks leading to a change in the mechanism of biomass thermal decomposition. As a result, bio-oils with quite different chemical compositions can be obtained, and these bio-oils may be used for varied applications. We determined the effect of six pretreatment processes (dilute phosphoric acid, dilute sulfuric acid, sodium hydroxide, calcium hydroxide, ammonium hydroxide and alkaline hydrogen peroxide) on the chemical and physical characteristics of bio-oils. Bio-oils were produced from pine wood feed stocks in an auger reactor at 450°C temperature. Proximate, ultimate and heating value analyses were performed for both biomass feedstock and the produced bio-oils. The chemical composition of the bio-oils were also determined by gas chromatography-mass spectrometry (GC-MS), gel-permeation chromatography (GPC) and Fourier Transform Infrared (FT-IR) techniques. Results showed that the chemical species produced were highly influenced by the biomass pretreatments applied. These chemical changes are compared and discussed in detail in this paper.

**Poster 3-72**  
**Antioxidant Activity of Low-molecular-weight Lignin Fraction from Organosolv Pretreatment of Wood for Ethanol Production**  
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Like other naturally occurring polyphenols, lignin possesses antioxidant activity. The radical scavenging capacity of lignin is dependent on the source and preparation method, specifically on the structural features of the lignin. Ethanol organosolv lignin samples were prepared from lodgepole pine under varied conditions (temperature, reaction time, ethanol concentration and catalyst dosage), which include water-insoluble organosolv lignin (high molecular weight fraction) and water-soluble lignin (low molecular weight fraction). Functional groups and molecular weight of the lignin samples were determined by means of NMR and GPC. Antioxidant activity of the lignin preparations was evaluated using DPPH (1,1-Diphenyl-2-picrylhydrazyl) method. Results indicated that the radical scavenging capacity of lignins was dependent on functional groups and molecular weight of the lignin. Low molecular weight and narrow polydispersity showed high antioxidant activity. Pretreatment conditions affected the functional groups and molecular weight of the extracted organosolv ethanol lignins, and thereby influenced the antioxidant activity of the lignins. In general, the lignins prepared at elevated temperature, longer reaction time, increased catalysts, and diluted ethanol possessed high antioxidant activity.
Lignocellulosic biomass and particularly hemicellulose from the forest products industry represents a large reservoir of sugars with the potential to be converted to higher value products through bioprocessing. This presentation will cover several projects regarding the fractionation and conversion of lignocellulose to succinic acid, a potentially important platform molecule in the synthesis of a number of commodity and specialty chemicals. The first of these investigates the feasibility of integrating a hardwood hemicellulose sugar extraction step into a Kraft pulping process with the intention of utilizing the hemicellulose as a fermentation feedstock. The requirements on processing configurations for hemicellulose extraction and recovery are compared, and a number of experimental parameters affecting the extraction (alkali, temperature, time) are investigated. Pulp quality is an important property and hemicellulose extraction can result in negatively affect the strength of the paper, which is also investigated. The second portion of the work deals with the fermentation requirements for microbial conversion of dilute acid hydrolyzed softwood to succinic acid. In particular, activated activated carbon and overliming detoxifications were tested for the ability to remove fermentation inhibitors and improve the fermentability of the hydrolyzates.
Chemical and biochemical conversion of sugarcane residues to obtain chemicals and products
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This work reports several chemical and biochemical processes for the fractionation of sugarcane residues to obtain organosolv- and NaOH/AQ pulp, cellulose-derivative polymers (CMC), cellulose- and lignin composites, PF-resins (resols), fermentable sugars, oxidized lignin and chelants. Biological and diluted acid pretreatment of sugarcane bagasse and straw in a laboratory- and industrial steam pretreatment are shown. The acid-catalyzed-organosolv-, dilute-alkaline- and NaOH/AQ delignification of raw residues and pretreated materials is also reported. Enzymatic treatment of pulps with xylanase and laccase was also carried out, providing about 50% lignin loss in straw organosolv pulps. NaOH/AQ pulping of straw shows that the delignification reach 5.3 kappa number (160°C 20 min). Kinetics of fungal pretreatment was studied and 15-day fermentation causes 30% total lignin loss, reducing the chemical requirement on delignification step. Polypropylene composites reinforced with cellulosic fibers (bagasse and straw) show good stress transference between fibers and matrix, excellent flexural properties and water absorption resistance. Straw pulps treated with xylanase provided an increasing of viscosity near to 10%. The steam pretreatment stage was carried out in 5 m³ reactor. This pretreated bagasse was submitted to dilute alkaline extraction followed by cellulase saccharification reaching 85% cellulose conversion to glucose. Oxidized lignin, obtained from enzymatic (Novozym S1003 and apple extract) and chemical (acetic acid, Co/Mn/Br, 50-115 °C, 5 h) has chelating properties removing about 20% of Cu²⁺ from aqueous solutions. Activation energy (16.4 kJ mol⁻¹) showed a reduction of approximately 50% in comparison with the non-catalyzed system. These results indicate that it is possible to produce several chemicals and products from biomass using the biorefinery concept. [Fapesp, Capes, CNPq, ALFA-Lignocarb].

Lignin recovery from pulping liquors ethanol-water of sugarcane straw: temperature and pH effects
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Currently the organosolv pulping has received most attention. Among these processes the ethanol/water mixture combines high efficiency, low cost and abundance of the ethanol in countries where sugarcane is economically important. Lignin is a by-product of pulping and also low-cost natural macromolecule of plants. Conditions for lignin precipitation on liquors from ethanol-water pulping of sugarcane straw, as temperature effect, acid concentration, type of acid and pH on the final solution were evaluated. Factorial design was held using 2⁴ factorial with 4 central points, which were evaluated 4 factors in 12 experiments determining the concentration of residual lignin by reading UV absorption at 280 nm. Absorbptivity value found for the lignin from sugar cane straw was 18.293 L g⁻¹ cm⁻¹. Temperature and pH are the factors that have a major influence on the amount of precipitated lignin in liquor, showing that thermodynamical factors are most important. The variables have to be fixed in the lower level to reduce the solubility of lignin and increase the content of lignin removed from the liquor (temperature, 5°C; acid concentration, 1N; and pH, 1). The isolated lignin was analyzed by FT-IR spectroscopy; the spectra were recorded from 4000 to 400cm⁻¹. The IR spectra showed GS absorption and a strong band in carbonyls groups that suggests an oxidation process during the ethanol-water pulping.

Acknowledgements: CNPq, Fapesp, Colciencias, ALFA program – LIGNOCARB project

Swine Manure Fermentation to Produce Biohydrogen
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Experiments were conducted to produce hydrogen through fermentation with liquid swine manure as substrate using a semi-continuously-fed fermenter (8 L in total volume and 4 L in working volume). A range of pH for the fermenter was tested (4.7 – 5.6) while temperature was controlled at 35 ± 1°C throughout the experiment. Three hydraulic retention times (16, 20, and 24 h) were investigated for their impact on the efficiency and performance of the fermenter in terms of hydrogen yields. The results indicate that hydraulic retention time (HRT) has a strong influence on the fermenter performance. An increasing HRT would increase the variation in hydrogen concentration in the offgas. To produce hydrogen with a fairly consistent concentration, the HRT of the fermenter should not exceed 16 h. A good hydrogen production was observed when pH was controlled at 5.0. When methane content in the offgas exceeded 2%, an inverse linear relationship between hydrogen and methane was observed with a correlation coefficient of 0.9699. The concentration of hydrogen in the offgas from the fermenter on average was around 15-20% with the highest reaching over 40% after the fermenter entered into steady-state operation.

Ethanol and initial state effect in analysis of viability and dynamic response to fermentation performance on Saccharomyces cerevisiae: intensification of ethanol production in a membrane two stages bioreactor
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The use of ethanol, particularly biomass-derived ethanol, can produce significant savings in carbon dioxide emissions. A two-stage bioreactor with cell recycle was developed; it is composed by two reactors with an external cell recycling loop. The first reactor dedicated to the growth reaction, second reactor (R2) was coupled to an ultrafiltration membrane in order to reach high cell concentration and/or high ethanol concentration. Saccharomyces cerevisiae CBS8066 yeast strain was used. Fed batch cultures were carried out at 30°C and pH controlled at 4 with addition of a 14%(v/v) NH₄ solution until the micro organism reached 50% of viability and 100g/L of produced ethanol concentration. This broth was used to inoculate bioreactors (10%(v/v) containing mineral media with an initial glucose concentration of 100gL⁻¹ and various initial concentrations of added ethanol (0, 38, 67, 88, 106gL⁻¹). The cultures were carried out first in batch mode until the substrate was depleted and then switched into a fed-batch mode. The impact of the initial ethanol concentration on the dynamic behaviour of S. cerevisiae was studied. This study showed that the yeast response to the stress conditions was a function of the initial cell viability and the ethanol concentration both in the inoculum and in the fermentative medium.
Platform chemical production from wheat-based biorefining strategy

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The increasing energy demand and cost of petroleum have spurred the need for alternative sustainable feedstocks. In the Sataké Centre for Grain Process Engineering, a novel cost-competitive wheat-based biorefining strategy for the production of generic fermentation feedstocks has been developed. These could be converted into platform chemicals, biodegradable polymers and biofuels via microbial bioconversion. Wheat is used as raw material and we aim to exploit all wheat components into both value-added end-products and precursors for chemical synthesis. Facultative anaerobe Actinobacillus succinogenes (ATCC 55618) efficiently utilised the nutrient-rich streams from cereal grains for succinic acid production without the need of enrichment. The bacterial fermentation involves CO₂ sequestration and promises a step towards acquiring a sustainable future.

This study presents different feedstock formulation strategies based on the production of wheat hydrolysates and fungal autolysate for the microbial production of succinic acid. Batch fermentations were conducted by using semi-defined medium containing commercial glucose and a complex medium containing wheat flour hydrolysate. Results showed that succinic acid could be successfully produced on the flour hydrolysate without inhibition by the complex medium. Also, yields were similar with both media (0.64 g succinic acid/g glucose) while a higher productivity (1.01 g/L.h) was obtained with the hydrolysate. When fermentations were carried out with wheat-derived feedstock and an addition of 30 g/L MgCO₃, 62.6 g/L succinic acid was produced from 100 g/L initial glucose. The results of these studies clearly demonstrate that the wheat-derived feedstock contains all the essential nutrients for A. succinogenes growth and succinic acid production.

Complete conversion of polymeric sugars and water to hydrogen and carbon dioxide for future power system

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Hydrogen production from less costly renewable abundant biomass can both decrease reliance on fossil fuels and achieve net greenhouse gas emissions, but current chemical and biological means suffer from low hydrogen yields or/and severe reaction conditions. We have invented a novel enzymatic reaction for producing hydrogen from starch and water as C H₂ O₆ (l) + 7 H₂O (l) → 2 H₂ (g) + 6 CO₂ (g) [PLoS ONE, 2007, 2:e456] The overall process is spontaneous and unidirectional mediated by 13 enzymes together. This new conversion can be catalyzed at 30°C and atmospheric pressure. Special features, such as, safe storage of solid carbohydrates, modest reaction conditions, easy separation of the products and reactants (gas/liquid), complete conversion, high hydrogen storage capacity (14.6 H₂ mass%), no toxic by-products generated (e.g., CO or sulfuric oxide), and no special infrastructure required, make carbohydrate as a hydrogen carrier more appealing, as compared to other hydrogen carriers, such as methanol and ammonia. Life cycle analysis suggests that the carbohydrate-hydrogen-fuel cell system would be the most energy efficiency power train system; system analysis suggests that it would have the same energy storage density as that of liquid fuel-internal combustion engine (MJ/kg); economic analysis suggests that it would be one of the cheapest fuel systems based on the final mechanical output. With technology improvement, we envision that we will drive vehicles powered by carbohydrates someday.
**Poster 4-22**

**Modeling of Biomass Gasifier Based on Thermodynamic Equilibrium**

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Biomass gasification is gaining increasing attention as one of the prominent thermochemical conversion methods to produce renewable fuels, energy and other products. In addition to producing energy for heat and power, syngas from biomass gasification can be subsequently converted into liquid transportation fuels such as methanol, DME, ethanol, diesel, gasoline etc. and other chemicals. The four main types of gasifier include fixed bed (updraft and downdraft, fluidized bed (bubbling and circulating flow) and entrained flow gasifier.

Here a thermodynamic equilibrium model of biomass gasification applicable to varying gasifier types is presented. Thermodynamic equilibrium model can be used to estimate the equilibrium composition of the syngas. Depending on the gasifier type and internal fluid flow, heat and mass transfer characteristics, with proper modification of the equilibrium model, a simple tool to simulate the operation and performance of the biomass gasifier can be developed. The objective of this presentation is to develop a thermodynamic equilibrium model of biomass gasification, and then to develop a modified model to simulate the performance of a downdraft gasifier. Simulation results show that the modified model can well describe the real downdraft gasifier.

**Poster 4-23**

**Integrated Forest Biorefining – A New Modeling Approach**

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In the near future, integrated forest biorefineries (IFBR) based on pulpign mills could become the most important lignocellulosic biorefineries because dedicated energy trees such as hybrid poplar have not been available in large quantity and most of the available wood resources have already been used in the pulpign mills and the facilities in the existing pulpign mills, if developed into integrated forest biorefineries, could lead to reduction in the capital investment cost. Therefore, in order to analyze the process techno-economic performance and environmental effect, it is necessary to perform process modeling of a whole IFBR.

The objective of the presentation is to develop a comprehensive approach for modeling IFBR, in order to achieve efficient design, simulation and optimization of the whole integrated forest biorefinery and to be able to perform the overall techno-economic analysis. An integrated method using two commercial process simulators Aspen Plus and WinGEMS and Microsoft Excel® as the communications interface between the simulators and a case study using the method are described. The comprehensive approach for modeling an integrated forest biorefinery and the mechanism of communications between Aspen Plus and WinGEMS with Excel as the communications interface is introduced with a case study.

The case study shows that the integrated method is efficient and reliable in modeling of the simplified IFBR. The method presented here is equally applicable for modeling the complex IFBR processes including biomass-based integrated gasification combined-cycle (BIGCC) or black liquor gasification combined-cycle (BLGCC), biosyngas conversions into liquid fuels and chemicals, and fiber separation, etc.

**Poster 4-24**

**Microbial Synthesis of Phloroglucinol and Xylitol**

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My group has been focused on the development and application of new protein engineering and metabolic engineering tools. Here I will present two specific examples. The first example concerns the development of a new bioprocess for synthesis of phloroglucinol, a specialty chemical that is currently produced at 140-200 tons/yr using chemical methods. Specifically, we discovered a novel type III polyketide synthase, PhID, that enables direct biosynthesis of phloroglucinol from D-glucose. Heterologous expression of PhID in E. coli led to the production of phloroglucinol in vivo, with an estimated amount of 10 g/L by using a continuous fermentation. To further improve the phloroglucinol yield, directed evolution was applied to enhance the activity of PhID. In addition, metabolic engineering was carried out to redirect the carbon flux inside E. coli to pathways responsible for the synthesis of phloroglucinol. The second example concerns the development of a new bioprocess for synthesis of xylitol, one of DOE’s top 12 platform chemicals for biorefinery. The current processes for xylitol manufacture, based on either chemical synthesis or fermentation, all rely on the use of pure D-xylene as a feedstock, resulting in relatively high cost of production. To address this limitation, we used protein engineering to create a xylose reductase (XR) mutant with decreased specificity toward L-arabinose, while maintaining its high activity toward D-xylene. Such engineered xylene-specific XR mutants will enable the direct use of inexpensive hemicellulose hydrolysates (mainly D-xylene and L-arabinose) as substrates in large-scale fermentation.

**Poster 4-25**

**Cellulosic Films obtained from the Treatment of Sugarcane Bagasse Fibers with N-methylmorpholine-N-oxide (NMMO)**

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Due to technological evolution and improved environmental awareness, it has always been necessary to keep abreast of new developments, trends and legislation. In recent years the N-methylmorpholine-N-oxide (NMMO) technology turned out to be a simple physical alternative to the yet dominant viscose-technology for producing regenerated cellulosic fibers, film, food casings, membranes, sponges and others, without hazardous byproducts. The NMMO is a solvent for cellulose that does not form a cellulose derivative. In the last few years it has gained increasing importance for the production of a new class of man-made cellulose fibers, the so-called lyocell fibers. The properties of lyocell include high tenacity as well as high fibrillation tendency. These properties are due to a high degree of crystallinity and a high orientation of the cellulose chains in the non-crystalline regions of the fibers. Sugarcane bagasse pulps were obtained by ethanol/water organosolv process under acid conditions (0.02mol/L sulfuric acid at 160°C for 1 h) and the obtained pulp was bleached using sodium chlorite. The main physicochemical characteristics in the pulp presented viscosity of 3.6 mPa.s and micro kappa of 1.1. The films were obtained with 0.5g of bleaching pulp, 2.5g H2O and 13.7g NMMO at 74°C for 1.5 h and 2.5 h. A good film formation was observed, which after FTIR, TGA/DSC analysis indicated no difference among the reaction times. The study showed to be an interesting and promising process, combining the prerequisites for a more efficient utilization of agro-industrial residues.

**Acknowledgements:** FAPESP, CNPq and CAPES (Brazil) and FCT (Portugal)
Application of Lignocellulosic Materials

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Considerable research activity is now in progress to use renewable carbon from biorefinery process streams for the production of new chemicals and materials. Carbohydrates being the primary process stream within the biorefinery can be sources of many novel, biologically important chemicals and materials. The diversity of substitution patterns, stereochemistry and chirality in carbohydrates offer challenges in developing a wide range of useful and selective methods for synthetic modification.

Glycals, which are carbohydrates with a double bond between carbons 1 and 2, provide scaffolds for a wide range of structural modification in addition to enhanced reactivity and versatility of these materials for construction of new nanoscale materials. We have synthesized a family of glycal based bolaamphiphiles by linking two glycal headgroups with a long, nonpolar hydrocarbon chain. These compounds exhibit diversity in head group structure, well-defined reactive sites as a result of the unique chiral centers and provide potential for β-stacking through the double bond. Upon dispersion in aqueous solution, these bolaforms undergo self-assembly to form new nanostructural materials. We will report our effort to synthesize a library of glycal based bolaformes by chemical modifications incorporating a wide range of head groups and varying hydrophobic spacer length. The effect of structural modification on the ultimate structure adopted by the supramolecular array will also be discussed. Eventually, structural analysis and modeling to convert bolaform library to macromolecular assemblies and develop a predictive model of how structural changes at molecular level are reflected at macromolecular level will be attempted.
**Poster 4-30**

**Determination of biodiesel physicochemical properties from six raw materials: an comparative study**


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Biodiesel is an alternative diesel fuel derived from a renewable feedstock such as vegetable oil or animal fat. It is biodegradable and produces lesser CO2, sulfur dioxide and unburned hydrocarbons than petroleum-based fuel. The physicochemical properties of biodiesel such as density, heat capacities and enthalpy may influence in the combustion and exhaustive emission. This work presents a comparative study of heat capacities, enthalpy and density of biodiesel from six raw materials. The biodiesel were made using bioethanol and sodium hydroxide as catalyst in laboratory scale. The raw materials used were crude soybean oil, castor oil, palm oil, animal fat, waste frying oil and coconut oil. The properties were measured at normal atmospheric pressure in the temperature range from 283 to 423K.

**Poster 4-31**

**Bioethanol production from different cellulose sources using Simultaneous Saccharification and Fermentation**

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The search for new technologies for plant biomass conversion into alternative fuels is leveraged by many social and environmental problems associated with the use of fossil fuels and their exploration. Cellulose, the major component of plant biomass, is a polysaccharide hydrolysable by three different cellulolytic enzymatic groups: endoglucanases, exoglucanases and b-glucosidases. This complex enzymatic system is inhibited by its final hydrolysis products, particularly glucose. To circumvent this problem and make the ethanol fermentation from cellulose technically feasible, a process known as Simultaneous Saccharification and Fermentation (SSF) should be adopted. It combines enzymatic hydrolysis and sugar fermentation to ethanol, and can be enhanced with the use of thermostable enzymes and yeasts. This study aimed at evaluating the enzymatic hydrolysis of three different cellulose sources (carboxymethyl cellulose, crystalline cellulose and sugar cane bagasse cellulignin) and comparing their fermentability between a recombinant Saccharomyces cerevisiae harboring the b-glucosidase gene from Humicola grisea with a commercial yeast strain used in bakery. The experiments were performed at 37°C, 20% (w/v) of cellulose source concentration and 25 FPU of commercial enzyme/g of cellulose material. The recombinant strain displayed superiority on the conversion of the resulting enzymatic hydrolysis products in comparison with the bakery yeast. The best results with the recombinant strain were obtained with the more complex cellulose substrate (bagasse cellulignin), achieving an ethanol concentration of 55 g.L⁻¹ after 60-hour SSF process. The studied system shows great biotechnological potential for bioethanol production from lignocellulosic feedstock.

**Poster 4-32**

**Direct Power Generation by Utilising Rhodopseudomonas palustris and Construction of a Photomicrobial Fuel Cell**

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It is understood that “Photomicrobial fuel cells (PMFCs)” can generate electricity from both photosynthesis and catabolism of endogenous carbohydrates in the light and from catabolism alone in the dark circumstance. In the present study, direct electron transfer from photomicrobial strain, *Rhodopseudomonas palustris*, to the anodes characterised with polymer-coated electrocatalytic composite were investigated. In addition, the examination of operation stability of a self-constructed photomicrobial fuel cell (PMFC) was also studied.
Biogas production by Bacillus sp. anaerobic cultivations: new trend of alternative energy source in urbanized areas

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Energy has been the base of society since the remote civilizations. Actually, energetic sources are each more necessary to promote the economic, social and cultural development. Alternative energy sources (wind, solar, biofuels) show capability to minimize the exhaustion and environmental problems of traditional energy sources. Industrialized and urbanized regions show a great environmental problem with the generation of organic residues. Anaerobic microorganism cultivations can degrade pollutants resulting in two kinds of by-products: activated sludge and biogas. The correct management of residual waste presents highest costs and the non-adequate treatment and destination can compromise this treatment. Environmental agencies have been stimulating the use of treated residual waste as fertilizer since removed pathogenic agents and reducing the humidity levels. Biogas can be utilized as an alternative energy source, basically formed by methane and hydrogen. Full-scale applications of anaerobic wastewater treatment technology have become widely accepted in the last decades due to the successfully development of several high-rate reactors. Great cities in development countries show the potential energy generation of 20 MW from the utilization of 50 m3/s of urban residue. Moreover, hydrogen production rate of 13.0 L/L.d (liters of hydrogen by liters of media per day) in a continuous reactor was attained when utilizing Bacillus subtilis in several plants. This work aims to evaluate the capability of production and application of biogas as alternative energy sources produced in urbanized areas by Bacillus subtilis cultivations and to compare the viability with other knowledge energy sources (oil, hydroelectricity, thermoelectricity, nuclear, wind and solar energies).

Influence of microwave pyrolysis conditions on char properties

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Microwave pyrolysis is a new process for converting biomass to bio-oil, syngas and solid char. In this study, pyrolysis of corn cob was carried out in an inert environment at atmospheric pressure and temperatures ranging from 300 to 600°C. The aim of this work was to study the effect of pyrolysis conditions on the characteristics of the solid char residue. The char was characterized using Scanning electron microscopy (SEM), Fourier transform infrared (FTIR) spectroscopy, and Elemental analyzer. The char yield from pyrolysis decreased significantly to 21% with an increase in temperature to 600°C. SEM analysis indicated that pyrolysis of corn cob led to a stepwise accumulation of inorganic matter onto the exposed surface, and some organic matter melted, resulting in the formation of hollow cavities by evolving volatile. FTIR results showed a continuous decrease in the intensity of the hydroxyl group stretch with temperature and the aromatic group to be at maximum at 600°C. Elemental analysis indicated the H/C ratio of the char decreased continuously with temperature, while the O/C ratio remained almost constant above 300°C.

Optimization Strategies for PHA production using Rhodopirillum rubrum Cultured on Synthesis Gas in a Continuous Reactor

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Synthesis gas (syngas) fermentation is a two stage process consisting of biomass gasification followed by fermentation to produce valuable fuel and chemical products. During gasification, biomass is thermochemically converted into a flammable gas mixture at high temperatures. Syngas, containing mostly hydrogen (H2), carbon monoxide (CO), and carbon dioxide (CO2), is produced from oxygen-blown gasification of biomass or other carbonaceous feedstocks. Rhodopirillum rubrum, a purple, non-sulfur photosynthetic bacterium, is capable of utilizing the CO in syngas to produce hydrogen, a high fuel value gas, and polyhydroxyalkanoate (PHA), a biodegradable plastic. R. rubrum accumulates PHA as an energy and carbon storage molecule under unbalanced or stressed growth conditions. The purpose of this investigation is to optimize R. rubrum growth conditions to increase PHA and H2 productivity. Different pH, temperature, acetic acid concentration, and buffering agent manipulations are evaluated in parallel with different cell growth stages for their effects on PHA and H2 productivity.

Production of the polysaccharide curdlan on the ethanol fermentation coproduct thin stillage

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The ability of the corn dry milling coproduct thin stillage derived from ethanol fermentation to support production of the polysaccharide curdlan was examined. A number of commercial uses exist for curdlan such as food and beverage applications. The bacterium Agrobacterium sp. ATCC 31749 synthesizes the polysaccharide in a nitrogen-limiting medium containing excess carbon source. In this study, the bacterium was grown in a phosphate-buffered minimal medium (pH 6.8) that contained thin stillage (from two different sources) as a source of carbon and nitrogen. With carbon limiting in the thin stillage, the effect of supplementing 3% corn syrup to the medium was also explored. The strain was grown in shake flask cultures for up to 120 hours at 30°C. To inoculate each culture, a culture containing the same medium grown for 48 hours was used. To determine curdlan and biomass production, gravimetric determinations were utilized. The strain could produce the polysaccharide from the medium containing the thin stillage alone. The highest curdlan concentration was observed after 96-120 hours at 30°C. Inoculating each culture, a culture containing the same medium grown for 48 hours was used. To determine curdlan and biomass production, gravimetric determinations were utilized. The strain could produce the polysaccharide from the medium containing the thin stillage alone. The highest curdlan concentration was observed after 96-120 hours at 30°C. Inoculating each culture, a culture containing the same medium grown for 48 hours was used. To determine curdlan and biomass production, gravimetric determinations were utilized. The strain could produce the polysaccharide from the medium containing the thin stillage alone. The highest curdlan concentration was observed after 96-120 hours at 30°C.
Poster 4-39
Sequential Process Production of Bioethanol from the Castor Bean Seed Cake (Ricinus communis L.) generated from the Biodiesel Process
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The solid residue arisen from the castor bean seed press, named castor bean cake (CBC) is rich in starch and bears a problem linked to the occurrence of a potent toxic protein (ricin). The challenge of this work is the use of this biomass for bioetanol production, optimizing, in a laboratorial scale, the starch hydrolysis process that, concomitantly, assures the detoxification of the CBC. The chemical hydrolysis was comprised of an acid pretreatment (ratio solid:liquid = 1:6; H2SO4=0.1 mol/L, 120°C, 40 minutes and 150 rpm), which assured the CBC detoxification, since the toxicity in vivo assays (DL50) pointed out a reduction of roughly 240 times in the CBC toxicity. This chemical stage generated a medium with 26 g/L of reducing sugars (hydrolysis efficiency=32%), which, when fermented, produced 11 g/L of ethanol. Another adopted strategy to increase the hydrolysis efficiency as well as the ethanol concentration in the fermented medium was to incorporate the enzymatic hydrolysis after the acid pretreatment, using commercial alpha-amylase (150 microL/g, 90°C, 4 h, pH=6) and glucoamylase (150 microL/g, 60°C, 4 h, pH=5). This resulted in a hydrolysate containing 73.0 g/L of reducing sugars (hydrolysis efficiency=92%), which were further fermented by a strain of Saccharomyces cerevisiae (10 g/L) in an instrumented bioreactor. At the end of fermentation, 34.5 g/L of ethanol were produced leading to a volumetric productivity of 4.25 g/L/h and an ethanol yield on substrate consumed of 0.46 g/g. These values indicate a production of 270 L of ethanol per ton of residual CBC.

Poster 4-40
ZrO2-SiO2-supported solid acid ClO4/Fe2O3-La2O3 as catalyst for straw liquefaction
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Today there has been an interest in the discovery a substitute- “green fuels” to replace the fossil fuel for its depleting reserves, continuously increase in price and growing environmental concern. Biomass is the most common renewable energy. Biomass converted to liquid fuel is one of the most promising sources of energy in the near future. However, the production of liquid fuels through the direct conversion of biomass is still challenging. Thus, it is needed to develop a more efficient and effective process of biomass conversion.

Poster 4-41
Effects of Treatment Variables on Supercritical Gasification of High-Diversity Grassland Perennials
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Low-input high-diversity (HLD) mixtures of native grassland perennials were subjected to a supercritical treatment process with the aim of obtaining hydrogen rich gases. The process was optimized based on the following treatment variables: reaction temperature (374-575 °C, corresponding to a pressure range of 22-40MPa), residence time (10-30 min), biomass content in the feed, and catalysts (0-4% NaOH and solid alkali CaO-ZrO2). The gaseous phase from gasification of HLD primarily consisted of hydrogen (H2), with a mixture of carbon monoxide (CO), methane (CH4), carbon dioxide (CO2), and a small amount of ethylene (C2H4) and ethane (C2H6). The statistical significance of treatment variables was evaluated using analysis of variance (ANOVA) F test. It showed that both temperature and catalysts significantly affected gas yields (P<10-5), while biomass content in the feed and residence time were not significant at the level of P<0.01.

Poster 4-42
Effect of yeast extract supplementation on curdlan production from condensed corn distillers solubles
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Curdlan is an alkali-soluble polysaccharide with several food applications. The polysaccharide is synthesized by the bacterium Agrobacterium sp. ATCC 31749 under growth conditions of excess carbon and limiting nitrogen. The objective of this study was to determine the effect of yeast extract supplementation on bacterial curdlan production using a coproduct resulting from corn dry-milling for ethanol production, namely condensed corn distillers solubles. A phosphate-buffered minimal medium (pH 6.8) containing selected solubles concentrations as its source of carbon and nitrogen was utilized. When supplemented, the yeast extract concentration in the medium was 0.5%. When testing whether supplementing additional carbon source increased curdlan production, corn syrup (3%) was added to the medium. After inoculation with a culture grown in the same medium (48 hours), the shake flask cultures were grown for up to 120 hours at 30°C. Gravimetric determinations were used to monitor bacterial curdlan and biomass production. For all solubles concentrations tested, it was determined that yeast extract supplementation increased curdlan production. Yeast extract supplementation decreased polysaccharide production after 120 hours compared to curdlan production on the unsupplemented medium. In general, bacterial curdlan production was higher after 120 hours when the medium contained corn syrup relative to production on the medium containing no additional carbon source. Biomass production by ATCC 31749 after 120 hours was found to be elevated when yeast extract was supplemented in the medium containing solubles alone or also containing corn syrup. Overall, yeast extract supplementation only increased bacterial curdlan production in the solubles medium containing corn syrup as an additional carbon source.
Saccharomyces cerevisiae needs both xylose reductase (XR) and xylitol dehydrogenase (XDH) for conversion of xylose to ethanol. Cofactor imbalance of the two enzymes often results in accumulation of xylitol, which is one of the main reasons for low ethanol yield. NADPH-dependent XR was engineered to change its cofactor specificity to NADH (J. Biol. Chem. (2005) 280, 10340). The mutated XR (NADH-dependent) and wild XDH (NADPH-dependent) were introduced into a host cell S. cerevisiae D452-2 to line up with cofactor specificity. The constructed S. cerevisiae was tested for ethanol production from a mixture of glucose and xylose. The recombinant yeast grown in aerobic condition was able to utilize xylose at 1.04 g/l h consumption rate without producing xylitol and ethanol. A change of growth condition to O2-limited allowed production of ethanol with final ethanol concentration 38 g/l and 25% (g ethanol per g xylose used) ethanol yield.

Studies on kinetics of the biodiesel fuel production process catalyzed by free and immobilized lipases

Alternative fuels for diesel engines are becoming increasingly important due to diminishing petroleum reserves and the environmental consequences of exhaust gases from petroleum-fuelled engines. In recent years, studies on biological systems for biodiesel synthesis have become an attractive alternative to classical methodologies mainly because chemical transesterification alkalification is energy intensive, the recovery of glycerol is difficult, and free fatty acids and water interfere with the reaction. Although the use of lipases can overcome the problems mentioned above it has some difficulties in the scale up of the process and the high costs of lipases have reduced industrial applications of these enzymes in transesterification reaction. The use of immobilized lipases is very advantageous since it can be easily recovered at the end of the reaction and continuously recycled. In this work experimental assays were carried out to study the biodiesel fuel production kinetics with ethanol from two different types of vegetable oils: soybean and coconut. The transesterification reactions were catalyzed by commercial lipases in free and immobilized forms, Lipzyme CALB L and Novozym 435, respectively, kindly supplied by Novozymes Latin America Ltda. The assays were carried out in a batch stirred glass reactor. A stirring of 150 rpm was kept constant throughout the progress of all experiments. The temperature of the reaction system was controlled heating metal jacket. Samples were withdrawn from the reactor at regular intervals for subsequent analysis. Biodiesel fuel concentrations were determined by gas chromatographic analysis. The results biodiesel yields of different runs were compared.
Poster 4-48
Fast biodiesel production by one-phase reaction
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Due to high oil price and Kyoto protocol, biodiesel became a fast growing market during the last years. To save the operation time and cost for biodiesel production, some fast biodiesel production processes have been suggested. Because oil is immiscible with methanol in the transesterification reaction, the contact of oil with methanol and catalyst is an important factor. Some researchers used a co-solvent such as tetrahydrofuran to enhance the mass transfer of oil and methanol. In this study, the feasibility of fatty acid methyl ester (FAME) as the co-solvent to increase the mass transfer was investigated. FAME as the co-solvent does not require its additional separation because FAME is the end product of the processes. To examine the intermediate phenomena of the reaction, the mixing speed was controlled at a slow rate. When the molar ratio of methanol to oil was 6:1, oil existed at the bottom and methanol and catalyst existed at the top. The top layer gradually became dark by the production of FAME and glycerol. After few minutes, the methanol layer fell down and began to be mixed with oil. When FAME was 5% of oil, FAME content increased more rapidly than the case without FAME addition. However, when FAME was 10% of oil, initial FAME content increased rapidly but final FAME content was lowered due to the reversible reaction by high concentration of FAME.

Poster 4-49
Kinetics of castor and waste cooking oils ethanolysis for biodiesel production
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This work presents the transesterification process of castor and waste cooking oils with bioethanol in the presence of sodium hydroxide as catalyst, because it leads to better conversion and smaller reaction time. A computer-aided tool of this system to model the kinetic of biodiesel production was developed to explore the impact of each strategy on the process behaviour which is an important issue to lead the process to be operated at high level of performance. An analysis was made of the temperature effects on the reaction rates, and it was determined the reaction rate constants and the activation energies derived from experimental observation. The kinetic data showed to be satisfactory for a wide range of operating conditions. The assessment of possible implementation difficulties are carefully considered and discussed.

Poster 4-50
New chemical building blocks and polymers from biorefinery process streams
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The use of renewable raw materials as feedstocks for chemical production is continually suggested as offering many advantages over conventional petrochemical feedstocks, including a lowered demand for crude oil, greater sustainability of the raw material, and the ability to recycle CO2. Renewables also provide a source of new, structurally interesting building blocks with novel properties and applications. Many well-known biochemical processes are rich sources of such compounds. For example, the citric acid cycle produces 2-ketogluutaric acid (2-KGA) as a key intermediate. Other pathways metabolize aromatics and produce 3-ketoapic acid. Together, these types of processes could be the basis of a biorefinery operating unit using hybrid chemical/biochemical processes to convert carbohydrates and lignin into a wide family of monomers and polymers. We will report recent results in our investigation to use 2-KGA as a component of new polysters. The reaction of 2-KGA with different diols leads to the production of new crosslinked polymeric materials whose properties can be controlled as a function of the polymerization conditions. We will also describe our current results using 2-KGA as a starting material for the synthesis of a broader family of renewables based chemical building blocks.

Poster 4-51
Growth and oil production studies of Botryococcus sudeticus (UTEX 2629) on secondarily treated wastewater
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Extensive studies of microalgae have demonstrated significant potential for developing processes that provide a means of bio-oil production with an accompanying benefit of carbon sequestration through carbon dioxide utilization as a primary feedstock. Production costs (particularly for defined medium constituents) can adversely affect economics for such processes. One means of reducing such costs is through the use of nutrients in municipal wastewater as the primary source for growth medium. Here, we report on preliminary work with the microalga Botryococcus sudeticus (UTEX 2629) growing on secondarily treated wastewater. Cell growth and bio-oil production results will be presented with initial studies in a flat-plate bioreactor scheme.

Poster 4-52
Production of Pyruvate by Metabolically Engineered Escherichia coli
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Although several microorganisms can produce pyruvate by fermentation of glucose and other renewable resources, Escherichia coli has many advantages including rapid growth, simple nutritional requirements and the ease of genetic manipulation. A series of steady-state (chemostat) experiments were conducted to evaluate the ability of metabolically engineered E. coli strains (pifB aceEF poxB pps ldhA) to produce pyruvate under several different nutrient-limited conditions. The greatest pyruvate formation rate was found under conditions of acetate-limited growth. Glucose consumption rate and therefore pyruvate productivity were further increased by introducing ATP synthase knockout, arcA knockout and NADH oxidase. High pyruvate concentration and productivity were achieved by well-controlled fed-batch fermentations (at a low specific growth rate).
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Succinic acid (succinate) and its derivatives have a wide range of applications in the food and chemical industries. Escherichia coli can sequester CO₂ through PEP carboxylase to convert PEP into oxaloacetate, and further into succinate. A readily available industrial source of CO₂ is flue gas from power plants which can contain several other gases such as NO₂, SO₂, CO and O₂. Two-phase fermentations (an aerobic growth phase followed by an anaerobic production phase) were conducted to produce succinate using E. coli AFP111, which contains mutations in pyruvate formate lyase (pdf) and the phosphotransferase system (ptsG). Gases normally found in flue gas (CO₂, NO₂, SO₂, CO, O₂) were studied. CO₂ concentration in gas phase affected succinate formation. Supplying 50-200 ppm NO₂ or 50-300 ppm SO₂ (commonly found in flue gas) during the anaerobic production phase did not deleteriously affect succinate production. The effect of other gas components (100-500 ppm CO or 1-10% O₂) on succinate production is also reported.

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The hydrothermal liquefaction of low-input high-diversity mixtures of native grassland perennials [1] was studied using the following treatment variables: heating rate, cooling rate, reaction temperature, reaction time, and catalysts. The liquefaction yields were found to be dependent on the final liquefaction temperature, the length of liquefaction time and the heating rate. The highest liquid yield of 82.1% was achieved within short residence time of 1 minute at 374°C.

A modified version of the previous model [2] is presented, which assumes that biomass first decomposes to gaseous products, tars, and chars via three competitive reactions and then tars go through a second cracking reaction to produce gases. Through the proposed model, the calculated data fit well with the experimental data obtained from liquefaction of prairie grasses. The dependence of the calculated kinetic coefficients on temperature was established using Arrhenius-type equations.


The microwave-assisted pyrolysis of low-input high-diversity mixtures of native grassland perennials was studied. The pyrolysis temperature ranged from 400 to 550°C depending on the power input. The pyrolysis process was evaluated when following catalysts were used: charcoal, solid superacid SO_4^2-/ZrO_2-Al_2O_3, Cl-/Fe-Zr-La, Cl-/Fe-O_2, solid alkali CaO-ZrO_2, La, and Rh. The pyrolytic oils were separated using solvent extraction, and their characteristics including elemental composition and calorific value were determined. The chemical profiles of the syngas and bio-oils were determined using GC and GC-MS. This study demonstrated that high-diversity grassland perennials could be a source for producing high value syngas and liquid fuels and activated carbons.

**Poster 5-07**

**Characterization of the Rheological Properties of Biomass Feedstocks**

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Agricultural residues, such as corn stover and wheat straw, and energy crops, such as switchgrass, represent significant sources of lignocellulosic biomass for use in biofuel production. However, due to their low bulk densities and fibrous constituents, they are difficult and expensive to process, handle, transport, and store. The results of an initial study to quantify the physical and rheological properties of herbaceous biomass materials, including corn stover, wheat straw, and switchgrass, are presented. The characteristics tested include the particle size and distribution, bulk density, compressibility, relaxation, springback, permeability, confined yield strength, and friction properties. These properties are a function of feedstock tissue structure, moisture content, consolidation pressure and history, and define the compactability and flowability of materials through various processes. This data is being used in the development of a uniform material format that is compatible with the use of low-cost, large-capacity feedstock handling, transportation, and storage systems.

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**Poster 4-57**

**Catalytic Microwave-Assisted Pyrolysis of High-diversity Grassland Perennials**

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The microwave-assisted pyrolysis of low-input high-diversity mixtures of native grassland perennials was studied. The pyrolysis temperature ranged from 400 to 550°C depending on the power input. The pyrolysis process was evaluated when following catalysts were used: charcoal, solid superacid SO_4^2-/ZrO_2-Al_2O_3, Cl-/Fe-Zr-La, Cl-/Fe-O_2, solid alkali CaO-ZrO_2, La, and Rh. The pyrolytic oils were separated using solvent extraction, and their characteristics including elemental composition and calorific value were determined. The chemical profiles of the syngas and bio-oils were determined using GC and GC-MS. This study demonstrated that high-diversity grassland perennials could be a source for producing high value syngas and liquid fuels and activated carbons.

**Poster 4-58**

**Biodiesel synthesis via enzymatic esterification of feed stock with high content of free fatty acids**

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The growing interest for renewable sources of energy is responsible for the worldwide efforts towards the development of biofuels. Biodiesel in particular is synthesized via transesterification of triglycerides from vegetable oils with ethanol or methanol. However the current production costs of biodiesel is not competitive when compared to petroleum diesel. An alternative route to biodiesel is based on esterification of free fatty acids (FFA) present in high concentration in certain feed stock. In this work, a distillate from soybean oil deodorization - a by-product from the production of soybean oil (FFA content: 73 wt%) - was used as feed stock for enzymatic synthesis of biodiesel. The local (Brazil) availability of ethanol from fermentation of sugar cane lead us to use this alcohol in all experiments. The esterification reactions were carried out in a batch reactor. Three commercially available immobilized lipases were used, namely, Novozyme 435, Lipzyme RM IM and Lipopzyme TL IM, all from Novozymes, in solvent free-media. We searched for optimum reaction parameters: temperature, enzyme concentration, amount of ethanol and its feeding technique to the reactor (stepwise ethanolysis). The enzyme reuse was also evaluated. Reaction was faster with Novozyme 435. The highest conversion (83.5%) was obtained after 90 min using 3 % wt. of Novozyme 435 and two stage stepwise addition of ethanol at 50°C. Regarding Lipzyme RM IM, the conversion was only 15% after 3 cycles.
Poster 5-10
Assessment of xylanase activity in dry storage as a potential method of reducing feedstock cost
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Enzymatic preprocessing of lignocellulosic biomass within the supply chain has potential to improve feedstock characteristics and reduce ethanol production costs at the biorefinery. While endo-β-1,4-xylanases improve fermentable sugar yields when used during enzymatic pretreatment in water-saturated systems, there are potential benefits to their application in low moisture environments such as dry storage where low water activity improves feedstock stability. To assess the feasibility of endoxylanase treatment in dry storage, endoxylanase activity was tested using wheat arabinoxylan and three commercial endoxylanases at various fixed water activities. Unbuffered solutions of a fixed enzyme to substrate ratio were prepared, flash-frozen, lyophilized, and incubated in chambers at 21% to 100% relative humidity (t=35±0.5°C). Replicates were sacrificed periodically and endoxylanase activity was quantified as an increase in reducing sugar relative to desiccant-stored controls. Endoxylanase activity was observed at water activities over 0.91 in all enzyme preparations in under four days, and at a water activity of 0.59 in under one week in two preparations. Endoxylanase activity after storage was confirmed for selected desiccant-stored controls by incubation at 100% relative humidity. Water content to water activity relationships were determined for three lignocellulosic substrates and results indicate that two endoxylanase preparations retained limited activity as low as 7 to 14 % water content (wet basis), which is well within the range of water contents representative of dry biomass storage. Future work will examine the effects of endoxylanase activity toward substrates such as corn stover, wheat straw and switchgrass in low-water content environments.

Poster 5-11
Wet harvested biomass: supply chain options and storage cost estimates
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Tremendous tonnage and diversity of biomass feedstocks for biofuel production are necessary to meet DOE identified petroleum offset goals. Agricultural residues represent a large quantity of lignocellulosic biomass potentially available for this purpose. Much of this biomass will be relatively wet at harvest, well over 30% moisture, and is produced in regions where field drying can be risky due to weather patterns. This biomass harvested at high moisture content poses significant cost barriers in the feedstock supply chain due to the excess weight of water and aerobic microbial instability. In response we identified seven pathways for delivery of biomass from the farm to a biochemical or thermochemical refinery. These pathways range from a dry “pioneer” bale system, where total 2007 storage costs were estimated at $11 and $35/DMT. Additionally, several innovative wet/dry hybrid systems were identified that could generate cost savings through co-product production from soluble sugars otherwise lost in silage fermentations. Wet/dry hybrid systems also allow the potential to process the biomass into an advanced uniform, commodity-type material for additional cost savings in transportation and handling. The storage pathways will be presented, a cost analysis provided, and cost barriers and process saving opportunities identified. Analyses of supply chain pathways with respect to a given geographical area may lead to a recommended system for handling wet harvested biomass.

Poster 6-07
Unnatural reactions to convert cellulosic biomass to fuels and chemicals
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Natural conversion of cellulosic biomass to energy by ruminants and fungi is slow partly because it must be compatible with living organisms. Industrial conversion of cellulosic biomass to energy can use a wider range of reaction conditions and intermediates. We propose the use of unnatural enzyme-catalyzed reactions. The unnatural part allows the use of faster more efficient reactions. The enzyme catalysis is efficient and allows multiple simultaneous reactions, which are needed to release sugars from a complex multi-component material like cellulose biomass. One example reaction is enzyme-catalyzed formation of peracetic acid. Peracetic acid is a strong oxidant that effectively alters lignin allowing subsequent release of sugars by cellulases and xylanases. Perhydrolases catalyze the formation of peracetic acid from hydrogen peroxide and acetic acid or acetate esters. The levels of peracetic acid formed are high enough effectively pretreat aspen wood. Another example is cofactor-independent hydrogenation using hydrogen directly. By replacing natural metals in the active site of an enzyme with unnatural hydrogenation catalysts, we have created an enzyme that uses hydrogen directly. The shape of the active site controls selectivity of the hydrogenation.

Poster 6-08
Acceleration of Enzymatic Conversion of Agricultural Waste Biomass into Bio-fuels by Low Intensity, Uniform Ultrasound Field
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One of the most critical stages of conversion of agricultural waste biomass into biofuels employs hydrolysis reactions between highly specific enzymes and matching substrates (e.g. corn stover cellulose with cellulase) that produce soluble sugars, which then could be converted into ethanol. Despite numerous advantages, a major limitation of enzymatic bio-processing is relatively slow reaction rates. Our research found that the introduction of a low energy, uniform ultrasound field into enzyme processing solutions greatly improved their effectiveness by significantly increasing their reaction rate. It has been established that the following specific features of combined enzyme/ultrasound bio-processing are critically important: a) the effect of cavitation is several hundred times greater in heterogeneous systems (solid-liquid) than in homogeneous, b) in water, maximum effects of cavitation occur at ~50 °C, which is the optimum temperature for many enzymes, c) cavitation effects caused by ultrasound greatly enhance the transport of enzyme macromolecules toward substrate surface and, d) mechanical impacts, produced by collapse of cavitation bubbles, provide an important benefit of “opening up” the surface of substrates to the action of enzymes. It appears that the introduction of ultrasound energy during enzymatic bio-processing of agricultural waste biomass could significantly accelerate this process and make it more suitable for widespread industrial implementation.
Poster 6-09
The production of recombinant cellobiohydrolase enzymes in transplastomic tobacco: challenges and prospects
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One promising strategy for reducing the cost of cellulolytic enzymes is to produce them in transgenic crop plants. We have shown that transplastomic tobacco expressing an endoglucanase (Acidothermus cellulolyticus E1 catalytic domain) can yield in excess of 10% of soluble leaf protein as the desired recombinant product. Furthermore, the recombinant enzyme is readily recovered from dried plant material (60–90% recovery) in an active form. We have applied the same strategy to the expression of 3 cellobiohydrolase genes: CBHI of Trichoderma reesei, Cel6B (formerly E3) of Thermobifida fusca, and Cel7D of Phanerochaete chrysosporium. In each case, the coding sequence was modified at its 5′ end by the addition of 30 bp encoding the N-terminal 10 amino acids of the highly expressed psbA gene product encoded by the plastid genome. The stability of the resulting fusion proteins was assessed in an E. coli expression system, with significant differences observed between the three cellobiohydrolases. In addition, significant growth rate inhibition was observed with some constructs when incorporated into the tobacco plastid genome. A comparison of mRNA and recombinant protein levels between transplastomic tobacco lines and E. coli expression strains will help us to gain insight into the factors affecting recombinant enzyme production in transplastomic tobacco.

Poster 6-10
Jatropha curcas L. seed lipase study
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Lipases are a group of enzymes defined as carboxylesterases able to carry out the ester bond hydrolysis in long chain acylglycerols. They generally act in the organic aqueous interface and acylglycerols are their natural substrates. Vegetable lipases, found in a great deal in nature and at a low cost, can be employed in an advantageous way for the enrichment or isolation of a fatty acid kind or class.

We investigated the catalytic lipase potential in germinated physic nut seed [acetone (AP), crude (CP) and lyophilized crude (LCP) powders] and dormant (DAP). In this research, substrate hydrolysis rates were analyzed (vegetable oils and lipid) under several reaction conditions. The germinated seeds enzyme showed great activity in pH 8.0 at 40°C. The AP hydrolyzed satisfactorily (>76%) all the evaluated substrates, except castor bean oil (23%), showing the soy oil the best result (97%), within two hours reaction. CP and LCP showed good conversion rates of soy oil (92% and 87%, respectively).

DAP did not show a significant hydrolytic activity. AP electrophoretic study showed only band in the zymogram and it did not present significant catalytic activity differences facing different chain size substrates (112U/g for tributyrin, 80U/g for tripalmitin and 100U/g for olive oil). Preliminary results showed that the AP of physic nut lipase was able to carry out the esters synthesis, revealing the biotechnological potential of this enzyme.

Poster 6-11
Withdrawn

Poster 6-12
Withdrawn

Poster 6-13
Cellulignin Hydrolysis Using Immobilized Cellulases
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Cellulign was covalently immobilized in a chitosan-alginate hybrid gel, after activation of the support with glycidol and/or glutaraldehyde. The best enzyme derivative showed half-life ten-fold higher than the soluble enzyme, with recovered activity of 20%. Temperature and pH for maximum enzyme activity were 55°C and 4.5, for soluble Cellulign, and 65°C and 3.5 for the immobilized enzyme. The maximum enzyme load was 68 FPU/g supernatant. A derivative containing 14.7 FPU/g support was used to catalyze the hydrolysis of sugar cane bagasse, previously submitted to alkaline treatment with sodium hydroxide. The performances of immobilized and soluble enzyme in the hydrolysis of 8.8% m/m of cellulignin, at 50°C, were compared. The total reducing sugar concentration reached with soluble Cellulign was much lower than with the enzyme derivative, for soluble enzyme concentrations in the reactor equal or higher than the apparent immobilized one.

The feasibility of the reuse of the immobilized enzyme was verified by running a set of three repeated batches. Immobilized enzyme, together with 5 FPU/g mannop of free enzyme, was used to hydrolyze 10% m/m of cellulignin. Each run was performed at 47°C for twelve hours, and at 37°C for 18 hours. After these reaction times, the solid was separated and returned to the reactor, being added more 5 FPU/g mannop of soluble enzyme and the same initial amount of cellulignin. The performance of the system was excellent, indicating that enzyme immobilization may be a good alternative to reduce costs of ethanol production from lignocellulosic materials.

Poster 6-14
Utilization of dairy manure for ligninolytic enzymes production by the white-rot fungus Phanerochaete chrysosporum
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Large amounts of animal manure from livestock industry have created great environmental concerns during the past decade. These manures can, however, be a potential resource for producing value-added bioproducts. In this study, dairy manure was used as sole substrate for ligninase production by the white-rot fungus Phanerochaete chrysosporum. Upon the characterization, raw manure contained a variety of nutrients including undigested crude fiber (12% of lignin, 11% of hemicellulase, 27% of cellulose), protein (18%), and notable amounts of minerals. Batch experiments were carried out in shaking flasks with P. chrysosporum pellets for ten days at 37°C without addition of veratryl alcohol as inducer. Effect of agitation (50-200 rpm) and manure concentration (5-20 g/L, dry basis) on the ligninase production were investigated respectively. The popular lignin-degrading enzyme, manganese peroxidase (MnP) production was found the highest at 100 rpm and 15 g/L manure. After 96 h incubation, the manure culture had a maximum MnP activity of 481 U/L. The result was further visualized with the total excreted proteins on Sodium Decylsulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE), suggesting that dairy manure is a suitable raw material for the production of ligninases and can be utilized for the mass production of the enzyme for the biotechnological applications. Future study is warranted to investigate the effect of pH, temperature, etc., to optimize the enzyme production system for continuous process. The expression analysis of total excreted proteins in the dairy manure should also be investigated to provide useful information for the identification of the associated proteins with ligninolytic enzymes.
**Poster 6-15**
Alkaline active hemicellulases from bacteria isolated from an extremely alkaline lake ecosystem

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Enzymes are critical for cost effective cellulosic ethanol production processes. The complexity and diversity of lignocellulose requires ancillary enzymes beyond cellulases for complete hydrolysis of cellulose to fermentable sugars. Enzymes that are optimally active above pH 9 are needed to complement alkaline pretreatment processes such as alkaline fiber explosion and alkali recirculation. Nutrient cycling and geochemistry of a lake system located in the Nebraska Sandhills was used to predict the presence of alkaline active enzymes. Extracellular hemicellulases have been isolated from no less than five species of Gram positive bacteria (*Bacillus wakoensis*, *Bacillus akhenensis* (2 isolates), *Bacillus alkalophilus* (2 isolates), *Jonsia sp.* and *Amphibacillus sedimentsii*) and two unidentified fungi. Supernatants from cultures grown on xylan contain enzymes that are active from pH 6 - 12. Growth profiles and zone of clearing assays indicate greater functional diversity between the isolates than is indicated by species identification. We report on the ongoing efforts to characterize and identify the enzymes from these microbial cultures.

**Poster 6-16**
Methods of Increasing β-glucosidase Expression in *Trichoderma reesei* for Improved Hydrolysis of Pretreated Corn Stover

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*Trichoderma reesei* produces two cellobiohydrolases (CBH I and CBH II), five endoglucanases (EGI-V), and two β-glucosidases (BG). Cellulose is hydrolyzed to cellobiose, a water-soluble beta-1,4-linked dimer of glucose, through synergistic action of cellobiohydrolases and endoglucanases. Cellobiose is then hydrolyzed to glucose by β-glucosidases. Wildtype *T. reesei* secretes levels of β-glucosidase insufficient to hydrolyze all the cellobiose under conditions of high substrate concentration, resulting in product inhibition of CBH and EG enzymes and a reduced enzymatic hydrolysis of cellulose. One method of economically alleviating this inhibition is by increasing the expression levels of β-glucosidase in *T. reesei* through molecular approaches. Initially the BG from *Aspergillus oryzae* was recombinantly expressed in *T. reesei*, but expression levels were still insufficient. The native signal sequence from the A. oryzae BG was replaced with a recombinant signal sequence from a *Humicola insolens* protein that is highly expressed in *T. reesei*. This swap effectively increased BG expression and resulted in a 2-fold improvement in the conversion of cellulose to glucose at the substrate levels tested. Further improvement in BG expression levels were obtained by creating a fusion protein comprised of an endoglucanase catalytic domain fused at the N-terminus of BG. This fusion resulted in additional expression and further improvement of the cellulase activity in PCS hydrolysis assays.

**Poster 6-17**
Cloning of Cellulase and Regulation Factor Genes in *Penicillium decumbens* and Analysis of the Mutation Mechanism of Strain JU-A10

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Cellulolytic fungus, *Penicillium decumbens* JU-A10 was a catabolite-repression-resistant mutant, which was obtained by physical and chemical mutagenesis of strain 114-2. Recently, strain JU-A10 has been successfully applied to cellulase preparations and cellulolytic ethanol production at an industrial scale in China. Cellulases from *Penicillium* and *Trichoderma* may have different compositions and regulation mechanisms of synthesis. Therefore, it is important to study the molecular biology of *P. decumbens*, which also would be helpful to improve cellulase production by engineering renovation of strain JU-A10.

Five cellulase genes (cbh1, cbh2, eg1, eg2 and bgl1) and two regulation factor genes (creA and ace1), encoding repressor of cellulase and xylanase expression) in *P. decumbens* were cloned by TAIL-PCR and degenerate PCR. Previous study shown that the catabolite repression-resistant character of strain JU-A10 may be caused by the change of energy-yielding metabolism or the mutation of catabolite repressors. By comparing the sequences and transcription of the seven genes in *P. decumbens* J114-2 with its mutant JU-A10, we found that the catabolite repression-resistant character of strain JU-A10 were caused by neither the mutation of these cellulase genes or upstream regulatory sequence, nor the mutation of catabolite repressors CRE A or ACE I. Based on comprehensive analysis, it is proposed that the catabolite repression-resistant character of strain JU-A10 is caused by the change of energy-yielding metabolism. Further verification is under way.

**Poster 6-18**
Biochemical characterization of β-glucosidases from different families

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β-glucosidases (BG) catalyze the hydrolysis of cellobiose to glucose, release the significant inhibition of cellobiose to cellulases, and thus are very important in the process of fuel ethanol production from cellulotic biomass. More than ten β-glucosidases from family 1, 3 and 5 are characterized by their substrate specificity, specific activity, thermostability, pH profile, temperature profile, and kinetic parameters, such as inhibition by glucose. The aim is to identify some active, thermostable, and high-glucose-tolerant BGs.

**Poster 6-19**
Fundamentals of Enzymatic Hydrolysis of Cellulose through a Restart Approach

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In order to develop better fundamental understanding of the mechanisms of enzymatic hydrolysis of cellulose, we explored how cellulose reactivity changes during enzymatic hydrolysis of cellulose and the relationship between major cellulase components and cellulose reactivity. In particular, our restart protocol, which was shown to be effective on pure cellulose hydrolysis, was applied to discern intermediate digestion and characteristics of reacted cellulose as the substrate reacted. In addition, the effect of enzyme-substrate interaction on reaction rates was studied using purified key cellulase components such as CBH1, CBHII, EGI, EGI2, and CBMs from wild type *Trichoderma reesei*. Enzyme synergism during interrupted enzymatic hydrolysis of pure cellulose with the restart protocol was compared with that during uninterrupted hydrolysis as well as with the hydrolysis behavior of the individual components. Important chemical and physical features, including accessible surface area, chemical moieties, chemical bonds, and cellulose reactivity with enzymes were characterized for each solid sample. Finally, a kinetic model was employed to clarify the effects of cellulose reactivity and cellulase processivity on enzymatic hydrolysis of cellulose.
**Poster 6-20**
The effect of corn stover pretreatment on enzyme inhibition
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The generation of soluble inhibitory compounds during biomass pretreatment has been well documented, and the extensive list of compounds includes aliphatic acids, furans, phenolic compounds and heavy metals. The characteristics of the pretreatment process directly affect the presence and concentration of inhibitors in the whole, pretreated biomass slurry. It is well known that certain inhibitory compounds affect the speed and productivity of fermentation, but, in addition, a subset of these compounds adversely affect cellulase activity. Cellulase inhibition, whether by specific or non-specific mechanisms, will increase the amount of enzyme required for complete cellulose hydrolysis, thereby driving the cost of the process upwards. This demonstrates the importance of integrating the pretreatment, enzyme hydrolysis and fermentation operations early during process development. Less research has been performed on the role of specific pretreatment processes in the generation of potent enzyme inhibitory compounds. This presentation will focus on the characterization of enzyme inhibition from corn stover pretreated by different pretreatment methods and will reveal the most potent cellulose-inhibiting substrates.

**Poster 6-21**
**Purification and characterization of a family 43 glycoside hydrolase from Geobacillus thermoleovorans IT-08 with dual function arabinofuranosidase/xylosidase activity**
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The gene from the thermophilic bacterium Geobacillus thermoleovorans strain IT-08 encoding a glycoside hydrolase family 43 enzyme based on the amino acid sequence was synthesized, and subsequently cloned with a C-terminal His-tag into a pET29b expression vector. The recombinant gene product termed GbtXA was expressed in E. coli and purified to apparent homogeneity. Michaelis-Menten kinetic parameters were obtained for the artificial substrates p-nitrophenyl-β-D-xlyopyranoside (kcat 0.2 sec-1 and Km 380 μM) and p-nitrophenyl-α-L-arabinofuranoside (kcat 1.2 sec-1 and Km 490 μM), indicating greater specificity for the arabinofuranose moiety. The enzyme exhibited end-product competitive inhibition with both xylose and arabinose. The pH maximum was pH 5.5, and the enzyme was not thermally stable above 33 °C, with a T1/2 on the order of 60 min at 56 °C under the conditions tested. GbtXA showed a broad substrate specificity when tested with natural substrates, and released xylose from beechwood arabinoxylan and arabinose from wheat arabinoxylan. GbtXA can thus be classified as a dual function arabinofuranosidase/xylosidase with respect to both artificial and natural substrate specificity.

**Poster 6-22**
Comparing Performance of Several Commercially Available Cellulases on Pretreated Corn Stover
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Cellulase plays a key role in biochemical conversion processes for producing fuels from lignocellulose biomass because it hydrolyzes cellulose to the fermentable sugar glucose. To further understand the performance of several commercially available enzymes, we tested the ability of Genencor’s Spezyme CP, GC220 and the recently available Accellerase 1000 to enzymatically saccharify dilute sulfite-acid pretreated corn stover solids that had been washed or left in the hydrolysate. The enzymes were tested at various temperatures (38-54°C), solids concentrations (5-30% w/w) and enzyme loadings (5-40 mg protein/g cellulose). A response surface methodology was used to develop an empirical model of monomeric glucose yield as a function of the four factors described above. Glucose yield was highly influenced by enzyme loading at low enzyme loadings and less severely affected at higher enzyme loadings. Increasing solids concentration had a large negative impact on glucose yields, and yields were even lower when the solids were left in the hydrolysate. Temperature was less important than the other factors. Overall, it is clear that enzymes are needed that perform better in the presence of inhibitors’ sugars being the most inhibitory compounds contained in dilute acid hydrolysates. The problem is further exacerbated at high solids concentrations.

**Poster 6-23**
Production and preliminary chemical and biochemical characterization of Aspergillus awamori β-glucosidase and xylanase
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The filamentous fungi Aspergillus awamori produces cellulolytic and hemicellulolytic enzymes that can be used for biomass degradation into sugars that can be used for bioethanol production. This microorganism also produces high levels of β-glucosidase (EC 3.2.1.21) that is critical for the biomass ethanol technology, as this enzyme hydrolyses cellobiose into its two glucose monomers. As such it minimizes end-product inhibition of cellulolytic enzymes and provides glucose for the ethanol fermentation. The same microorganism also produces the enzyme xylanase (EC 3.2.1.8), that hydrolyzes the xyllosidic linkages in xylan that is the major component of hemicellulose. The present study aims the chemical and biochemical characterization of Aspergillus awamori β-glucosidase and xylanase. Enzymes were produced under optimized conditions using wheat bran as carbon source. The culture supernatant was fractionated by ultrafiltration, gel filtration and ion-exchange chromatography. SDS-PAGE was used to evaluate the extent and nature of the purification. Protein separation through gel filtration resulted in the identification of two peaks with β-glucosidase activity and one with xylanase activity. Further fractionation of a selected β-glucosidase peak using CM-Sepharose, resulted in two peaks showing enzyme activity. The peak with xylanase activity was further separated into three peaks with xylanase activity using a Q-Sepharose column. The study for the characterization of the enzymes produced by Aspergillus awamori aims the formulation of an enzymes cocktail to be used for the hydrolysis of sugar cane bagasse.
Poster 6-24
Effects of xylanases addition on enzymatic hydrolysis of biomass
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Hemicellulose is a major component of lignocellulosic biomass. The content of xylan left in the pretreated biomass varies dramatically, depending on the source of the biomass, the pretreatment method used, and the severity of pretreatment. For instance, dilute acid pretreated corn-stover contains typically 3% of xylan in the washed solids, while steam expanded corn-stover has about 10% xylan. From an economic perspective, the hydrolysis of xylan, and converting the sugars contained in the hemicellulose fraction of pretreated biomass to ethanol is critical to biorefinery. As the structure of xylan is variable, including not only linear β-1,4-linked xylose chains, but also highly branched heteropolysaccharides, the hydrolysis of xylan requires the synergistic action of many enzymes. The effects of adding a range of Genencor xylanases on the enzymatic hydrolysis of the xylan, and glucan, of differently-pretreated lignocellulosic biomass will be reported.

Poster 6-25
Enzyme Pools for Sugar Cane Bagasse Hydrolysis
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Complete biomass hydrolysis to sugars of six and five carbons is carried out by cellulolytic enzymes (endoglucanases and exoglucanases) and β-glucosidase plus a collection of enzymes that are able to hydrolyze xylan, pectin and the linkages between the biomass polysaccharide moiety and lignin (auxiliary enzyme activities). The collective action of the auxiliary activities acts synergistically to facilitate the hydrolysis of cellulose. In this work enzyme mixtures produced by \textit{Trichoderma reesei} RUT C30 and a selected strain of \textit{Aspergillus awamori} were assessed for their efficiency to hydrolyze untreated and treated sugar cane bagasse that is a potential raw material for biomass ethanol production in Brazil. The crude enzyme preparations (culture supernatants) were individually concentrated using ultrafiltration and blended to obtain preparations with different profiles of cellulases and auxiliary enzymes. The blends were used to hydrolyze untreated and steam-explosion treated sugar cane bagasse. Experiments were performed at 40, 45 and 50°C. For comparison, the efficiency of the enzyme pools was evaluated “vis-a-vis” to the results that were obtained using commercial enzyme preparations. In all cases it was used a load of 10 FPU per gram of dry biomass. All enzymatic preparations were stable during the 72 hours of the hydrolysis experiments. A selected enzyme blend was as effective as the commercial enzyme preparations that were evaluated. Preliminary results show that a biomass syrup presenting glucose 100 g/L was fermented to ethanol, with high efficiency, by the yeasts \textit{Saccharomyces cerevisiae}.

Poster 6-26
An α-glucuronidase enzyme activity assay adaptable for solid phase screening
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Glucuronic acid is a common chemical moiety that decorates the xylan polymer of hemicellulose. This chemical substituent impairs both enzymatic and acidic hydrolysis of xylosidic bonds. The α-glucuronidase enzyme hydrolyzes the 1,2-linked glucuronic acid from the terminal, non-reducing xylose of xylo-oligosaccharides. There are relatively few a-glucuronidase genes in the public databases. We have developed an assay with commercially-available reagents that can be used to search DNA libraries for a-glucuronidase genes in a high throughput, solid phase activity screen.

Poster 6-27
Improved Cellulase Performance on Pulp and Paper Substrates
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One of the important steps in developing the nascent Biorefinery industry is the co-optimisation of ligno-cellulosic substrates and the enzymes required for their conversion to simple sugars. Of the many potential substrates being worked on by process developers, by-products of wood processing are of particular interest as the infrastructure necessary for the biomass supply and even, in some cases, pre-treatment for Biorefinery use already exists in the wood processing industries. This work will present the results of our applications research to develop ligno-cellulolytic enzymes for such substrates. One major component has been our involvement in a French consortium of academic and industrial groups - including the Institut du Pin (Bordeaux), the Laboratory of Biotechnology & Bioprocessing (INSA, Toulouse) and Tembek - funded through ADÉME. We will present our enzyme performance results on a wide range of cellulose fiber samples generated within this French Pulp & Paper project, focusing on the identification of important parameters affecting enzyme conversion.

Poster 6-28
Enzymatic activity of cellulase immobilized on glass using layer-by-layer self-assembly
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Results from activity studies of cellulase immobilized on glass and silicon will be presented. Polyethyleneimine (PEI) and polystryrenesulfonate (PSS) were the polyelectrolytes used for the nano self-assembly process. Conversion of celllobiose to glucose was monitored in a batch reactor to determine a first order rate constant. The efficacy of the enzyme layers were monitored over a period of days to weeks. Surface charge modification using Self-Assembled Monolayers (SAM) was utilized to enhance the layering on glass.

Poster 6-29
Enzymatic Hydrolysis of Lignocellulosic Biomass for Bioethanol Production – A Study on Its Degradation Mechanisms, Kinetic models and Economical Impact
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The potential importance of cellulose hydrolysis in the context of conversion of plant biomass to fuels and chemicals is widely recognized. Cellulose hydrolysis also represents one of the largest material flows in the global carbon cycle. The kinetics of cellulose hydrolysis is been widely studied, and Michaelis-Menten type of rate expressions with substrate or product inhibition terms have been proposed to describe the observed reaction kinetics. Also measured parameters for cellulase components and substrates could in principle be incorporated into models used to predict the behavior of multicomponent cellulase enzyme systems. Once a quantitative model is validated, it can be used to rapidly formulate new hypotheses of significance in both fundamental and applied contexts. The current work presents a detailed state of arte study on the degradation mechanisms of the enzymatic hydrolysis of lignocellulosic residues for bioethanol production, where different kinetic models for enzymatic hydrolysis are presented. Parameters such as enzyme adsorption, enzymatic inhibition and Beta-glucosidase adsorption are also studied. We suggest that more studies needed to be achieved concerning the functional modeling of enzymatic hydrolysis of cellulose. Models based on application of these and other methodologies to relate changes in substrate properties to rates of primary and secondary mediated by various cellulases and cellulose systems over the course of reaction appear to be a promising direction for future research.

Acknowledgments: FAPESP and CNPq
Poster 6-30
Transglycosylation reactions by a purified β-glucosidase from the thermophilic fungus, *Thermoascus aurantiacus*, using glucose, cellobiose and maltose as substrates: Potential applications
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β-Glucosidases (EC3.2.1.21) are responsible for catalyzing the final step in the enzymatic saccharification process of cellulose into glucose. These enzymes are also known to catalyze transglycosylation reactions and under certain environmental conditions produce gluco-oligosaccharides that find applications as prebiotic and nutraceutical molecules. In this study, the β-glucosidase purified from *Thermoascus aurantiacus* strain 179-5 was used to catalyze the reverse synthesis of gluco-oligosaccharides from glucose, cellobiose and maltose. The β-glucosidase was precipitated from the extracellular fluid with 80% ammonium sulfate, and applied to a DEAE-Sephadex-A-50 ion-exchange column and eluted with a linear NaCl (0-1.0M) gradient. Fractions showing β-glucosidase activity (assayed against p-nitrophenyl-β-D-glucoside) were applied to a Sephadex-G-50 column and eluted with 50mM sodium-acetate buffer (pH5.0). The purified β-glucosidase preparation was concentrated by ultrafiltration. Transglycosylation reactions were performed using glucose, cellobiose and maltose (50mM) in 5ml solution containing 1.0-unit of β-glucosidase, and the mixture incubated at 40°C and pH6.0 for glucose, 60°C and pH4.0 for cellobiose, and 40°C and pH4.0 for maltose. The reverse synthesis products produced over various time intervals were identified by HPAEC-PAD. The main products arising from glucose transglycosylation were gluco-oligosaccharides of DP≥2 (31%), and 12% unidentified with TR7.5 min (retention time). With cellobiose, the products included glucose (T, 3.03 min; 15%), and gluco-oligosaccharides of DP≥2 (11%). The action of β-glucosidase on maltose resulted in 38% conversion that comprised glucose (13%), and an array of gluco-oligosaccharides (25%). The results are important in elucidating the mode of action of β-glucosidases from *T. aurantiacus* in transglycosylation reactions, and their potential applications as nutraceuticals.

Poster 6-31
Purification and Characterization of *Saccharophagus degradans* 2-40 Cel5G and Cel15G
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The marine bacterium *Saccharophagus degradans* 2-40 produces a multicomponent cellulosylotic system composed of ten annotated GHS endoglucanases, two GH9 endoglucanases and several cellodextrinas, cellobiohydrolase, glucanases and phosphorylases during growth on cellulose. To establish their biochemical activities, two apparent paralogous CBM6&GH5-carrying β-1,4-endoglucanases, Cel5G and Cel15G, were cloned into pET28b to create N and C terminal 6x His tags to the full length polypeptide as well as to ΔCBM derivatives. Each was expressed in E. coli Rosetta™ and purified to apparent homogeneity using a combination of affinity and size exclusion chromatography. The specific activity of each expressed protein was determined on soluble and partially crystalline substrates, such as 4-nitrophenol β-D cellobiose, carboxymethylcellulose (CMC), phosphoric acid swollen cellulose, Avicel and filter paper. Typical of CBM6-carrying endoglucanases, activity was maximal on low crystallinity substrates and was stimulated by ionic strength but significant activity was also detected on microcrystalline substrates, such as Avicel and filter paper. The activity of the full length and the ΔCBM derivatives were compared to evaluate the role of the CBM in degradation of crystalline substrates.

Poster 6-32
Selecting a Cellulase for Biomass
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Not all cellulase mixtures are created the same. This is true for cellulases from different organisms, but also for cellulases produced by the same organism under different conditions. Genencor produces cellulases for a number of industries, with performance criteria set accordingly. To evaluate the range of biomass conversion performance that could be expected from T. reesei-produced cellulosases, we collected 61 independent cellulase samples. Whole cellulase (from seven T. reesei strains, three production processes, 1996-2006 production lots of both commercial and experimental samples, and several product formulations) were used to convert 4 cellulosic substrates to sugars. The results will show a significant range of cellulose conversion efficiency and that no one production parameter is solely responsible for generating a cellulase with good or poor biomass performance. The results show that repeated production of cellulase under the same controlled conditions produces a cellulase with predictable and reproducible performance. No one substrate was predictive of the others but good (or poor) conversion of one substrate did correlate with good (or poor) conversion of another substrate. These results emphasize the importance of optimizing and controlling cellulase production specifically for the biomass industry. To that end, Genencor developed and commercialized the first cellulase product specifically for biomass conversion.

Poster 6-33
Synergies of Integrating Enzyme Development and Substrate Understanding
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Understanding processes for utilization of lignocellulosic substrates is a focus area for Novozymes North America, Inc. in order to generate valuable information for development of next generation cellulase enzymes and to provide our customers with an excellent level of technical support. A vast amount of research has been directed towards development of processes involving either combined or separate hydrolysis and fermentation, and lately various hybrid models have been proposed.

Development of new generation enzymes in combination with different pretreatments has an impact on the hydrolysis process. We will illustrate how mathematical models can be used as powerful tools in optimizing enzymatic hydrolysis and understanding substrate-enzyme interactions.
**Poster 6-34**

**Enzymatic Saccharification of Xylooligomeric Compounds Present in Hydrolysates from Neutral and Acidic Pretreatments of Lignocellulosic Biomass**

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The complete removal of xylan from the cell wall matrix has long been a key target for improving the efficacy of enzymatic saccharification of lignocellulosic biomass. In consideration of this, a number of high-temperature dilute acid and neutral pretreatment schemes have been employed. Typically, the high pretreatment severities needed to achieve complete conversion of xylan to monomeric sugars result in some sugar degradation. It is also known that some of these degradation products have inhibitory effects on subsequent fermentation processes; often requiring costly conditioning steps to address. In this work, we looked towards lower severity pretreatment conditions to reduce sugar degradation losses and the production of toxic by-products. At lower severities, the efficiency of xylan removal can remain high, although the bulk of hydrolyzed xylan remains in more complex xylooligomeric (XO) forms. In this study, we utilized LCMs to determine the numerous simple and branched XO forms that can exist in low severity pretreatment hydrolysates. We also investigated the manner in which the XOAs can be hydrolyzed enzymatically to achieve complete conversion to xylose. We demonstrated that many commercially available enzyme preparations can hydrolyze the majority of XO compounds, although these preparations often have only trace amounts of the activities necessary for optimal conversions; leading to slow conversion times and increased protein requirements. Additionally, we will present xylooligoglycose studies using purified hemicellulolytic enzymes to better understand which activities are crucial to complete XO hydrolysis; these include endoxyylanase, beta-xylosidase, acetyl xylan esterase, feryl acid esterase, arabinofuranosidase and xyloglucanase activities.

**Poster 6-35**

**Chlorella Algae and Their Viruses Are a Source of Xyloglucan Hydrolyzing Enzymes**

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Certain eukaryotic Chlorella-like green microalgae are hosts for large (>310 kb), plaque-forming, dsDNA viruses (chlorella viruses or chloroviruses) found in fresh water throughout the world. Chloroviruses, family Phycoconstaviridae, are a rich source of enzymes with unique structural and genetic features. The prototype of the family, PBCV-1, has a 331 Kb genome, that encodes ~365 proteins and 11 tRNAs. The algae-virus system is a model for studying DNA virus/algae interactions and has been the focus of our laboratory’s research for the past 25+ years. Cell wall degrading enzymes are crucial at two points during PBCV-1 replication. First, the virus infects its host by attaching specifically to the external surface of the algal cell wall. Attachment results in the release of virion-packaged cell wall digesting enzyme(s) that allow entry of virus DNA, leading to replication and release of progeny virus particles in 6 – 8 hours post infection. Second, progeny viruses are released from the host by cell wall lysis. Six viruses have been sequenced and annotated (Fitzgerald et al., 2007a, b, c). Fifteen genes with cellulose binding domains were identified, nine of which have predicted cellulase/xylanase functions, including a b-1,4-glucuron lyase, an exo-1,4-b-D-glycanase, and a b-1,3-glycanase. These genes were cloned, expressed and determined to be enzymatically active. The host, Chlorella sp. NC64A, is also predicted to encode its own wall-degrading enzymes to aid in progeny release during cell replication. The Chlorella sp. NC64A genome was sequenced by the Joint Genome Institute (USDOE) and predicted to encode at least 5 xyloglucan-specific endoglucanases.

**Poster 6-36**

**Cellulases production by Aspergillus fumigatus FBSPE-05 isolated from sugar cane bagasse**

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Aspergillus fumigatus, one of the wide range cellulase-producing organisms available, is ubiquitous in Brazil, being found in soil, as well as in agro-industrial residues. In our studies aiming at isolating cellulolytic fungi from sugar cane bagasse, a promising strain was selected and identified as Aspergillus fumigatus FBSPE-05. This strain was tested for cellulases production in submerged fermentation in a mineral medium using two main carbon sources, sugar cane bagasse and wheat bran, and corn steep liquor as nitrogen source. Sugarcane bagasse was the best substrate for CMCase production in these conditions, giving values of 360 U/L after six days fermentation, at 30°C and pH 4.8. For FPase production both carbon sources were good substrates, giving values around 46U/L after two or three days fermentation in the same conditions. Solid state fermentation was also tested, using brewer’s spent grain, sugar cane bagasse and wheat bran as carbon sources and corn steep liquor and sodium nitrate as nitrogen source. After enzyme extraction, the highest level of extracellular CMCase (21.06 U/g substrate) was obtained in 1:2 solid:liquid ratio, sugar cane bagasse as carbon source and corn steep liquor as nitrogen source, at 30°C after 4 days fermentation. The partial characterisation of enzyme activity have shown the best activity at 65°C and pH 2.0. More studies will be carried out to better characterize cellulase activity by Aspergillus fumigatus, but our previous results indicate that this organism produces a thermophilic acidic-cellulase.

**Poster 6-37**

**β-D-Xylosidase from Selenomonas ruminantium: Thermodynamics of Enzyme-Catalyzed and Noncatalyzed Reactions**

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β-D-Xylosidase from Selenomonas ruminantium is the best catalyst known for promoting hydrolysis of 1,4-β-D-xylooligosaccharides and it has potential utility in industrial saccharification processes. Kinetic parameters, kcat and K_m, are more than 10-fold larger than those reported for the enzyme isolated from other organisms. In cleaving 1,4 glycosidic bonds, the family 43 glycoside hydrolase acts through an inversion mechanism and cleaves a single xylene residue from the nonreducing end of xylooligosaccharides per catalytic cycle without processivity. Three-dimensional structures of homologous GH43 β-xylosidases indicate that the enzyme active site has only two subsites for recognition of substrate, the two terminal xylosyl residues that share the scissile glycosidic bond. In addition to its β-xylosidase activity, the enzyme efficiently catalyzes hydrolysis of 4-nitrophenyl-β-xyloside and 4-nitrophenyl-α-L-arabinofuranoside using the same active site as for its β-xylosidase activity. Temperature dependence of kinetic parameters of enzyme-catalyzed reactions and noncatalyzed reactions were determined.
**Poster 6-38**

**β-D-Xylosidase from Selenomonas ruminantium: Catalyzed Reactions with Natural Substrates**

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β-D-xylanase from *Selenomonas ruminantium* is the best catalyst known for promoting hydrolysis of 1,4-β-D-xylooligosaccharides and it has potential utility in industrial saccharification processes. Kinetic parameters, $K_m$ and $k_{cat}/K_m$, are greater than 10-fold larger than those reported for the enzyme isolated from other organisms. In cleaving 1,4 glycosidic bonds, the family 43 glycoside hydrolase acts through an inversion mechanism and cleaves a single xylose residue from the nonreducing end of xylooligosaccharides per catalytic cycle without processivity. Three-dimensional structures of homologous GH43 xylanases indicate that the enzyme active site has only two subsites for recognition of substrate, the two terminal xylosyl residues that share the scissile glycosidic bond. In addition to its xylanase activity, the enzyme efficiently catalyzes hydrolysis of 4-nitrophenyl-α-L-arabinofuranoside. Interestingly, 1,4-β-D-xylanase is a better substrate than 4-nitrophenyl-β-D-xylopyranoside, indicating that subsite +1 offers considerably more transition state stabilization to the natural substrate than to the artificial substrate. Reactions with xylan and arabinoxylan substrates were characterized.

**Poster 6-39**

**Crystalline Cellulose Hydrolysis Proceeds with A Transition from Substrate Excess to Substrate Limited**

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Heterogeneous cellulose accessibility is an important substrate characteristic, but all methods for determining cellulose accessibility to the large-size cellulase molecule have some limitations. Characterization of cellulose accessibility to cellulase (CAC) is vital for better understanding the enzymatic cellulose hydrolysis mechanism [Zhang and Lynd, Biotechnol. Bioeng. 2006, 94: 888-898]. Quantitative determination of cellulose accessibility to cellulase (m2/g cellulose) was established based on the Langmuir adsorption of the fusion protein containing a cellulose-binding module (CBM) and a green fluorescent protein (GFP) [Hong et al. 2007. Langmuir 23: 12535]. One molecule of the recombinant fusion protein occupied 21.2 cellulose lattices on the 110 face of bacterial cellulose nano-fibers. The CAC values of several cellulotic materials — regenered amorphous cellulose (RAC), bacterial microcrystalline cellulose (BMCC), Whatman No. 1 filter paper, fibrous cellulose powder (CF1), and microcrystalline cellulose (Avicel) — are 41.9, 33.5, 9.76, 4.53, and 2.38 m2/g, respectively. The CAC value of amorphous cellulose from Avicel is 17.6-fold larger than that of crystalline cellulose - Avicel. Avicel enzymatic hydrolysis proceeds with a transition from substrate excess to substrate limited. The declining hydrolysis rates over conversion are mainly attributed to a combination of substrate consumption and a decrease in substrate reactivity. Declining heterogeneous cellulose reactivity is significantly attributed to a loss of CAC where the easily-hydrolyzed cellulose fraction is digested first.

**Poster 6-40**

**Investigation of the role of Trichoderma reesei cellulolytic enzymes in lignocellulose hydrolysis using reconstituted enzymatic pools**

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Enzymatic hydrolysis of lignocellulose is generally considered as the major economic bottleneck of the bioethanol from lignocellulose production process. Among the strains that can produce cellulolytic enzymes, *Trichoderma reesei* is the fungal strain commonly used for the industrial production of enzymes for lignocellulose hydrolysis, especially because of its high capacity of enzyme secretion (>30 g L-1 protein for industrial strains). Understanding the complexity of the synergistic mechanisms involved will result in defining the optimal ratios between processive and non-processive cellulolytic enzymes. The present approach allows a rapid investigation of multiple cellulase ratios not naturally found in fungal secretomes. This analysis is based on the separation of the main cellulolytic activities present in the secretome of the cellulolytic strain *T. reesei*. The fractionation is carried out by Fast Protein Liquid Chromatography (FPLC) and the separation method used leads to the purification of the main active enzymes, i.e. CBHI, CBHII, EG I and EGI, up to 94% without loss of activity. The separated enzymatic fractions are used in a miniaturized degradation test of pre-treated lignocellulosic substrates. The degradation capacities are determined by the final sugar production and hydrolysis yields using HPLC. These tests were used to evaluate the effects of these reconstituted pools on the degradation of a steam-exploded wheat straw. Multiple combinations of different purified enzymes simulated *in vitro* variations of each cellulase and provided new information on their respective roles in lignocellulose degradation.

**Poster 6-41**

**Response Surface Methodological Approach for Optimization of Cellobiose Production**

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Cellobiose has biotechnological potential for value-added products in food, cosmetic, and pharmaceutical industries. This work utilized experimental design methodology to improve cellobiose production from cellulase by cellulase in the presence of gluconolactone. Preliminary tests were employed to obtain the favorable conditions for cellulase activity analysis and found that the optimal temperature and pH were 37°C and 5.2, respectively. Moreover, the inhibition pattern of gluconolactone on cellulase was investigated. The optimal conditions for cellobiose production were determined by response surface methodology (RSM). A three-level design of 29 experiments was conducted with four factors, each of which was designed to study reaction time, cellulose concentration, cellulase concentration, and gluconolactone concentration. Our statistical model predicted that the highest yield of cellobiose would be 9.4 g/L under the following optimized reaction condition of 1.5% of cellulose, cellulase 3.8 FPU, 3.0% of gluconolactone, and 20 hr reaction time.
Sugar cane bagasse enzymatic hydrolysis by *Trichoderma reesei* cellulases

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Four billion gallons of alcohol fuel were produced in Brazil, in 2006, from a total sugar cane production of 427 million tons. The residue (crushed cane stalk – bagasse) formed after the extraction of the sucrose juice amounts to 75 million tons of dry biomass annually produced. Although each sugar and/or alcohol mill burns the bagasse for the production of steam (heat) and for power/electricity generation, there is a 12% surplus available for the production of biomass ethanol, that increases fuel production per planted area. In this study, *Trichoderma reesei* RUT C30 was cultivated in lactose, wheat bran and steam treated bagasse (STB) (used in Brazil as cow feed) for the production of cellulolytic enzymes and beta-glucosidase.

The performance of the crude enzyme preparations were evaluated for the hydrolysis of “STB” and “STBL”, a steam treated bagasse at Lund University in presence of an acid catalizer. For comparison, the performance of the commercial enzymes GC220 and Spezye CP (both from GENENCOR) were also evaluated. In all cases, reaction medium for the enzymatic hydrolysis contained 25 g/L bagasse and 10 FPU/g dry biomass. Hydrolyses were performed at 50 ºC, pH 5.0 and 48 hours. The hydrolysis of “STB”, for all enzyme preparations, resulted on 7.8 g/L glucose excepted for GC 220 that resulted on 6.5 g/L. The hydrolysis of “STBL” using Spezye CP and the other enzyme preparations resulted approximately on 14 g/L and 12.4 g/L glucose respectively, showing that “STBL” is more accessible for the attack of *Trichoderma* cellulases.

Enzymatic conversion of sucrose to invert sugar using a membrane reactor

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The aim of this work was to evaluate the performance of soluble and insoluble invertase for the conversion of sucrose to invert sugar through a continuous process using the membrane reactor (MR) coupled with a 100kDa-cutoff ultrafiltration membrane (UF-MR) or a 5μm-pore-size microfiltration membrane (MF-MR). Invertase was immobilized by adsorption on anionic polystyrene-divinylbenzene beads. The 10mL-membrane reactor (UF-MR or MF-MR) was fed continuously with a 2.5mM sucrose solution at feeding rate of 1.6 h⁻¹, being the temperature and agitation maintained at 30°C and 100 rpm, respectively. At least, a continuous 20h-steady-state regime was attained in all tests. The yields (expressed as percent of sucrose converted) and reaction rates (expressed as mmol/h.menzyme⁻¹) for sucrose conversion to invert sugar were 100%, 84% and 99% and 0.23, 0.88 and 1.02, respectively, for soluble–UF-MR, insoluble–UF-MR and insoluble–MF-MR. No leakage of enzyme from the support was detected. Insoluble–MF-MR had similar yield and reaction rate 77% higher than that of soluble–UF-MR. The improved performance of insoluble–MF-MR over soluble–UF-MR would probably be due to differences of structural and/or chemical nature of the membranes, leading to different internal flux patterns, which, at the end, would affect the reaction rate and the yield. The data showed that the membrane reactor was suitable for sucrose hydrolysis.
Poster 6-47
Utilization of Canola Cake in the Production of Poligalacturonase and Protease by Semi-Solid Fermentation

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Agroindustrial residue had received increasing attention once can be used as a raw material for obtaining products with highest aggregate value. In this context, fermentation in solid stage (FSS) plays an important role in the utilization and bioconversion of these residues in products of interest. The aims of this work was to evaluate its potential of canola cake for poligalacturonase synthesis by semi-solid fermentation. The centesimal characterization of the cake was done, as well as starch contents, water activity and granulometric distribution of particles. The culture medium was composed of 75ml of water in 100g of canola cake. After strong homogenization, 40g of medium were dispensed in500ml-Erlenmeyers, autoclaved and inoculated with 107spores/g of medium. Fermentations were incubated at 30°C for 96 hours. Tested microorganisms were Aspergillus niger CNPAT001, IOC207, IOC4220, IOC4222, IOC3883 and CCT0916 and A. oryzae IV. Samples were taken in 24 hours intervals, in order to determine poligalacturonase activity (PG) protease. The synthesis of this enzyme by the selected strains was evaluated in media prepared with the addition of different water volumes to 100g of canola cake. The evaluated culture media demonstrated satisfactory ability in maintain microorganism growth and all the analyzed strains presented visual growth. A. niger CNPAT001 strain was the more appropriated for PG production, with 26.0U/g obtained after 24h in the medium composed by 75mL of water in 100g of cake. For the protease determination, A. oryzae IV strain presented the best activity after 48 hours under fermentation, showing 25.5U/g, in the same culture medium.

Poster 6-48
Cell-Free Ethanol Production

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Theoretical work has suggested that a cell-free process, consisting of the twelve enzymes involved in the yeast anaerobic glycolysis pathway, is capable of making ethanol much faster than a microorganism based fermentation. This and other potential advantages have suggested that a cell-free process may improve the economy of fuel ethanol production. We have attempted to further investigate the potential of this process with lab scale studies. Glycolytic enzymes were purified from yeast and a micro scale assay was developed to evaluate varying enzyme, substrate and cofactor concentrations. Progress of the reactions was followed by monitoring ethanol produced as well as observing phosphorylated metabolite profiles using 31P NMR. Correlation of the results to theoretical predictions will be detailed.

Poster 6-49
Influence of Formic Acid in a Pre-Purified Xylose Reductase Aiming an Enzymatic NAD(P)H Regeneration System

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Oxireductive enzymes are playing an important role in the current biotechnology and organic chemistry, since they are capable to reduce or oxidize different types of compounds, which could result in a less costly process than a chemical way. NAD(P)H-dependent D-Xylose reductase (XR) belongs to the monomeric aldo–keto reductase superfamily. This enzyme catalyzes the reaction of xylose to xylitol, with aid of the co-enzyme NAD(P)H. The XR gene is used to construct recombinant S. cerevisiae strain for utilizing xylose and producing ethanol; it can be used, also, for xylitol production and in other process that needs an enzymatic NAD(P)H regeneration system (ENRS). However, there are not many studies of the influence of different substances in the XR kinetic, although these studies are very important to better understand the behavior of this enzyme in different reactional media. In this context, the present work had as objective to determinate the formic acid (a hypothetical ENRS in XR catalyzed reactions) influence in the pre-purified NADPH-dependent XR from Candida guilliermondii. The enzymatic extract produced by the cultivation of the yeast cells and was pre-purified by the reversed micelles technique. The enzymatic assays were performed at 30°C using a constant concentration of NADPH and formic acid, only varying the concentration of xylose. The results showed that formic acid has an uncompetitive inhibitory effect under XR, the apparent inhibition constant was 0.14±0.1 M. This result showed that acid formic is a good alternative for an ENRS in XR catalyzed reactions.

Acknowledgements: FAPESP, CNPq and CAPES.

Poster 7-07
Production of Ligninase from Wheat Straw Using a Pelletized Phanerochaete chrysosporium Culture

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White rot fungus Phanerochaete chrysosporium is a well known filamentous fungus that can deconstruct lignocellulosic materials by excreting extracellular oxidative enzymes, such as lignin peroxidase, manganese peroxidase. As a filamentous fungus, the morphology of P. chrysosporium could be clump-type or pellet-type. The clump morphology increases the viscosity of the medium, wraps around baffles and impellers which influence the nutrient mass transfer and reactor performance. The situation would be more significant for ligninase production using P. chrysosporium because ligninase synthesis needs high O2 tension and low shear stress. Using pelletized fungi can alleviate the problems. Pelletization of filamentous fungi makes it possible not only to improve the nutrients mass transfer, but also to increase significantly oxygen concentration and to reduce the shear stress. In addition, another obstacle to commercial production of ligninases is the cost of chemical defined cultural medium. Our results indicated that ligninase can be produced from pelletized morphology using wheat straw as sole nutrient. In this paper, ligninase production from clump morphology and pellet morphology is first compared. The effects of medium composition such as wheat straw, mineral salts, pH, and nitrogen sources are then studied on pelletized fungal culture in order to maximize the ligninase production from wheat straw.
Poster 7-09

Importance of morphology of Trichoderma reesei for production of cellulases

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A major bottleneck in developing an economically feasible process for enzymatic hydrolysis of cellulose is the high cost of the enzyme production. *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 (e.g. Celluclast). However, further improvements in the enzyme production process are necessary if the cost of the enzymes is to be lowered enough to make second generation bioethanol production economically feasible. *T. reesei* has been 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* focusing on the enzyme profile and levels produced in relation to conditions with relevance to those found during full scale production is needed. Enzyme productivity may be strongly connected to fungal morphology in *T. reesei* and investigating this relationship could be a major step towards designing improved fermentation processes for cellulase production. In this presentation the effect of pH and agitation on morphology and enzyme production of *T. reesei* Rut-C30 in batch fermentations is described. Enzyme activity has been investigated by measuring the total cellulolytic activity as well as investigating the detailed composition of the enzyme mixture.

Poster 7-10

Changes in proliferation characteristics and a filter paper degrading ability of the *Trichoderma* strain, B5, after the second colchicine treatment

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In this study, we attempted to observe the changes in proliferation characteristics and a filter paper degrading ability of the *Trichoderma* strain B5 after the second colchicine treatment. The strain B5 is selected from the colchicine-treated conidia of *T. reesei* Rut C-30 using the double layer selection medium and this strain possesses superior proliferation characteristics and degrading ability of a filter paper comparing with the original strain. The green mature conidia of the strain B5 were swollen and treated with colchicine solution. These treated conidia were incubated in the double layer selection medium followed by isolation of several strains. Every selected strain appeared on the surface of the selection medium earlier than those derived from *T. reesei* Rut C-30. These selected strains showed superior proliferation characteristics on the Avicel plates comparing with the strain B5 and the original strain. Moreover, these selected strains showed superior degrading ability of a filter paper comparing with the strain B5 and the original strain. From these results, we concluded that additional colchicine treatment can cause increase in proliferation rate on the Avicel plates and the degrading ability of a filter paper.

Poster 7-11

Simultaneous Saccharification and Fermentation of Pretreated Olive Pruning for Bioethanol Production

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Olive tree pruning is a largely available renewable agricultural residue in the Mediterranean countries with no industrial applications. Apart from a limited application as domestic firewood, it is nowadays disposed in the field causing economic and environmental problems. As an alternative use, this residue could provide a promising feedstock for bioethanol industry, due to its carbohydrate content (about 50% on dry matter basis). However, research is needed to determine the feasibility to produce ethanol from this raw material by technologies developed so far.

Among biomass to ethanol available technologies, enzyme-based conversion technologies present important advantages because they catalyze only specific reactions and consequently there are no side reactions or by-products and the hydrolysis can potentially be performed at very high yields. The pre-treatment step required to break the lignocellulose structure has been studied in depth and several process technologies, as those based in the use of hot water or dilute-acid, have been proved to be very effective to improve enzymatic digestibility of raw material.

In the present study olive tree pruning biomass pretreated by both Liquid Hot Water (LHW) and dilute sulfuric acid was tested as substrate for ethanol production by the Simultaneous Saccharification and Fermentation (SSF) process. Three different process configurations, separate hydrolysis and fermentation (SHF), simultaneous saccharification and fermentation (SSF) and presaccharification and simultaneous saccharification (PSSF), regarding ethanol production from pretreated olive tree pruning were compared at different water insoluble solids concentration. Main results of this research will be reported.
Poster 7-12

Systems-level analysis and engineering of Escherichia coli for the production of L-valine

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The L-valine producing strain of Escherichia coli was constructed by rational metabolic engineering and stepwise improvement based on transcriptome analysis and in silico gene knock-out simulation. Feedback inhibition of acetohydroxy acid synthase isoenzyme III by L-valine was removed by site-directed mutagenesis and the native promoter containing the transcriptional attenuator leader regions of the ilvGMDA and ilvBN operon were replaced with the tac promoter. The ilvA, leuA and panB genes were deleted to make more precursors available for L-valine biosynthesis. This engineered Val strain harboring pKKilvBN, which overexpresses the ilvBN genes, produced 1.31 g/liter L-valine. Comparative transcriptome profiling combined with in silico gene knock-out simulation was used for the enhanced production of L-valine. The VAMF strain (Val DaceF Dmdh DpfkA) harboring pKBRilvBNCED and pTrc184yga zHlrp was able to produce 7.55 g/liter L-valine from 20 g/liter glucose, resulting in a high yield of 0.378 g L-valine per g glucose. The approaches described here can be a good example of systematically engineering strains for the enhanced production of amino acids. [This work was supported by the Korean Systems Biology Project of the Ministry of Science and Technology (M10309020000-03B5002-00000). Further supports by the LG Chem Chair Professorship and KOSEF through the CUPS are appreciated].

Poster 7-13

Screening of microorganisms for bioconversion of limonene to a-terpineol

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The limonene, a cheap and readily available natural substrate, which can be used for the production of more valuable natural flavour compounds of interest for the industries of cosmetics, druggist and foods. The a-terpineol is the most important of the monocyclic monoterpenes alcohols. The objective of this work was to carry through screening of microorganisms, from rejects of the citric juice industry, and other sources of limonene, for bioconversion of this. The election was carried through with the isolation of diverse microorganisms in plates with Potato Dextrose Agar. To verify the enzyme presence that degrades limonene in the isolated microorganisms, it was used minimum media more limonene, in electromagnetic agitation. Fermentation in orbital agitator was become fulfilled, using minimum media and limonene (1.5%, v/v), with the microorganisms that had presented resulted positive in the previous test. The products gotten in the bioconversion process (a-terpineol), had been characterized by GC/MS with yield of 30 %.

Poster 7-14

Systems metabolic engineering of Escherichia coli for L-threonine production

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Amino acid producers have traditionally been developed by repeated random mutagenesis owing to the difficulty in rationally engineering the complex and highly regulated metabolic network. By combined genome engineering, transcriptome analysis, and genome-scale metabolic flux analysis, we report the development of the first genetically-defined L-threonine (Thr) overproducing Escherichia coli strain. All known feedback inhibitions, transcriptional attenuation regulations, and those pathways that negatively affect Thr production were removed by genome engineering. Several target genes were identified by transcriptome profiling combined with flux response analysis, and were engineered accordingly. The final engineered E. coli strain was able to produce 82.4 g/l Thr by fed-batch culture. The strategy of systems metabolic engineering reported here can be employed for developing genetically-defined organisms for the efficient production of bioproducts. [This work was supported by the Korean Systems Biology Project of the Ministry of Science and Technology (M10309020000-03B5002-00000). Further supports by the LG Chem Chair Professorship and KOSEF through the CUPS are appreciated].

Poster 7-15

Development of shuttle vectors for succinic acid producing rumen bacteria

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Shuttle vectors carrying the origins of replication that function in Escherichia coli and two succinic acid producing capnophilic rumen bacteria, Mannheimia succinicivorans and Acidobacillus succinogens, were constructed. They were found to be stably maintained with 10 copies number in rumen bacteria during the serial subcultures in the absence of antibiotic pressure for 120 generations. By optimizing the electroporation condition, the transformation efficiencies of 3.0×10^6 and 7.1×10^6 transformants/μgDNA were obtained in M. succinicivorans and A. succinogens, respectively. The 1.7 kb minimal replicon was identified that consists of the rep gene, four iterons, A-T rich regions and a dnaA box. It was found that the shuttle vector replicates via the theta mode, which was conformed by sequence analysis and southern hybridization. These shuttle vectors were found to be suitable as expression vectors as the homologous lumC gene encoding fumarase and the heterologous genes encoding green fluorescence protein and red fluorescence protein could be expressed successfully. Thus, the shuttle vectors developed in this study should be useful for genetic and metabolic engineering of succinic acid producing rumen bacteria. [This work was supported by the Korea Science and Engineering Foundation (KOSEF) grant funded by the Korea government (MOST) (2005-01294). Further supports by the LG Chem Chair Professorship, IBM SUR program, Microsoft, and by the KOSEF through the Center for Ultramicrochemical Process Systems are appreciated].
**Poster 7-16**

**Effects of dissolved CO₂ levels on the growth of *Mannheimia succiniciproducens* and succinic acid production**

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This study presents the metabolic responses of *Mannheimia succiniciproducens* to the different dissolved CO₂ concentrations (0 to 260 mM). The cell growth and succinic acid production rates increased proportionally as the dissolved CO₂ concentration increased from 8.74 to 141 mM. Also, the yields of biomass and succinic acid on glucose obtained at the dissolved CO₂ concentration of 141 mM were 1.49 and 1.52 times higher, respectively, than those obtained at the dissolved CO₂ concentration of 8.74 mM. The additional CO₂ sources provided in the form of NaHCO₃, MgCO₃, or CaCO₃ had positive effects on cell growth and succinic acid production. While, cell growth inhibition was observed when excessive bicarbonate salts were added. We also found that PEP carboxylation by PEP carboxykinase (PCKA) is the most important for succinic acid production as well as the growth of *M. succiniciproducens* since it provides the additional ATP. [This work was supported by the Korea Science and Engineering Foundation (KOSEF) grant funded by the Korea government (MOST) (2005-01294). Further supports by the LG Chem Chair Professorship, IBM SUR program, Microsoft, and by the KOSEF through the Center for Ultramicrochemical Process Systems are appreciated.]

**Poster 7-17**

**Nisin biosynthesis by *Lactococcus lactis* in bioreactor using diluted skimmed milk as culture media**

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Nisin, a bacteriocin composed by 34 amino acids with a molecular mass of 3.3kDa, is produced by *L. lactis*, this biomolecule is active against Gram-positive organisms including bacterial spores, but it is not generally active against Gram-negative, yeasts or fungi. Nisin is widely used as a natural preservative in the food industry and is being considered for use in health care products, for human and veterinary uses. This study has been developed to optimized large-scale nisin production in skimmed milk aiming low-costs process and stimulating its utilization. *L. lactis* ATCC11454 was assayed in a fermentor with 1.5L of diluted skimmed milk at 25% of standard concentration (2.27g total solids) at: (i) airflow 0.5 L.min⁻¹ (30°C/200rpm/36h) and (ii) airflow of 1L min⁻¹ (30°C/100 rpm/36h), both without pH control. The titers of nisin expressed and released in culture media were quantified and expressed in arbitrary units (AU.mL⁻¹ of medium) by the agar diffusion assay utilizing *L. sakei* ATCC15521 as sensitive indicator microorganism. The highest nisin activity was detected after 12h (7298.61AU.mL⁻¹) and 4h (4049.39 AU.mL⁻¹) of fermentation process, respectively. The results show that the dilution of skimmed milk supplied the nisin expression from cells into media and it was strongly affected by fermentation process conditions. The utilization of diluted skimmed milk as cultivation substrate is an important factor to reduce the production costs of this biomolecule high value-added and increase the commercial application of nisin. Furthermore, the utilization of milk subproducts as milk whey (industrial dispose) can be exploited to reduce the environment pollution.

**Poster 7-18**

**Kinetic modeling of batch fermentation for succinic acid production by *Mannheimia succiniciproducens***

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This study presents kinetic models that described the batch production of succinic acid from glucose by *Mannheimia succiniciproducens* MBELS5. Experimental data collected from batch fermentations with different initial glucose concentrations were used to estimate parameters. The optimal values of the parameters were determined by minimizing the discrepancy between predictions and experimental results. The growth of *M. succiniciproducens* could be described by a modified Monod model combining inhibitions of glucose and organic acids accumulated in the culture broth. The Luedeking-Piret model well presented the formation of organic acids, in which succinic, acetic, and formic acids followed a mixed-growth-associated pattern. Interestingly, lactic acid fermentation by *M. succiniciproducens* was nearly nongrowth-associated unlike most lactic acid producing-bacteria. In all cases, the models agreed well with the experimental observations, and therefore, enabled to explain the fermentative characteristics of *M. succiniciproducens* in production of succinic acid from glucose. The models developed in this study can be used as good tools for development and optimization of a biobased succinic acid production process in *M. succiniciproducens*. [This work was supported by the Korea Science and Engineering Foundation (KOSEF) grant funded by the Korea government (MOST) (2005-01294). Further supports by the LG Chem Chair Professorship, IBM SUR program, Microsoft, and by the KOSEF through the Center for Ultramicrochemical Process Systems are appreciated.]

**Poster 7-19**

**Proteome characteristics of succinic acid overproducing bacterium, *Mannheimia succiniciproducens***

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A capnophilic rumen bacterium, *Mannheimia succiniciproducens* MBELS5, isolated from bovine rumen is an efficient succinic acid producer. Recently, a genetically engineered succinic acid overproducing mutant, *M. succiniciproducens* LPK7, was developed based on full genome sequence. 2-DE and LC-MS/MS were used to analyze proteome of the mutant cells at the exponential and stationary phases. The results were compared with those of the wild type strain (MBELS5) to elucidate the global physiological and metabolic changes responsible for succinic acid overproduction. Comparative proteomic analyses between the MBELS5 and the mutant strain showed the apparent differences in 87 and 69 protein spots at the exponential and stationary phases, respectively. As the mutant cells grow, the expression levels of 58 proteins also changed. The results could allow us to understand the global protein changes related with succinic acid overproduction. Moreover, several features, indispensable for further improving the succinic acid producers by rational metabolic engineering, will be described in detail. [This work was supported by the Korea Science and Engineering Foundation (KOSEF) grant funded by the Korea government (MOST) (2005-01294). Further supports by the LG Chem Chair Professorship, IBM SUR program, Microsoft, and by the KOSEF through the Center for Ultramicrochemical Process Systems are appreciated.];
**Poster 7-20**

**Biological Hydrogen Production by Bacillus smithii: A Kinetic Study**

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The objective of the national hydrogen and fuel cell R&D program is to help industry develop technologies to produce, store and use hydrogen made from renewable resources in quantities large enough, and at costs low enough, to compete with traditional energy sources such as coal, oil and natural gas. The bacterium Bacillus smithii, ATCC 55404, produces H2 and CO2 from CO and water through the well-known water-gas shift reaction. B. smithii does not require light as an energy source for growth, and does not utilize CO as a growth substrate. Thus, the bacterium can channel essentially all of the CO (with water) to H2 production. Batch serum bottle experiments were conducted under kinetically limited conditions to obtain cell (X), growth-limiting substrate (glucose, S), reaction substrate (CO, S1) and product (H2, P) concentrations with time. The data were used to determine and model the specific growth rate (μ), the specific hydrogen production rate (v), the specific carbon monoxide uptake rate (q), the yield of hydrogen from carbon monoxide (YH2/C) and the yield of cells from glucose (Yx/s).

**Poster 7-21**

**Characterization of a phosphoglucose isomerase homoethanologenic Escherichia coli mutant**

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Ethanologenic Escherichia coli KO11 was engineered to channel glucose through the Entner-Doudoroff (ED) and pentose phosphate (PP) pathways using a phosphoglucose isomerase interruption into the glycolytic pathway. KO11 pgi was evolved to recover the capacity to grow in mineral media with 4% glucose under anaerobic conditions, and a homoethanologenic derivative was obtained deleting the pta, ack and idh genes, obtaining KO11 PPAL. Main results show that activity values for glucose-6-phosphate dehydrogenase (ZWF) and the Entner-Doudoroff (ED) pathway enzymes increased 17-fold and 2-fold (respectively) in KO11 PPAL in comparison with KO11. Increased expression of pyruvate decarboxylase and alcohol dehydrogenase in KO11 PPAL allowed to obtain specific ethanol formation rates similar to those found in KO11, but with half the cell mass and without carbon flux to acetate, formate and lactate, i.e. a large ethanol/glucone yield in mineral media. These results demonstrate that it is possible to obtain the same carbon flux using the PP and the ED pathways as alternative routes for the Embden-Meyerhoff-Parnas pathway for the glucose catabolism.

**Poster 7-22**

**Banana as Adjunct in Beer Production: Applicability and Performance of Fermentative Parameters**

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Traditionally the raw materials to beer production are barley, hops, water, and yeast, but most brewers use also different adjuncts. During the alcoholic fermentation, the contribution of aroma compounds from other ingredients to the final beer flavour depends on the wort composition, on the yeast strain and mainly on the process conditions. The banana can also be a raw material favorable to alcoholic fermentation being rich in carbohydrates, minerals and providing low acidity. Besides, its use in the brewing process is also considered because of its low price and high level of production in specific regions from producing countries like Brazil. In this work, the objective was to evaluate the performance of wort adjusted with banana juice in different concentrations. For this, static fermentations were conducted at 15°C in pilot scale (120 L of medium). The study evaluated the addition of banana to change the concentration of the malt wort from 10ºP to 12 and 15ºP (ºP is the weight of the extract or the sugar equivalent in 100 g solution, 20ºC). The results showed an increment of approximately 20% in the ethanol concentration (42 to 50 g/L), with 0.50 g/L of ethanol yield and 0.60 g/L(h) of volumetric productivity. Thus, it was concluded that the banana can be used as adjunct in brewing methods, helping in the development of new products as well as in the elaboration of concentrated worts.

**Acknowledgements:** CAPES, FAPESP, Malteria do Vale, Corn Products Brasil, Wallerstein Industrial e Comercial, and DiverseyLever (Brasil); FCT and GRICES (Portugal)

**Poster 7-23**

**Continuous fermentation of all corn fibre sugars using Thermoanaerobacter BG1**

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Corn fibre, a co-product from the production of ethanol from corn kernels, is produced in greater and greater amounts as the production of ethanol increases. The corn fibre is very rich in xylose and arabinose and ethanol production from these sugars is therefore instrumental if the fibre is to be used efficiently for ethanol production. Thermoanaerobacter BG1 has been shown to be a very efficient ethanol producer in wheat straw hydrolysates containing mostly glucose and xylose, with ethanol yields of up to 0.42 g/g from glucose and xylose combined. The strain is able to grow in non-detoxified wheat straw hydrolysates and to produce ethanol from a wide range of sugars in continuous reactor systems. The growth temperature of 70 degrees C efficiently prevents contamination and thereby reactor downtime due to cleaning and sterilisation. Recently we have shown that BG1 mutants are able to efficiently and simultaneously convert glucose, xylose and arabinose from corn fibre efficiently into ethanol in continuous reactor systems. This opens the possibility of generating more ethanol from corn kernels, and at the same time maintain a by-product rich in protein.

**Poster 7-24**

**A New Process for the Conversion of Lignocellulosic Feedstocks to Ethanol**

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One of the major challenges to a successful commercial application of a second generation bioethanol process utilising ligno-cellulosic feedstocks is to find an ethanologenic strain with the ability to convert efficiently the diverse range of sugars found in biomass feedstocks. TMO Renewables Ltd, a UK-based technology company, has developed such a strain (TM242) which sponsors a number of process advantages including rapid feedstock conversion, low enzyme requirements for biomass hydrolysis, low microbial contamination, and lower capital and operating requirements. TM242 is a thermostable bacterium able to convert C5 and C6 monomers and oligomers from a wide range of biomass feedstocks to ethanol at high yields and at high temperatures. One of the first commercial opportunities for this technology will be a side-door application on existing corn to ethanol operations adding value by converting the residual sugars in the distiller’s grains or fibre fraction co-products, thereby increasing plant ethanol yields by 10-15%. The TMO ethanol process is outlined from pretreatment and hydrolysis through to fermentation. The ability of TM242 to realise a commercially viable cellulosic ethanol process from feedstock to fuel is illustrated using distiller’s grains at high dry solids.
**Poster 7-25**

**SSCF of Paper Sludge Using Recombinant Xylose-Fermenting Microbes**

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As a cellulosic waste that in many cases does not require pretreatment, paper sludge is a potentially attractive commercial substrate as well as a model substrate for the investigation of simultaneous saccharification and co-fermentation (SSCF) featuring cellulose hydrolysis, hemicellulose hydrolysis, and fermentation of resulting sugars in an single process step. Two recombinant xylose-utilizing strains, *Zymomonas mobilis* 8b and *Saccharomyces cerevisiae* RWB222, were studied for performance in paper sludge SSCF with a commercial *Trichoderma reesei* cellulase preparation under conditions producing > 40 g/L ethanol. Substrate conversion, ethanol production, and cell viability were evaluated, and the role of ethanol inhibition, mass transfer limitation, and inhibition by compounds present in paper sludge was investigated. Formation of ethyl b-xylopyranoside, a previously-unreported byproduct, was observed in substantial amounts (corresponding to approximately 25% of hemicellulose hydrolyzed) during paper sludge SSCF. A comprehensive mathematic model was developed for SSCF, the first such model known to us. Model validation data and use of the model for hypothesis testing will be presented, and overall conclusions about paper sludge conversion and SSCF will be drawn.

**Poster 7-26**

**DOM – Domestication of Microorganisms to solve environmental problems**

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Microorganisms can reduce environmental problems e.g. by converting plant biomass to biofuels, replacing chemical pesticides, reducing nutrient leakage in plant growth promotion, and preventing toxic compounds from contaminating the environment in biophylaxis. The DOM programme provides sustainable microbial solutions to environmental problems through research and cooperation with partners. This includes fermentor production of microbial inoculants, followed by formulation steps for long-term stability and ease of application. Safe use of microorganisms requires careful consequence analyses, which for commercial products may be followed by lengthy registration procedures. Presently, growth of novel biotechnological industries that can solve environmental problems is hampered by lack of knowledge about fermentation and formulation techniques. The absence of relevant safety assessment systems for microorganisms, suited for decision making by regulatory authorities, presents even more serious obstacles to sustainable development. The research programme forms a "DOM Center of excellence" that harness the metabolic power of the natural microbial diversity through a domestication programme, focusing on safety and formulation to generate stable products with high efficacy and production economy. Through communication with regulatory authorities at early stages of development, the registration process will be facilitated, allowing earlier product commercialisation. Since process and product safety are assessed at an early stage, potential risks for humans and the environment can be minimised. The DOM Center supports implementation of solutions to environmental problems by connecting university research, industrial development and regulatory processes. DOM has an annual budget exceeding 1.9 M USD for the years 2006-2010 and welcomes international cooperation!

**Poster 7-27**

**Heterologous expression of the XynA endoxylanase from Thermomyces lanuginosus 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 has been successfully used to express heterologous proteins. We describe here, the use of *Z. mobilis* to heterologously express the XynA endoxylanase from *Thermomyces lanuginosus*. A plasmid (pJL101) was constructed that contains a chimeric gene where the *T. lanuginosus* XynA exons I and II are driven by the *Z. mobilis* pgAP promoter, and terminated by the bacteriophage T7 terminator sequence. Transformants of *Z. mobilis*, grown both aerobically and anaerobically, were demonstrated to express detectable levels of endo-xylanase activity using AZCL-xylan. Furthermore, XynA expression in *Z. mobilis* has no adverse effect on cell growth rates. While we are currently unable to visualize the XynA protein via Coomassie and silver-stained polyacrylamide gels, its detectable activity on AZCL-xylan suggests that the small amount of protein being expressed is indeed very active. Additionally, we show that this plasmid when maintained in *E. coli*, expresses active XynA as detected by an AZCL-xylan zymogram. Our preliminary work provides support that *Z. mobilis* may eventually function as a CBP organism.

**Poster 7-28**

**Improved Production of Isopentenol Using Isopentenol Biosynthetic Genes from Bacillus subtilis**

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Naturally derived isoprenoids are used in nutraceuticals, flavors, fragrances, polymers, drugs and other manufacturing processes. Commercial production of these molecules is often limited by natural resources, sometimes with environmentally deleterious effects. Microbial production of isoprenoids would relieve the supply limitation; however there are relatively few terpene synthases whose function has been well characterized. One isoprenoid of particular interest is the alcohol form of isoprene (isopentenol) which has potential for a wide variety of commercial chemistry applications. Two genes (*yhfR* and *nudF*) whose protein products acted directly on prenyl diphosphate precursors and produced isopentenol, were discovered through enrichment of a library of genomic DNA from the isoprene-producing bacterium *Bacillus subtilis* strain 6051 in *E. coli* engineered to produce elevated levels of isopentenyl diphosphate and dimethylallyl diphosphate. Expression of *yhfR* and *nudF* in *E. coli* engineered with the mevalonate-based isopentenyl pyrophosphate biosynthetic pathway resulted in the production of isopentenol. Improvements in the mevalonate pathway, including replacement of the original yeast HMG-CoA reductase increased isopentenol titers.
**Poster 7-29**

**Evaluation of Different Carbon Sources to Surfactin Production by Bacillus subtilis LAM1007**

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Bio-surfactant are amphiphilic compounds with considerable potential in commercial applications within various industries. Although they have advantages over their chemical counterparts, they are not widely utilized due to high production costs associated with use of expensive substrates and inefficient product recovery methods. Surfactin, one of the most effective cyclic lipopeptide bio-surfactant produced for Bacillus subtilis can lower the surface tension of water from 72 to 27 mN m⁻¹. It can be produced by B. subtilis cultures using sugars, vegetable oils or starch. Therefore, the aim of this work was to develop a growth medium able to stimulate bio-surfactant production by B. subtilis LAM1007. A mineral medium (MM) was used as base and it was supplemented with different carbon sources: glucose, fructose, sucrose or Cashew Apple Juice (CAJ), which is rich in reducing sugar (fructose and glucose), fibers, vitamins and minerals salts. Furthermore, the influence of the yeast extract (YE) was also evaluated in the production of surfactin. Batch cultivations were carried out at 180 rpm and 30°C for 96 h. No reduction in the surface tension of the medium free of cells was observed without supplementation with YE. B. subtilis LAM1007 consumed all the carbon sources studied after 48 h. Best result of surfactin concentration (3.50 ± 8.0 x 10⁻³ mg L⁻¹) was achieved when mineral media was supplemented with CAJ and YE. The obtained results indicate that cashew apple juice, a co-product of the cashew nut agro-industry, is a suitable and cheap ($0.5/kg) raw material for bio-surfactant production.

**Poster 7-30**

**Simultaneous saccharification and fermentation (SSF) of steam pretreated hemp**

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This work was focused on industrial hemp, which is rarely investigated as possible substrate for bioethanol production. Preliminary results, presented at the 29th Symposium, showed that even though enzymatic accessibility of the cellulose rich component in hemp was increased by chemical pretreatment, unfortunately the enzymatic conversion was still rather low. According to these experiments a more effective pretreatment method (steam explosion) was adressed for further studies. The aim of the present work was to optimize this pretreatment method of hemp with a view on maximizing ethanol production by two different yeast strains.

Hemp was grown and harvested in Hungary in the fall of 2006. Steam explosion (SE) was carried out in a batch pilot plant according to a factorial design, where the effect of residence time, temperature and use of acid catalyst were investigated. Pretreated material (slurry) was separated into liquid (prehydrolysate) and solid fraction and analyzed for carbohydrates and toxic compounds.

Liquid fractions were used to test the effect of inhibitory compounds of steam pretreated hemp produced during pretreatment on Saccharomyces cerevisiae and Kluyveromyces marxianus CECT 10875. Fermentation of the SE solids was performed to determine the ethanol yield from the cellulose fraction of hemp by baker’s yeast and K. marxianus. SSF was performed in 50 ml at 10 (w/v)% solid content at pH 5.0. Commercial enzymes at loading dosage of 25 FPU/g cellulose were applied. The flasks were incubated for 3 days at 32°C and 42°C, respectively. Results from this study will be presented and discussed at the conference.

**Poster 7-31**

**Co-Culture Fermentation to Produce Ethanol from Dilute Acid Pretreated Corn Stover**

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Successful implementation of biochemical conversion technology for producing fuels from lignocellulose depends on achieving good conversion of biomass derived sugars to ethanol. It may be possible to reach better overall conversion of these sugars by using more than one fermentative microorganism. We investigated the ability of a co-culture fermentation using a recombinant glucose-xylose fermenting bacteria, Zymomonas mobilis 8b, and a glucose fermenting yeast, Saccharomyces pastorianus, to convert all of the cellulolic and hemicellulosic sugars available in dilute-acid pretreated corn stover slurries. The whole pretreated stover slurry was conditioned with ammonium hydroxide and inoculated with Z. mobilis 8b to ferment glucose and xylose produced during pretreatment, and then later, S. pastorianus and cellulase were added to convert the cellulose by a simultaneous saccharification and fermentation process. We also tested a process, in which, a fraction of the liquor was removed from the slurry, separately conditioned by ammonium hydroxide, and then added back to the concentrated solids, then continued with the procedure just described. Both processes were tested at a 10% and 20% total solids concentration. The difference between the two processes is that in the latter configuration the cellulotic solids were not exposed to a high pH. The whole slurry process achieved a theoretical ethanol yield of 87% at 10% total solids from all fermentable sugars, the yield dropped to 52% at 20% total solids. Ethanol yield was about 30% greater when liquor was separately conditioned. Future work will investigate the cause of the performance difference.

**Poster 7-32**

**High-throughput isolation of extreme thermophiles that produce ethanol from switchgrass and Populus**

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Switchgrass and Populus could provide energy-rich feedstocks for the consolidated bioprocessing of cellulotic biomass to ethanol. However, advances in overcoming the recalcitrance of converting plant cell walls to fermentable sugars are needed for an economically viable process. In order to investigate the diversity of microorganisms that can efficiently hydrolyze the cellulotic fraction of switchgrass and Populus at elevated temperatures, we collected water, sediment and decaying wood samples from thermal features located in Yellowstone National Park, USA. Enrichment cultures using acid pretreated switchgrass and Populus as the primary carbon and energy source supported the anaerobic growth of microorganisms at temperatures between 60 to 80 deg. C. Cloning and sequencing of amplified 16S rDNA from several enrichments verified the presence of known cellulolytic, extremely thermophilic bacteria including Caldicellulosiruptor and Thermosphaerobacter spp. Clones representing uncultivated clades of Dictyoglomus, Thermosedulivoibrio, and Nitrospira were also recovered. In order to characterize individual organisms in detail, a high-throughput (HT) isolation system based on flow cytometry was developed that allowed rapid separation and growth of cellulolytic, extreme thermophiles. Coupled to modern screening and phenotyping approaches, the HT isolation system will be used to assemble a large collection of novel cellulolytic, ethanol producing microorganisms from diverse environments.

**Poster 7-33**

Withdrawn
Poster 7-34
Improvement of yield and productivity of ethanol production in yeast through artificial transcriptional factors

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Ethanol production not from sugar or starch-based substrates but from the hydrolyzate of lignocellulosic biomass faces outstanding problems for commercialization. Among these problems, the capability of fermenting pentose and resistance to various inhibitors from pretreatment processes of recalcitrant lignocellulose are critically required traits for optimal strains. Previous studies suggested that simultaneous perturbation of multiple genes might be needed to implement such traits. Moreover, a set of gene targets responsible for the traits is not determined yet. As such, we employed a combinatorial approach based on artificial transcriptional factor (ATF) libraries which were previously developed for genome-wide perturbation of multiple genes (1, 2). Specifically, ATF libraries consisting of DNA binding domains and an activating domain were introduced into the engineered Saccharomyces cerevisiae and improved strains were isolated under various selection conditions. As a result, recombinant Saccharomyces cerevisiae the engineered

References:

Poster 7-35
The YMR315W gene from Saccharomyces cerevisiae codes for an alcohol dehydrogenase and is required for full resistance to oxidative stress

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Ymr315w protein levels have been shown to increase in cells grown on xylose. The mRNA level for the YMR315W gene was also seen to increase in cells grown on xylose, indicating an important function for YMR315W during growth on xylose. YMR315W encodes for a highly conserved protein of unknown function and, based on sequence similarity to known motifs, is predicted to be a dehydrogenase. The YMR315W gene is also co-regulated under numerous stress conditions with six other oxidoreductases, including the GRE3 gene, which has been shown to function in xylose metabolism.

In this study, the YMR315W gene was cloned and the protein was over expressed in Saccharomyces cerevisiae. Lysate from cells expressing elevated levels of the Ymr315w protein were analyzed for enzymatic activity using various substrates and compared to activities from cells that were deleted for the YMR315W gene. Lysate from cells with elevated levels of Ymr315w protein showed increased enzymatic activity toward multiple substrates. Other labs have shown that mRNA levels for the YMR315W gene also increase when cells are exposed to oxidative damage by chemicals such as H2O2. During exposure to oxidative damage, cells require increased amounts of NADPH to defend against reactive oxygen species. We found that cells lacking the YMR315W gene were sensitive to H2O2. This result suggests that YMR315W, through its NAD(P)H-dependent dehydrogenase activity, may function to maintain cellular redox balance, a problem also encountered by cells grown on xylose.

Poster 7-36
Changes on performance and microbial population induced by hydraulic retention time in a pilot plant thermophilic anaerobic digester

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Bacterial and archaeal communities, as well as the digester performances, were analyzed in order to evaluate the influence of hydraulic retention time. A 40m3 pilot plant anaerobic thermophilic bioreactor was used for the experiments and fed with poultry litter. Chemical composition of feed, effluent and biogas were monitored throughout the experiment. Mass and energy balances were used to evaluate the performance of the biodigester. Samples of microbial community DNA were collected each week during the experiment. The relative abundances of dominant microbial populations were measured by terminal restriction fragment length polymorphism (T-RFLP) using PCR primers targeting the bacteria and archaea. Results indicate that loading rate plays an important role in biogas production and reduction of COD and BOD. These changes were associated with variable microbial population dynamics in the bioreactor. T-RFLP profiles of the bacteria indicate that 4 ribotypes dominated the profile throughout the experiment. The abundance of 2 of the 4 ribotypes changed during the course of the experiment while 2 ribotypes remained relatively constant. Archaeal T-RFLP profiles revealed 1 dominant ribotype which does not appear to change in abundance. The results of this experiment, both power input and biodigester performance, indicate that the 13d HRT was optimal for the anaerobic digestion of poultry litter in the experimental conditions evaluated here.

Poster 7-37
Simultaneous saccharification and fermentation of Kanlow switchgrass using Kluyveromyces marxianus IMB4

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Simultaneous saccharification and fermentation (SSF) has long been known to have benefits such as reduced cellulase product inhibition and reduced capital costs; however, the incompatibility between ideal cellulase temperatures and temperatures for ethanolgenic microorganisms reduces SSFs effectiveness. In this study, a thermotolerant yeast strain called Kluyveromyces marxianus IMB4 was used in an SSF process using Kanlow switchgrass as a feedstock. Switchgrass was pretreated using pressurized, liquid hot water at 200°C for 10 min. After pretreatment, insoluble solids were separated by filtration from the liquid prehydrolysate and washed with deionized water to remove soluble sugars and inhibitors. The insoluble solids were then hydrolyzed using a commercial cellulase preparation and fermented by K. marxianus IMB4 using SSF. SSF was done at 37, 41, or 45°C and pH 4.8 or 5.5 for 7 days. Results were compared with a control of Saccharomyces cerevisiae DSA at 37°C and pH 4.8. Fermentation by IMB4 at 45°C ceased after 3 and 4 days, respectively, when a pH 4.8 citrate buffer was used. Fermentation continued for all 7 days using IMB4 at 37°C and the control. When pH 5.5 citrate buffer was used, fermentation ceased after 4 days using IMB4 at 45°C. IMB4 at 45°C, pH 5.5 achieved 78% theoretical ethanol yield after 4 days, which was 3 days faster than when the control achieved 78% yield.
Examining the activity optimum. SSF of cellulose with Bacillus coagulans as microbial biocatalyst that grows at 50-55°C matching the fungal enzyme activity optimum. SSF of cellulose with B. coagulans strain 36D1 was compared with that of Saccharomyces cerevisiae, Zymomonas mobilis and Lactococcus lactis, microbial biocatalysts used by ethanol and lactic acid industries. SSF of cellulose with fungal cellulases was conducted with pH control at conditions that are optimal for each organism. Based on volumetric productivity, SSF of cellulose at 50-55°C with B. coagulans required about 4-times less cellulases in comparison to SSF at 35°C with the other three organisms. B. coagulans as the microbial biocatalyst in SSF of cellulose at 55°C can significantly reduce the cost of fungal cellulases in optically pure Li+-lactic acid production. The results consequently Engineering of B. coagulans or other thermophilic bacteria for ethanol production can reduce the cost of cellulosic ethanol simply by matching the microbial biocatalyst to the optimum for fungal cellulase activity.

Influence of nutrient supplementation on ethanol batch production rates


Ethanol is a renewable energy source produced through fermentation of sugars unlike the fossil fuels. On account of limited global supply of oil, ethanol has reemerged as an alternative to petroleum-based liquid fuels. During fermentation, activities of Saccharomyces cerevisiae closely respond to changes in the environmental conditions, which are accompanied by variations in the mass transfer around and the metabolic behavior of the microorganisms. To ethanol production the yeast requires not only a simple carbon source but also vitamins and others nutrients mainly nitrogen, phosphorus, potassium among others. The nutritional necessities of the yeast during alcoholic fermentation processes 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 different sucrose initial concentrations of cane molasses and sugar cane musts as the sole carbon source for S. cerevisiae. The nutrients supplementation was done with some commercial products with different nutrients in it and at least three initial concentrations of the commercial nutrients formulation were used. Fermentation flasks were then shaken in the incubator at 200 rpm and 32°C. 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, productivity and yield were calculated. The results consequently provide a better understanding of nutrients supplementation effects on the cell activities for further development of the process.

Global Gene Expression Analysis in Pichia stipitis: Examining the Effect of Carbon Source and Oxygen Level Using Batch Cultivation and Continuous Culture

J.R.H. Van Vleet, C. Lu and T.W. Jeffries

Pichia stipitis is a haploid yeast closely related to yeast endosymbionts of passalid beetles that inhabit and degrade white-rotted hardwood. It is capable of using all of the major sugars found in wood and has the highest native capacity for xylose fermentation of any known microbe. Xylose is the second most abundant sugar in nature, second only to glucose, and is therefore extremely significant as biofuels become increasingly important. With the recent sequencing of P. stipitis CB56054, gene chip studies have become possible to examine global gene expression in this yeast.

To study the effect of carbon source on gene expression levels, P. stipitis has been cultivated aerobically in well controlled bioreactors using either glucose or xylose as a carbon source. The effect of oxygen level has on gene expression has also been examined. Unlike S. cerevisiae, which regulates fermentation by sensing the presence of fermentable sugars, P. stipitis induces fermentation in response to oxygen limitation. P. stipitis has been cultivated on both glucose and xylose under oxygen limited conditions. Comparison of gene chip results from these fermentations with the aerobic 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. These studies will provide invaluable insight into growth on xylose by P. stipitis and the genes responsible for fermentation of this extremely abundant sugar. Identification of these genes will yield targets for the future engineering of improved xylose fermenting yeasts.

Exploring the applicability of crude glycerol from biodiesel production as substrate for obtaining biomolecules by Hansenula anomala CCT 2648

J.D. Rivaldi, B.F. Sarrouh, R.F. Branco and S.S. da Silva

The world’s biodiesel marked is in constant increasing. As resulted of its high volume production, large amount of glycerol, major byproduct in the biodiesel industry, is being generating. Normally, glycerol valorization includes direct chemical transformation to useful compounds as feedstock in food, cosmetic, pharmaceutical and chemical industries. However, the purification treatments are too expensive for small and medium size biodiesel producers, due to this fact; more and more quantity of glycerol could be accumulated or discharged in the environment without any treatment. The microbial conversion of glycerol to different products, such as ribonucleotides, single cell protein, citric acid or ethanol, could constitute an alternative to turn biodiesel production economically feasible. In this work, crude glycerol (25 g/L) was used as substrate for the growth of the yeast Hansenula anomala (pH 5.5: 200 rpm; 30°C), which regulates fermentation by sensing the presence of fermentable sugars, was cultivated aerobically in well controlled bioreactors using CBS6054, gene chip studies have become possible to examine global gene expression in this yeast.

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Modeling oxygen uptake rates in a rhamnolipid type biosurfactant production

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The rhamnolipids have been pointed out as promising biosurfactants. The most studied microorganisms for the aerobic production of this molecules are the bacteria of the genus Pseudomonas. The aim of this actual work was to evaluate and model the oxygen uptake rates during the rhamnolipid type biosurfactant production in a bench scale bioreactor by one strain of Pseudomonas aeruginosa isolated from oil environments. In a previous work, a non dispersive oxygenation device was developed. In order to study the microorganism dependency on oxygen, a programmable logic controller (PLC) was used to set the dissolved oxygen concentration in a constant value. Using the data stored in a computer connected to the PLC and the predetermined characteristics of the oxygenation device, it was possible to evaluate the oxygen uptake rate (OUR) and the specific oxygen uptake rate (SOUR) of this microorganism. These rates, obtained for some different dissolved oxygen concentrations, were than compared to the theoretical ones. When the exponential growth phase begins, there is a linear rise in this rate, which varies between 9,0 and 14,0 mgO2.(gDW.h)-1 per hour. This rise last until the end of the exponential phase. After that, the SOUR reduces to 2,0 mgO2.(gDW.h)-1, remaining constant until the end of the fermentation. The simulation results were close to the experimental ones. Thus, they can be used to estimate the total oxygen demand in a fermentation for the rhamnolipid production.

Bioethanol production from xylose using recombinant Saccharomyces cerevisiae expressing protein engineered NADP+-dependent xylitol dehydrogenase

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Saccharomyces cerevisiae is used widely for industrial ethanol production. However, S. cerevisiae is naturally unable to metabolize xylose, which is the second major sugar present in hard woods and herbs, so its fermentation is essential for the economic conversion of lignocellulose to ethanol. Accordingly, heterologous expression of genes for xylose reductase (XR) and xylitol dehydrogenase (XDH) cloned from Pichia stipitis (PsXR and PsXDH, respectively) in S. cerevisiae has been studied extensively with regard to ethanol fermentation from xylose. In addition, overexpression of the xylulokinase (XK) gene from S. cerevisiae has been shown to aid xylose utilization. However, it has not yet been applied to the industrial bio-process, mainly due to the unfavorable excretion of xylitol which occurs during xylose fermentation. Intracellular redox imbalance caused by the different coenzyme specificities between PsXR (with NADPH) and PsXDH (with NAD+) has been thought to be one of the main factors that promote xylitol excretion. To reduce xyitol formation, we have already generated several PsXDH mutants (e.g., ARSdR mutant) with complete reversal of coenzyme specificity toward NADP+ by site-directed mutagenesis. In this study, we constructed a set of recombinant S. cerevisiae strains with xylose-fermenting ability and measured the efficiency of ethanol fermentation from xylose. The results of such fermentation analyses indicated that improved fermentation performance, as seen by the increased ethanol production, decreased xylitol excretion, and faster xylose consumption, was observed in a strain expressing genes of PsXR and NADP+-dependent PsXDH and endogenous XK as compared with the reference strain expressing the wild-type XDH.

Photobiological production of hydrogen from water by Cyanobacterial hydrogenase

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One of the most potentially promising approaches to convert renewable solar energy to hydrogen biofuel is through photosynthetic water splitting by Cyanobacteria. Efficient and economical bioconversion of sunlight requires comprehensive understanding and optimization of the biological processes. Synechocystis sp. PCC 6803 evolves hydrogen via a bidirectional hydrogenase in the dark under anoxic conditions. Our work seeks to investigate and optimize metabolic and regulatory interactions between hydrogenase regulation and hydrogen production, photosynthesis and respiration, and nutrient status. We have designed and validated whole-genome oligonucleotide (70-mer) microarray as a platform to probe the genome-wide transcriptional regulation under macronutrient limiting conditions such as CO2, nitrogen, phosphorus and sulfur. As a first step the physiological and transcriptional responses to sulfur deprivation by Synechocystis were investigated. Our data showed that sulfur deprivation conditions down-regulate photosystems, Cytochrome b6/f complement and ATP generation, while sulfate transport, phycobilisome degradation protein genes, along with many other genes of unknown function, were up-regulated. This transcriptional reprogramming was confirmed not due to a growth-stage related response, but sulfur element starvation which significantly improved hydrogen production. The metabolic and regulatory information derived from this study will be used for improving the hydrogen productivity and sustainability by Cyanobacteria.

Simultaneous saccharification and fermentation of oxalic acid pretreated corn cob to ethanol using xylose-fermenting Pichia stipitis CBS 6054

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Biomass has received special attention as a resource for renewable energy. In particular, woody biomass and agronomic such as corn stover, corn cob, sugarcane waste, wheat or rice straw and forestry residues are on the rise as potential materials that can be converted into fuel ethanol. This work evaluated oxalic acid pretreatment of corn cob to produce ethanol by simultaneous saccharification and fermentation (SSF). The pretreatment was optimized by application of 2 full factorial design and response surface methodology. Samples pretreated by different combination of temperature, oxalic acid concentration and residence time were subjected to enzymatic hydrolysis, using preparations of Novozyme 50013 and Accellerase 1000. The enzymatic saccharification of the cellulose fraction to glucose was favored using pretreated corn cob under conditions that yielded optimal hemi-cellulose solubilization (72.27 g/kg dry matter) of oxalic acid, at 180°C for 50 min). The cellulose saccharification rate was inversely related to the amount of residual hemicellulose in the pretreated corn cob. The highest total sugar concentration (34.5 g/L) obtained by enzymatic saccharification was attained at 55°C, pH 5.5 (medium citrate buffer) after 96 h using 10 FPU/g dry matter at 10% dry solids. The saccharification, which depends on the enzyme loading rate and reaction time, had different yields of glucose from the cellulose and xylose from residual hemicelluloses. We will present results on simultaneous saccharification and fermentation of oxalic acid pretreated corn cob to ethanol using xylose- and cellobiose-fermenting Pichia stipitis CBS 6054.

Photobiological production of hydrogen from water by Cyanobacterial hydrogenase

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One of the most potentially promising approaches to convert renewable solar energy to hydrogen biofuel is through photosynthetic water splitting by Cyanobacteria. Efficient and economical bioconversion of sunlight requires comprehensive understanding and optimization of the biological processes. Synechocystis sp. PCC 6803 evolves hydrogen via a bidirectional hydrogenase in the dark under anoxic conditions. Our work seeks to investigate and optimize metabolic and regulatory interactions between hydrogenase regulation and hydrogen production, photosynthesis and respiration, and nutrient status. We have designed and validated whole-genome oligonucleotide (70-mer) microarray as a platform to probe the genome-wide transcriptional regulation under macronutrient limiting conditions such as CO2, nitrogen, phosphorus and sulfur. As a first step the physiological and transcriptional responses to sulfur deprivation by Synechocystis were investigated. Our data showed that sulfur deprivation conditions down-regulate photosystems, Cytochrome b6/f complement and ATP generation, while sulfate transport, phycobilisome degradation protein genes, along with many other genes of unknown function, were up-regulated. This transcriptional reprogramming was confirmed not due to a growth-stage related response, but sulfur element starvation which significantly improved hydrogen production. The metabolic and regulatory information derived from this study will be used for improving the hydrogen productivity and sustainability by Cyanobacteria.

Simultaneous saccharification and fermentation of oxalic acid pretreated corn cob to ethanol using xylose-fermenting Pichia stipitis CBS 6054

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Biomass has received special attention as a resource for renewable energy. In particular, woody biomass and agronomic such as corn stover, corn cob, sugarcane waste, wheat or rice straw and forestry residues are on the rise as potential materials that can be converted into fuel ethanol. This work evaluated oxalic acid pretreatment of corn cob to produce ethanol by simultaneous saccharification and fermentation (SSF). The pretreatment was optimized by application of 2 full factorial design and response surface methodology. Samples pretreated by different combination of temperature, oxalic acid concentration and residence time were subjected to enzymatic hydrolysis, using preparations of Novozyme 50013 and Accellerase 1000. The enzymatic saccharification of the cellulose fraction to glucose was favored using pretreated corn cob under conditions that yielded optimal hemi-cellulose solubilization (72.27 g/kg dry matter) of oxalic acid, at 180°C for 50 min). The cellulose saccharification rate was inversely related to the amount of residual hemicellulose in the pretreated corn cob. The highest total sugar concentration (34.5 g/L) obtained by enzymatic saccharification was attained at 55°C, pH 5.5 (medium citrate buffer) after 96 h using 10 FPU/g dry matter at 10% dry solids. The saccharification, which depends on the enzyme loading rate and reaction time, had different yields of glucose from the cellulose and xylose from residual hemicelluloses. We will present results on simultaneous saccharification and fermentation of oxalic acid pretreated corn cob to ethanol using xylose- and cellobiose-fermenting Pichia stipitis CBS 6054.
In recent years, there has been growing interest in fermentative hydrogen production using a variety of carbonaceous renewable resources as feedstocks. Among the various bacteria able to produce hydrogen through dark (i.e., non-photosynthetic) fermentation, particular attention has been given to *Clostridium* species. Extensive biodiversity has been observed among uncultured and as-yet uncharacterized clostridia. Experiments were conducted to assess the rate and yield of hydrogen production by two phylogenetically novel groundwater isolates belonging to the genus *Clostridium*. For comparison purposes, two *Clostridium* species previously studied in hydrogen production research, *C. acetobutylicum* (DSM 792T) and *C. butyricum* (DSM 10702T), were also studied. Static batch experiments were carried out at 30°C in anaerobic, buffered, glucose medium at pH 6. Hydrogen, volatile suspended solids (VSS), and glucose concentrations were measured as a function of time following inoculation. Cumulative hydrogen production and VSS data were fitted to a modified Gompertz equation to calculate maximum rates of hydrogen production in terms of mL hydrogen produced per gram VSS per hour. The environmental isolates exhibited higher maximum hydrogen production rates (248 and 245 mL·g⁻¹·h⁻¹) than *C. acetobutylicum* (216 mL·g⁻¹·h⁻¹) or *C. butyricum* (279 mL·g⁻¹·h⁻¹) under the conditions tested. Hydrogen yield (mmol hydrogen produced per mmol of glucose consumed) was also higher for these novel isolates (1.40 ±0.04 and 1.35 ±0.06 mmol/mmol) than for *C. acetobutylicum* (1.20 ±0.18 mmol/mmol) or *C. butyricum* (0.73 ±0.10 mmol/mmol). Results demonstrate that previously uncharacterized hydrogen-producing clostridia hold promise for improved rate and yield in biological hydrogen production processes.

**ZWF1 overexpression in Saccharomyces cerevisiae protects cells from furfural-induced damage to cellular membranes, chromatin, and the actin cytoskeleton**

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Bio-ethanol is a leading alternative to fossil fuels due to environmental and economical reasons. To reach bio-ethanol goals, it is essential to develop efficient strategies to use various lignocellulosic substrates for ethanol production (e.g. agricultural and industrial waste products). The pretreatment process used to generate fermentable sugars from lignocellulosic biomass also generates growth inhibitors, such as furfural and 5-hydroxymethylfurfural. Thus a robust strain tolerant to lignocellulosic biomass also generates growth inhibitors, such as the pretreatment process used to generate fermentable sugars from lignocellulosic biomass also generates growth inhibitors, such as lanolin and thymol which severely limits the usefulness of the castor seed after the oil extraction. Solid state fermentation (SSF) of castor bean waste, a byproduct of biodiesel production, was carried out for ricin detoxification, allergenic problems reduction and lipase production. The fungus Penicillium simplicissimum, an excellent lipase producer, was able to grow and produce the enzyme in this waste. The maximum lipase activity achieved was 17 U/g, without optimization of culture conditions. Furthermore, the fermentation by the fungus *P. simplicissimum* was as able to reduce the ricin content to non detectable values as to decrease the allergenic potential of the castor bean waste in approximately 16%. In this way, the SSF of castor bean waste by *P. simplicissimum* aggregated value to the residue, by the production of a enzyme with greater biotechnological potential at low cost and also by solving the principal disposal problem of this residue, the ricin...
Poster 7-50
Fed-batch Simultaneous Saccharification and Fermentation for bioethanol production by a thermotolerant yeast
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Among all processes for bioethanol production, Simultaneous Saccharification and Fermentation (SSF) appears as a promising alternative. This process performs the enzymatic hydrolysis and fermentation in one single step but it presents an important drawback. Whereas saccharification has an optimum temperature around 50 °C, most of fermenting yeast have an optimum temperature ranging from 30 to 37 °C. Therefore, it would be advantageous to use a microorganism such as Kluyveromyces marxianus, capable to grow and ferment with good yields at temperatures above 40 °C.

In order to reach higher ethanol concentration, solids content in the SSF broth should be as high as possible. However, using too high dry matter contents can cause difficulties in stirring the viscous medium and inhibition of yeast metabolism due to the increase of toxic compounds derived from sugar or lignin during pretreatment.

Both disadvantages together with the end-product inhibition due to glucose accumulation in the broth could be overcome by performing a fed-batch configuration. By the addition of slurry to the SSF broth from time to time yeast improves its ability to detoxify some inhibitors and the viscosity is diminished because the substrate is continuously hydrolysed.

Ethanol production by Kluyveromyces marxianus in a fed-batch SSF at 42 °C and 15 FPU/g of celluose of commercial cellulose is tested. Starting substrate loading is 10% (w/v) and it will be increased gradually up to 14% (w/v). Different addition times and different substrate pulses are going to be also studied in order to figure out the best configuration process.

Poster 7-51
Expression of a heterologous xylose transporter in a Saccharomyces cerevisiae strain engineered to utilize xylose increases xylose uptake and improves xylose/glucose co-consumption
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Strains of Saccharomyces cerevisiae have been engineered to utilize xylose by expressing either the genes for xylose reductase and xylitol dehydrogenase, or for xylose isomerase. These strains still use xylose at sub-optimal rates for industrial fermentation. Unlike natural xylose fermenting yeasts such as Pichia stipitis, S. cerevisiae does not contain xylose-specific transport systems. Analysis of strains that have been adapted for enhanced growth on xylose indicates increased expression of hexasaccharides transporters and suggests that xylose transport is one of the limiting steps in xylose utilization. Since the increase in xylose transport in these strains results from increases in hexose transporters, xylose uptake remains inhibited by glucose, thus limiting xylose and glucose co-consumption.

The goal of this study was to determine the effect of a xylose transport system on xylose/glucose co-consumption and total xylose consumption. We expressed two heterologous transporters from Arabidopsis thaliana in xylose-utilizing S. cerevisiae strains. Strains expressing the heterologous transporters were grown aerobically on glucose and xylose mixtures. Sugar consumption rates and product accumulation were determined and compared to a control strain not expressing the A. thaliana xylose transport genes. Our results from aerobic cultivation with glucose/xylose mixtures indicate that expression of the transporters increased xylose co-consumption rates (prior to glucose depletion) by up to 100%. Increased xylose co-consumption also correlated with increased ethanol concentration, yield and productivity. It was concluded that in these strains, xylose transport is a limiting factor for xylose utilization and that increasing xylose/glucose co-consumption is a viable strategy for improving xylose fermentation.

Poster 7-52
Ethanol Impact on Xylose Metabolism in S. cerevisiae 424A(LNH-ST)
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Ethanol toxicity could be a significant bottleneck in industrial ethanol fermentation of sugars from lignocellulose. To understand ethanol impact on xylose fermentation, batch fermentations were carried out using S. cerevisiae 424A (LNH-ST), an engineered strain capable of co-fermenting glucose and xylose. The fermentation of xylose was carried out in YEP growth media, using largely non-growing cells in the presence of initial ethanol concentrations between 4 - 8% (w/v).

The effects of extraneously added ethanol (pure xylose fermentation) and ethanol generated from glucose equivalent (co-fermentation) are compared. This yeast strain was found to cease fermentation of xylose at an extraneously added ethanol concentration of 9% (w/v). However, co-fermentation of glucose and xylose was capable of achieving a final ethanol titer over 11% (w/v). A preliminary unstructured, Monod-type model of these batch fermentations that include ethanol inhibition is presented.

Poster 7-53
Combined Effect of Acetic Acid and Controlled pH on the Co-fermentation of Glucose and Xylose by Recombinant Yeast
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Lignocellulosic biomass, primarily comprised of cellulose, hemicellulose, and lignin, is a promising renewable feedstock for the microbial production of chemicals, especially ethanol. The major fermentable sugars (hydrolysates) released from the processing of the lignocellulosic 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 during the pretreatment of the biomass. In this poster, we report the effect of acetic acid on glucose/xylose co-fermentation by the genetically modified S. cerevisiae 424A(LNH-ST). The co-fermentation of glucose and xylose was performed under acetic acid conditions of 5, 10, 15 g/L, over a pH range of 5 – 6. To maintain the pH at the specified initial value, the fermentations were carried out in a 1L New Brunswick BioFlow 110 benchtop fermentor equipped with a pH controller. Results showed that the fermentation of both sugars was affected by the presence of acetic acid. The inhibitory effect of acetic acid increased as the pH decreased. The results also indicate that the utilization of xylose is more influenced by acetic acid concentration and pH than the utilization of glucose.
Performance of Zymomonas mobilis 8b on Ammonium Hydroxide Conditioned Dilute Acid Pretreated Corn Stover in Various Process Configurations

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Efficient processes for converting lignocellulosic hydrolysates to ethanol require optimal utilization of the biomass-derived sugars present in these materials. This study evaluated fermentation performance of the glucose-xylose fermenting recombinant Zymomonas mobilis strain 8b using various process configurations. The goal was to determine the microorganism’s ability to produce high ethanol concentrations and yields at high hydrolysate solids loadings. Dilute acid pretreated corn stover slurries were produced in a pilot scale pretreatment reactor and the whole slurry or liquor fraction was subsequently conditioned by ammonium hydroxide. When processing whole slurries, a simultaneous saccharification and fermentation process (SSF) or a separate hydrolysis and fermentation (SHF) process was tested. The liquor fraction was also removed from the solids and conditioned, and then fermented at various dilutions to assess the microorganism’s tolerance to high concentrations of inhibitors. A whole slurry SHF at a 20% (w/w) total solids concentration achieved an ethanol yield of 55% and used 40% of the available xylose, while an SSF using this microorganism performed poorly. However, fermentation of the separated liquor fraction at the same equivalent strength achieved an 89% ethanol yield and used 95% of the available xylose. At a higher liquor strength equivalent to whole slurry at 25% total solids, the ethanol yield dropped to 75% and 82% of the available xylose was used. This study showed that better ethanol yields can be achieved in the liquor only fraction, but further exploration is necessary to explain this behavior.

Lipase production of Aspergillus parasiticus by solid state fermentation

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The solid-state fermentation (SSF) appears as an interesting low-cost alternative for the production of enzymes. Additionally, low-cost, abundant materials of vegetal origin, such as agroindustrial wastes (soy cake, wheat bran, sugar cane bagasse, etc.) can be employed as raw materials. The present investigation reports the production, and characterization of lipase of an Aspergillus parasiticus strain. Initially, the kinetic study experiments were carried out using babassu cake as basal solid medium moisturized with water (70%, w/v) without supplementation or with 3.3% (w/w) sugar cane molasses showing higher activities on babassu cake without supplementation in 72h of fermentation at 30°C. The influence of 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 activities could be achieved at lower temperatures and moisture. The maximum lipase activity was obtained at the temperature of 28°C and moisture of 65% (w/v). The substrate specificity of the crude lipase was determined using several p-nitrophenyl as substrate. Higher Vmax and lower Km were obtained with p-nitrophenyl butyrate indicating that this enzyme presents high affinity for short-chain esters. The immobilized lipase in hydrophobic support (acurel) also catalyzes the synthesis of ethyl oleate. Thus the lipase production by SSF using an abundant and low cost agroindustrial residue as culture medium, showed high biotechnological potential.

Zymomonas mobilis has many desirable ethanologen attributes including producing near theoretical yields of ethanol and recombinants strains are able to ferment both C-5 and C-6 sugars. The availability of the Z. mobilis ZM4 (ZM4) genome sequence and U.S. Department of Energy Joint Genome Institute plans to sequence the genomes of additional strains in the near future provide opportunities to gain fundamental insights into the organism’s physiology leading to strain improvements. Towards this aim of strain improvements, we are using systems biology and genetic tools to elucidate ZM4 oxygen stress responses and process inhibitor tolerance mechanisms. Comparative genome resequencing of a classically derived acetate tolerant ZM4 strain (AcR) combined with transcriptomics, quantitative proteomics, metabolomics and genome reannotation form the basis of ongoing studies to elucidate key loci and targets for strain improvements. We have established a research platform that integrates the advantages of classical selection strategies and systems biology technologies to establish a new paradigm in industrial strain characterization and development.

Pretreatment of feedstock is critical to achieve satisfactory conversion of lignocellulose. Several pretreatment processes have been developed and achieved some level of success. However, most existing pretreatment processes have two major pitfalls. First, the pretreatments are effective only on size reduced biomass feedstocks. As a result, these processes are unable to take the advantage of the softening effect of pretreatment on wood structure to reduce energy consumption for size reduction. The second, the existing processes produced low enzymatic hydrolysis (cellulose-to-glucose conversion) efficiency (<75%) when they are applied to softwood. Unfortunately, forestry is a significant source of biomass feedstock for biorefining, and softwood is the major species in several parts of the US, Canada and Scandinavia. This study demonstrated a novel pretreatment process (Patent application pending) for robust bioconversion of lignocellulose including softwood. Enzymatic cellulose hydrolysis of 90% was achieved within 24 hours with commercial enzymes for spruce (softwood) and short rotation eucalyptus (hardwood) after the pretreatment under mild conditions of chemical dosage, reaction time, and temperature. No significant delignification is required. Furthermore, the pretreatment provided near complete separation of hemicellulose form cellulose, which promised separate utilization of hemicellulose sugars. In addition, the pretreatment reduced the overall energy consumption for size reduction by 70%, to only about 150 Wh/kg for softwood.
Poster 8-08
High Solid Loading Enzymatic Hydrolysis and Fermentation of AFEX Treated Corn Stover
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The production of ethanol from lignocellulosic biomass is now becoming a reality and conversion processes are improving. Process economic studies for production of lignocellulosic ethanol indicate that a minimum ethanol concentration of at least 4% (w/w) prior to distillation is required. Taking this fact into consideration, the total non-soluble lignocellulosic solids loaded for enzymatic hydrolysis must be above 15% (w/w) for most biomass materials. Enzymatic hydrolysis at higher solid loadings is significantly inhibited by: (1) higher concentration of inhibitors (e.g. organic acids, furans and phenolic compounds) (2) free monomeric sugars and (3) mass transfer limitations. Ammonia Fiber Expansion (AFEX) is a novel alkaline pre-treatment technology used to pretreat lignocellulosic biomass that may produce reduced inhibitor levels compared to other pretreatments. It is the aim of this study to understand how high non-soluble solid loadings can affect the enzymatic hydrolysis of AFEX treated corn stover. In this study, corn stover was pretreated using optimal AFEX conditions. The pretreated samples were hydrolyzed using commercial enzymes with and without water washing under high solid loadings. In addition, protein rich substrates were also used as additives to improve enzymatic hydrolysis. Sugar conversions after enzymatic hydrolysis and ethanol fermentation profiles using *Pichia stipitis* were determined for a range of glucan loadings (1-12%). Details of these findings will be discussed.

Poster 8-09
Pretreatment of reed by wet oxidation and applicability of the pretreated material in ethanol production and cellulase enzyme fermentation
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Reed (*Phragmites australis*) is a large perennial grass native to wetland sites at temperate and tropical latitudes. Due to its relatively high cellulose (36%) and hemicellulose (21%) content, reed is often recognized as a potential substrate of various lignocellulose based bioprocesses. However, at the moment it is among the least characterized residues considered for such purpose. The production of second generation bioethanol (often coupled with the fermentation of cellulase enzymes needed in large quantities within the same process) is predicted to become the largest bulk technology to utilize lignocellulose biomass soon. In order to remain sustainable no residue available locally with sufficiently high ethanol potential can be left unutilized and therefore the evaluation of reed for its convertibility to ethanol is a must at the geographical sites under discussion.

Among the available physico-chemical methods developed for the pretreatment of lignocellulosics, wet oxidation is a relatively novel development, which was successfully applied to reed in the present study. Wet oxidation employing various reaction temperatures (185°C - 200°C) for 12 min using 2 g/L Na\(_2\)CO\(_3\) as the catalyst was evaluated via the characteristics of produced solids obtained after various treatments. Enzymatic convertibility of the cellulose content of the pretreated material by cellulases, fermentability of the hydrolyzed cellulose to ethanol by *Saccharomyces cerevisiae*, and applicability of pretreated material in cellulase enzyme production by *Trichoderma reesei* were investigated in the present study. Results will be presented and discussed at the conference as the measure of obtained conversion, ethanol yield, and cellulase activity, respectively.

Poster 8-10
Pretreatment of Waste Licorice by Aqueous Ammonia for Bioethanol Production
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Production of bioethanol from renewable lignocellulosic materials and identification of appropriate feedstocks have been the subject of many recent research efforts. Waste licorice is a waste material generated from oriental herb pharmaceutical production. It is a potential feedstock for bioconversion to ethanol. In this study, pretreatment using aqueous ammonia was applied to this feedstock and its effectiveness on bioconversion was investigated. From batch pretreatment experiments conducted using aqueous ammonia, the optimum conditions of pretreatment were determined in terms of the reaction temperature, time, and ammonia concentration. The criteria of optimum condition were based on lignin removal, carbohydrate retention, and glucose yield upon enzymatic hydrolysis of the treated waste licorice by cellulase. The optimum conditions determined as such were: 150°C, 10 minutes, and 17% aqueous ammonia concentration. The 72-h glucose yield obtained from enzymatic hydrolysis of the waste licorice treated under the optimum condition was 88% of theoretical maximum with 15 FPU/g-glucan enzyme loading.

Poster 8-11
The Effect of Lignin Removal by Alkaline Peroxide Pretreatment on the Susceptibility of Lignocellulosic Biomass to Individual Cellulolytic and Hemicellulolytic Enzymes
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Increased demand worldwide for alternative biomass derived fuels to reduce nations’ dependence on the currently volatile petroleum market has created a renewed interest in studies of plant cell wall structure and deconstruction. Traditionally, the lignin and xylan components of the cell-wall matrix are thought to be key targets for removal in order to improve the accessibility of the cellulose fraction to whole fungal cellulase systems. These enzymatic systems often contain a number of cellulytic, xylanolytic and other hemicellulolytic enzyme activities working in concert, making it difficult to determine how the removal of a particular cell-wall fraction improves the saccharification of the residual structural carbohydrates. In this study, we have utilized a suite of purified cell wall hydrolyzing enzymes in order to investigate the role that lignin removal plays in improving the accessibility of the cell wall microfibrils. Using individual as well as combinations of specific enzyme activities, we examined the effect that lignin removal by alkaline peroxide treatment has on the ability of individual cellulases and hemicellulases to hydrolyze their respective substrates. Using this approach, we were able to identify which types of activities are most affected by the removal of lignin. We propose that this simplified strategy will permit us to understand how specific enzyme components in traditional fungal cellulase preparations function to deconstruct plant cell walls.
Poster 8-12

Enzymatic Hydrolysis and Fermentation of Pretreated Cashew Apple Bagasse with Diluted Sulfuric Acid for Bioethanol Production

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There has been an increasing in the worldwide interest in alternative, non-petroleum-based sources of energy. The most common renewable fuel today is ethanol produced by fermentation of sucrose in Brazil or corn glucose in the United States. However, these raw bases will not be sufficient to satisfy the international demand. Consequently, future large-scale use of ethanol will most certainly have to be based on the production from lignocellulosic materials. In the state of Ceará (northeast of Brazil), the cashew agroindustry has an outstanding role in the local economy and cashew apple bagasse (CAB) appears as an alternative raw material for ethanol production. The aim of this work was to optimize the enzymatic hydrolysis of the cellulose fraction of CAB after diluted acid pretreatment, and to evaluate its fermentation to ethanol using Saccharomyces cerevisiae. Some variables were investigated, such as: temperature (30, 37 and 45°C) and enzyme source (Celluclast 1.5L and Cellulase – Sigma) and enzyme loading (15 and 30 FPU/g Bagasse). The enzymatic hydrolysis was carried out in 250 mL flasks at 150rpm and pH 5.0. The hydrolyzed products were used as fermentation medium without any nutritional supplements. Fermentation assay were carried in batch with 200 mL working volume, at 37°C, 150rpm, pH 5.0, and an initial cell concentration of 5.0 (dry weight/L). The fermentation of the hydrolyzate of the pretreated CAB with diluted sulfuric acid stands as an excellent alternative for the production of fuel ethanol from lignocellulosic biomass.

Poster 8-13

Achieving high xylose yields from dilute acid pretreatment of corn stover under process relevant conditions

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Pretreatment experiments were carried out with the goal of achieving high xylose yields in current high solids pretreatment batch reactors to serve as a benchmark for further increases in xylose conversion. Corn stover was pretreated with dilute sulfuric acid using a four liter Steam Gun and a two liter stirred ZipperClave® reactor at an initial solids loading of 45% and with nominal particle sizes of either 6 or 18 millimeters. Acid impregnation was performed prior to pretreatment by soaking the biomass in an acid bath at 60°C for four hours and then dewatering the biomass to 45% total solids in a hydraulic press. Pretreatment was carried out at temperatures between 180°C and 200°C for residence times of either 90 or 105 seconds. Results demonstrate an ability to achieve high xylose yields over a range of pretreatment conditions, with performance showing little dependence on particle size or pretreatment reactor type. The highest yield of xylan to total soluble xylose (monomer and oligomer) was 67% at 180°C for 90 seconds using the steam gun, with 82% recovered as monomers. The total xylan removal was 93% under these conditions, with a xylan loss to furfural of 4%. High xylose yields reported in this study were attributed to effective catalyst impregnation, along with operating at high solids loadings that permit effective steam penetration. The pretreatment results obtained are among the highest published for dilute acid pretreatment and high solids concentrations, and provide a benchmark for achieving higher xylan-to-xylose yields in continuous reactors.

Poster 8-14

Optimization of Pre-Treatment for Ethanol Production from Post-Harvest Sugarcane Residue

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Sugar production is a major industry in Louisiana. In 2004, 804 producers from 23 parishes produced just over 1.2 million tons of sugar. One problem sugarcane farmers face is the post-harvest sugarcane residue in the form of leaf litter that is left after harvesting. At 3 to 10 tons residue per acre, it is a major impediment to farming practices. Currently farmers use open-air burning techniques to get rid of the residue. The open-air burning accounts for up to 21% of total air pollution in Louisiana, which is known to cause public health problems such as asthma and emphysema. Farmers are now under increasing pressure to find alternatives to open air burning. Research at Nicholls State University explores the possibilities of making alcohol from the sugarcane residue. A chemical pre-treatment process using alkaline peroxide was applied to remove lignin, which acts as physical barrier to cellulo-lytic enzymes. Two yeast strains including Saccharomyces cerevisiae ATCC strains 765 and 918 were used in the experiment. The pre-treatment process effectively removed lignin. Alcohol production in the culture sample was monitored using gas chromatography. The results indicate that ethanol can be made from the sugarcane residue. The fermentation system needs to be optimized for evaluating the economics of producing ethanol from the sugarcane residue.

Poster 8-15

Effect of Corn Stover Fractions and Harvesting Period on Overall Sugar Yields following AFEX Pretreatment and Enzymatic Hydrolysis

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Corn stover cell wall composition changes considerably throughout the yearly growth period and also varies significantly between the various fractions of the plant (i.e. leaf vs. stem). Previous studies have shown that variations in cell wall composition (i.e. cellulose, hemicellulose and lignin) can have a substantial influence on pretreatment recalcitrance, enzymatic digestibility, and maximum achievable sugar yields. It is reasonable to assume that, due to differences in composition, different stover fractions could have different optimal pretreatment conditions and corresponding sugar yields. Maximum sugar yields from individual fractions would be one criterion for determining which fractions should be left on the field for erosion control following harvest. Assuming no other factors, it would be most logical to harvest the least recalcitrant biomass and leave the remainder.

Ammonia Fiber Expansion (AFEX) followed by enzymatic hydrolysis was performed on four different corn stover fractions (stem, leaf, husk and cob) from both September (early) and November (late) harvests. The objective of this project was to determine: 1) whether individual stover fractions have different optimal AFEX conditions and whether this is different from previously optimized values for homogeneously milled corn stover and 2) which fractions give the highest glucose and xylose yields. Based on the maximum glucose, xylose and total sugar yields from the individual fractions, optimal harvest scenarios, assuming 30% and 60% dry matter left on-field, were determined.
Poster 8-16
Comparative study of corn stover pretreated by dilute acid and cellulose-solvent-based lignocellulose fractionation
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Breaking biomass recalcitrance is among the largest obstacles to cellulosic ethanol production because natural biomass and even pretreated biomass have a limited accessibility to cellulase. Quantitative determination of cellulose accessibility to cellulase (CAC) was established based on adsorption of the non-hydrolytic protein TGC containing a cellulose binding domain (CBM) and green fluorescence protein (GFP) (Hong et al. 2007. Langmuir 23: 12535). Here we apply this new technology to measure surface area of cellulose and lignin fractionations of pretreated corn stover by dilute acid and cellulose-solvent-based lignocellulose fractionation (CSLF). The quantitative surface area data show that CSLF can break lignocellulose structure more efficiently than dilute acid, resulting much more surface area of cellulose fractionation. Therefore, the pretreated corn stover by CSLF was hydrolyzed faster with higher sugar yields than dilute-acid-treated corn stover.

Poster 8-17
Enhanced enzymatic hydrolysis of rice straw biomass by an electron beam irradiation pretreatment process
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The feasibility of using electron beam irradiation (EBI) as a pretreatment process to increase the enzymatic digestibility of lignocellulosic biomass by cellulose hydrolysis enzymes. Depending on strength of the EBI, the pretreated rice straw by EBI demonstrated the different magnitudes physical and chemical changes. In particular, the EBI pretreatment resulted in an increase of enzyme digestibility using cellulose hydrolysis enzymes (Trichoderma reesei cellulase and Asperillus niger beta-glucosidase). However, there was no significant change in the carbohydrate and lignin composition of the rice straw after the pretreatment by EBI. The optimum EBI pretreatment condition (1 MeV-80 KGy) yielded 8.6 fold improvement in enzymatic digestibility compared to that of the control which was not pretreated at all. The possible causes of the enhanced the enzyme digestibility were further investigated by using scanning electron microscope (SEM) and X-ray diffraction. The relation between crystallinity index (CrI) and enzyme digestibility indicates strong correlation by giving an R² value of 0.9052. When certain EBI pretreatment conditions such as 1 MeV-92.4 KGy, 1.5 MeV-92.4 KGy and 2 MeV-92.4 KGy were excluded, the CrI and enzyme digestibility yielded a higher R² value of 0.9555. The three dimensional plot established by the relations of enzyme digestibility, electron beam energy, and irradiation dose successfully indicated the optimum ranges of EBI pretreatment conditions for achieving the maximum value of enzyme digestibility.

Poster 8-18
Optimization of lignin-degrading enzyme production of white-rot fungi, Phanerochaete chrisosporium and Dichomitus squalens, for the biological pretreatment of lignocellulose
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Unlike various thermal or chemical processes for pretreating biomass, which are usually carried out at an extreme condition, the biological pretreatment using white rot basidiomycetous fungi could bring potential improvement in the current pretreatment and deconstruction of biomass by minimizing inhibitors and energy consumptions. In our study, a lignin-degrading enzyme was selected as targets for the primary tool for disabling the natural recalcitrance of lignocellulosic biomass. The production of a fungal lignin-degrading enzyme, manganese peroxidase (MnP) by Phanerochaete chrisosporium and Dichomitus squalens was optimized. Various culture conditions such as carbon, nitrogen, trace minerals, and vitamin sources were optimized against the activity of MnP in both cell-envelop-bound and intact-cell-bound forms by the response surface methodology (RSM) including the Plackett-Burman design (PBD), Box-Behnken design (BBD), and Ridge Analysis (RA). For the direct measurement of change in the enzyme digestibility by using cellulose hydrolysis enzymes (Trichoderma reesei cellulase and Asperillus niger beta-glucosidase) of biomass in the fungal culture media, the rice straw samples were periodically taken from the culture media, and the enzyme digestibility of the cellulose in the rice straw was measured. The increase of enzyme digestibility exhibited a strong dependency on the activity of MnP in the cultivation medium of the fungi. The result indicates that fungal fermentation of lignocellulose could be a potent alternative to the conventional thermochemical or chemical pretreatment processes.

Poster 9-07
Study of the production of biodiesel in supercritical medium using reactive distillation
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Biodiesel has become attractive mostly due to its environmental benefits, but the high production cost is still a point of concerns to make this product a commercial long term alternative diesel fuel. The combination of chemical reaction plus distillation in a single piece of equipment is advantageous when the reaction rate decreases with the chemical equilibrium rendering low conversion and selectivity of liquid phase reactions.

The application of the reactive distillation technique for the production of biodiesel via enzymatic alcoholysis (transesterification) of sunflower oil with ethanol in supercritical CO₂, which generates glycerol and a mixture of ethyl esters (biodiesel), was investigated. Kinetic parameters for the overall reaction were fit to experimental data [Madras et al. (Fuel 83, 2004, 2029-2033)] according to empirical and reversible reaction models. A fed-batch reactor was modeled with and without distillation approach. The results suggested that it is not attractive to apply reactive distillation to this reaction under the specified conditions since the products formed in this reaction are not volatile enough to be distilled off and dislocate the equilibrium toward the desired products. Moreover, the characteristics of the compounds involved, such as high viscosity, offer additional mass and heat transfer problems and may preclude the use of this technique for practical purposes.

Keywords: biodiesel, enzymatic alcoholysis, reactive distillation, sunflower oil
**Poster 9-08**

**Effect of BSA treatment of cellulosic biomass on continuous enzymatic hydrolysis and SSF**

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The addition of BSA as a model non-catalytic protein has been shown to enhance cellulose hydrolysis rate and reduce the amount of enzyme needed to reach a certain conversion. Furthermore, continuous processes are generally favored for producing fuels such as ethanol as well as commodity chemicals, but data on continuous biological processing of lignocellulosic biomass are scarce. The objective of this work was to characterize growth of C. thermocellum, quantify H2 production, and determine soluble end-product synthesis patterns during fermentation of powdered α-cellulose under continuous culture conditions. A 5 L fermentor was established and growth was maintained for over 3000 hours. During this time, the cellulose was introduced continuously with continuous nitrogen gas sparging to prevent clogging of the feed-line. The concentration of cellulose was increased stepwise from 1 to 4 g per L. The pH (7) and temperature (60°C) of the reactor were maintained throughout the experiment. At concentrations above 4 g per L, the delivery of α-cellulose was impaired due to feed-line clogging and it became difficult to maintain a homogenous suspension. The highest total gas (H2 plus CO2) production rate, 56.6 mL per L per hr, was observed at a dilution rate of 0.042 L per hour and substrate concentration of 4 g per L. Under these conditions, the H2 production rate was 5.06 mmol per hr. Acetate and ethanol were the major soluble end-products, while lactate and formate were greatly reduced compared to production in batch cultures. Concentrations of all metabolites increased with increasing substrate concentration, with the exception of lactate. These results show that H2 production is proportional to substrate concentration, but product ratios remain constant within the loading rates tested.

**Poster 9-09**

**Continuous hydrogen production during fermentation of alpha-cellulose by the thermophilic bacterium Clostridium thermocellum**

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Continuous hydrogen (H2) production during fermentation of α-cellulose was established using the thermophilic, anaerobic, bacterium Clostridium thermocellum ATCC 27405. The objectives of this work were to characterize growth of C. thermocellum, quantify H2 production, and determine soluble end-product synthesis patterns during fermentation of powdered α-cellulose under continuous culture conditions. A 5 L fermentor was established and growth was maintained for over 3000 hours. During this time, the cellulose was introduced continuously with continuous nitrogen gas sparging to prevent clogging of the feed-line. The concentration of cellulose was increased stepwise from 1 to 4 g per L. The pH (7) and temperature (60°C) of the reactor were maintained throughout the experiment. At concentrations above 4 g per L, the delivery of α-cellulose was impaired due to feed-line clogging and it became difficult to maintain a homogenous suspension. The highest total gas (H2 plus CO2) production rate, 56.6 mL per L per hr, was observed at a dilution rate of 0.042 L per hour and substrate concentration of 4 g per L. Under these conditions, the H2 production rate was 5.06 mmol per hr. Acetate and ethanol were the major soluble end-products, while lactate and formate were greatly reduced compared to production in batch cultures. Concentrations of all metabolites increased with increasing substrate concentration, with the exception of lactate. These results show that H2 production is proportional to substrate concentration, but product ratios remain constant within the loading rates tested.

**Poster 9-10**

**Towards Optimal Operation of Distillation Column for Bioethanol Production**

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Bioethanol represents an important option for the exploitation of an alternative source of energy and the reduction of polluting gases. As a means to increase its economic efficiency as fuel, optimization proposals are essential, mainly on ethanol concentration and dehydration, which usually require a significant amount of energy. The focus of this work is the separation of a mixture proceeding from the fermentation stage. This mixture is sent to a conventional distillation column in order to concentrate ethanol near the azetroptic value. This column was optimized by minimizing the product loss and the demanded energy.

A great number of components, like ethanol, water, acetaldehyde and furfural, and the strong interaction between them make the column’s simulation more complex. These components are present in industrial large scale units but rarely are taken into account. Therefore, a deep study about this interaction is indispensable as well as how such components impact the general column behavior including the energy consumption.

The work was carried out in the Aspen Plus® simulator and comparison with experimental data was considered. An extensive evaluation of the thermodynamic models adequacy was made to represent suitably the separation process units. The results have shown that a significant improvement in terms of energy consumption can be achieved with optimal operation definition and also it is depicted that is important to take into consideration components usually neglected in the separation process synthesis.

**Poster 9-11**

**Biofuels Process Model Development with SuperPro Designer**

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Pretreatment processes have been identified as one of the significant cost prohibitive and inefficient steps to the development of cellulosic feedstock based fermentations in order to produce liquid fuels. Previous research has emphasized the need for individual feedstock process optimization, typically specific to pretreatment methods. More recent work has extended pretreatment to include more efficient and complementary coupled methods. With the use of SuperPro Designer, we have evaluated and performed mass and energy balances for coupled pretreatment pilot plant designs. We highlight some of our experiences while using SuperPro Designer and present comparisons of varied pretreatment designs.

**Poster 9-12**

**Rhamnolipids-enabled affinity foam fractionation of Trichoderma β-glucosidase**

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Rhamnolipids-enabled affinity foam fractionation of Trichoderma β-glucosidase

Affinity foam fractionation involving selective binding between enzymes and substrates/analogs can be a powerful and economical bioseparation tool. In this study we showed that the biosurfactant rhamnolipids (RLs) can be used as the affinity foaming agent to selectively separate β-glucosidase from cellulase of Trichoderma reesei. Produced by Pseudomonas species, RLs consist of one or two molecules of rhamnose and one or two molecules of β-hydroxyalkanoic acids, with pKa ranging from 4.8 to 5.6. According to our HPLC-MS analysis, the RLs used in the study had two predominant components: one di-rhamnose (molecular weight = 650) and the other mono-rhamnose (MW = 530), in a molar ratio of 52:48. Both RLs had a chain of two inter-esterified β-hydroxydecanoic acids. When added to fermentation broth of T. reesei or the solutions of commercial cellulase, the RLs significantly improved the foaming and selectively brought β-glucosidase into the foamate, reaching up to 20-fold enrichment ratios (without enriching the endo-glucanase and exo-glucanase). The selectivity is attributed to the di-rhamnose of RLs, which resembles the disaccharide substrate (cellobiose) to β-glucosidase. The effects of operating conditions (pH, air flowrate, RLs concentration, and the ratio of RL concentration to cellulase FPU) on the affinity foam fractionation were systematically investigated and an empirical model established.
Poster 9-13
Liquid-liquid equilibria for castor oil+glycerol+alcohol
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Biodiesel can be produced through transesterification reaction of vegetable oils or animal fats with alcohol, generally methanol or ethanol, in the presence of a catalyst. Besides the alkyl esters, this reaction forms glycerol along with mono and diglycerides. For most vegetable oils, the products form two liquid phases, one rich in glycerol and the other in biodiesel with alcohol distributed in both phases. The mixture can easily be separated by decantation. When castor oil is used as a feedstock the mutual solubility of the products is high and only one phase is obtained due to the fact that the corresponding esters have a hydroxyl group in their chains causing an increasing in solubility. The objective of the present work is to study the phase equilibrium behavior of the system biodiesel+glycerol+alcohol to provide experimental data for the optimization of the separation down stream processes. The measurements of liquid-liquid equilibrium were carried out for the ternary systems containing biodiesel derived from castor+glycerol+methanol at 25°C and +ethanol at 25 and 60°C. An increase of the system mutual solubility was observed with temperature. The phase boundary was determined by turbidimetric analysis using the titration method under isothermal conditions. The tie lines were indirectly measured by analyzing the mixture density. **Keywords:** biodiesel, ternary systems, methanol, ethanol

Poster 9-14
Continuous flash fermentation applied for the production of biobutanol
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The objective of this work is to demonstrate the technical feasibility of the continuous flash fermentation for the production of butanol using mathematical modelling and computer simulation. The process consists of three interconnected units, as follows: fermentor, the cell retention system (tangential microfiltration) and vacuum flash vessel (responsible for the continuous recovery of butanol from the broth). The concentration of butanol in the fermentor lowered from 11.3 to 7.8 gL-1, what represents a significant reduction of the inhibitory effect. As a result, the final concentration of butanol was 28.2 gL-1, for a broth with 140 gL-1 of glucose. Butanol productivity and yield were, respectively, 7.0 gL-1h-1 and 20.2 % and sugar conversion, 95.6 %. Compared to the conventional continuous fermentation process, the gains of the flash fermentation in terms of productivity, yield and conversion were approximately 150 %.

Poster 9-15
Real-time optimization of the biotechnological process for acrylic acid synthesis: determination of the optimality conditions
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The acrylic acid is one of the most important industrial chemical products. Usually it is obtained by oxidation of propylene that means using petrochemical feedstocks. The biotechnological route for it is production may be quite attractive compared to the conventional chemical processes, depending upon the level of conversion, so that it is necessary to work out towards biotechnological route based process improvement. This work introduces an investigation study of the optimization and control of an alternative process for the acrylic acid production, to know, the biotechnological synthesis. The idea is to define the optimal operational conditions or the set points in the optimization layer and then to use them in the advanced control layer. The process integration is carried out with the two-layer approach. Initially, the task is to find optima values to maximize the acrylic acid yield. Posteriorly, it was seen that is interesting to maximize the yield of the intermediates products. In such study, it was possible to realize that the optimized value of the manipulated variable to maximize the intermediates products is different of the optimum value to maximize the acrylic acid concentration. This is an important feature to be understood in the definition of the operation and control strategies, since the intermediates products can be on-line monitored a long the process running time.

Poster 9-16
Production of biodiesel and potential pharmaceuticals from cottonseed oil
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Transesterification of cottonseed oil was carried out using ethanol and potassium hydroxide. A central composite design with six center and six axial points was used to study the effect of catalyst concentration (0.5 to 1.5 % wt/wt), molar ratio of ethanol to cottonseed oil (3:1 to 20:1) and reaction temperature (25 to 75 °C) for percentage yield and percentage initial absorbance (%A385nm) of the biodiesel. Maximum predicted percentage yield of 98 % was obtained at a catalyst concentration of 1.07 % (wt/wt) and ethanol to cottonseed oil molar ratio of 20:1 at reaction temperature of 25°C. Optimal yield in the range of 95–98 % were obtained along a ridge extending over the entire range of molar ratios studied and over a range of 1.07-1.5 % (wt/wt) for catalyst concentration. Along this high-yielding ridge, very low %A385nm were obtained with a maximum of 24 % at a catalyst concentration of 1.5 % (wt/wt) and ethanol to cottonseed oil molar ratio of 3:1. Maximum predicted %A385nm of more than 85 % was obtained at 0.5 % (wt/wt) catalyst concentration and molar ratio of 3:1 at 25°C. The response surfaces describing % yield and %A385nm were inverse with the antioxidant, gossypol, contributing to the absorption. Gossypol is currently under investigation as an antioxidant in biodiesel fuels as well as a potential anticancer compound. The glycerol fraction also contains the remaining fraction of gossypol that may be purified for further exploitation.
Poster 9-17
A continuous plug-flow system for hydrothermal processing of aqueous biomass
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Dwinding supplies and rising fuel costs spell changes in the way we as a society will view energy in the future. Maximizing the energy value in renewable waste products is one way to supplement some of the energy which we might have historically had to derive from fossil resources.

We have been working to develop a continuous system for the hydrothermal processing of biomass slurries. This process is expected to be very fast and save the energy that is otherwise spent on pre-drying of biomass for most of the current thermochemical processes. This presentation will provide an update on the progress of the design and construction of the reactor system and preliminary test results with the system. A description of the system’s operation and resultant products will be given. The energy balance for the system will be analyzed in terms of energy input compared to the energy value of the products. Potential applications of the technology in producing energy from a number of renewable sources will be discussed.

Poster 9-18
Effects of neutralising agent, organic acids, and osmolarity on succinic acid production by Escherichia coli AFP184
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Using a low-cost medium Escherichia coli AFP184 has previously been reported to produce succinic acid with volumetric productivities close to 3 g L⁻¹ h⁻¹. At a total organic acid concentration of 30 g L⁻¹ the productivity decreased drastically resulting in final succinate concentrations of 40 g L⁻¹. The economical viability of biochemical succinic acid production would benefit from higher final succinic acid concentrations and volumetric productivities maintained at >2.5 g L⁻¹ h⁻¹ for an extended period of time. In the present work the effects of osmolarity and neutralising agent (NH₄OH, KOH, NaOH, K₂CO₃, and Na₂CO₃) on succinic acid production by AFP184 were investigated. Highest concentration of succinic acid was obtained with Na₂CO₃, 75 g L⁻¹. It was also found that the osmolarity resulting from succinate production and subsequent base addition, only marginally affected the productivity per viable cell. Organic acid inhibition due to the produced succinic acid on the other hand significantly reduced succinic acid productivity per viable cell. When using NH₄OH productivity completely ceased at approximately 40 g L⁻¹. Volumetric productivities remained at 2.5 g L⁻¹ h⁻¹ for 5 to 10 hours longer when using K- or Na-bases than when using NH₄OH. However, loss of cell viability occurred, and together with the acid inhibition decreased the volumetric productivities. In this study it was demonstrated that by altering the neutralising agent it was possible to increase the period of high volumetric productivity in the anaerobic phase and improve the final succinic acid concentration by almost 100%.

Poster 9-19
Biodiesel production from various oils in supercritical fluid condition by Candida antarctica lipase B and its analysis using solubility of fatty acids
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In this study, we considered the reaction factors, such as pressure, temperature, agitation speed, enzyme concentration, and water contents to increase biodiesel production in supercritical fluid condition. In addition, to establish enzymatic process on biodiesel production, it was produced from various oils. Optimal points were as follows: pressure 130 bar, temperature 45 °C, agitation speed 200 rpm, enzyme concentration 20% and water contents 10%. Among various oils such as soybean, palm, rapeseed, sunflower and olive oils, olive oil showed the highest yield on transesterification and its conversion yield was 65.18% because of high solubility in supercritical carbon dioxide. However, in batch system, conversion yield of biodiesel was not increased over 65%. Therefore, stepwise reaction was carried out for the improvement of biodiesel production. When initial concentration of methanol was 90 mmol in reaction medium and then 90 mmol of methanol was added every 2 h during biodiesel production, conversion yield of biodiesel was 98.92% at 6 h. Finally, reusability was carried out using immobilized lipase. In repeated biodiesel production, biodiesel conversion was maintained at over 85% after 8 reuses.

Poster 9-20
Evaluation of Green Fluorescent Protein Purification Using Different Purification Methods
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Due to the ability to emit fluorescence in a wide range of environmental conditions without use of substrates, the Green Fluorescent Protein (GFP) is a potential tool for monitoring industrial processes comparing its fluorescence intensity to the process by a rapid and low cost method. Nevertheless, the purification step is crucial for uses in applied biotechnology. This work aims to evaluate the purification of GFPsv* and GFP obtained from distinct sources (E. coli DHS-α and Bacillus subtilis W1012) by two different methods (hydrophobic interaction and ion exchanger chromatography columns). GFPsv* and GFP were extracted from cells by a three-phase-purification method. Aliquots of 0.5 mL were loaded onto 1 mL N-Butil Column or a HiTrap ion exchanger chromatography columns (Q Sepharose XL, DEAE and ANX resins) (GE Healthcare Biosciences’, Uppsala, Sweden) pre-equilibrated with 10mM tris-EDTA buffer (pH 8.0). GFP elution range was tested with fractions of a salt gradient from 0.05 to 0.30 M NaCl in 10 mM phosphate buffer (pH 7.0). High concentrations of protein were eluted in fractions between 0.2 M and 0.3 M NaCl for all resins evaluated. Results demonstrated columns have capability to recovery GFPsv* and GFP between 25% and 140% according the kind of column and source of GFP. Nevertheless, N-Butil and Q XL resin resulted in a high loading capacity and better purification, showing that it is well suited for GFPsv* (139.4%) or GFPsv* (90%) purification, providing a final product with high purity plus high fluorescence intensity.
Soft-lens Properties of Poly-sorbitan Methacrylate Hydrogel

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Hydrogels are generally characterized by their hydrophilicity. It is the most important aspect being the absorbed amount of water, often defined as the equilibrium water content (EWC). In biomedical fields, high water content is an attractive attribute related to higher oxygen permeability, low cell adhesion and protein absorption. Recently, many researchers have noted the myriad possible applications of sugar-containing polymeric materials, which can synthesize from sugar esters. Sugars constitute an attractive group of multifunctional compounds, since they are biologically relevant and they harbor multiple hydroxyl groups. Sugar esters, which contain sugar molecules, have been receiving increased amount of interest, and are already being utilized in a variety of applications as contact lens materials. In this study, the properties of poly-sorbitan methacrylate hydrogel as soft-lens material were investigated. The tested properties are moisture content, oxygen permeability, and light transmittance.

Acknowledgments: This work supported by the Korean Ministry of Education and Human Resource Development through the Second Stage of BK21 Program.

Screening and Optimization of Medium Composition for Poly(γ-glutamic acid) Production by Bacillus sp. RKY3 through Statistical Approaches

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Poly(γ-glutamic acid) (γ-PGA) is an unusual anionic, naturally occurring homo-polyamide that is made of D- and L-glutamic acid units connected by amide linkages between α-amino and γ-carboxylic acid groups. It is produced by microorganisms and chemical synthesis methods. In this study, to optimize the medium composition for γ-PGA production, Plackett-Burman factorial design and response surface methodology were studied.

We applied Plackett-Burman factorial design to screen and select factors affecting γ-PGA production by Bacillus sp. RKY3. Based on analysis of regression coefficients and t-value of 15 variables, we selected four components such as glycerol, glutamic acid, yeast extract, and K₂HPO₄, as significant factors increasing γ-PGA production. These four variables were chosen to obtain the optimum levels by response surface methodology. The maximum production of γ-PGA predicted when 60 g/L of glutamic acid, 3 g/L of K₂HPO₄, 20 g/L of glycerol, and 2 g/L of yeast extract were used. This is obviously in close agreement with both the value of model prediction and results of previous conventional optimization of medium components.

Development of Efficient Purification Process for Poly(γ-glutamic acid) from Newly Isolated Bacillus sp. RKY3.

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Recently, there has been an increasing interest in the development of biopolymer materials, such as polyactic acid, poly-3-hydroxybutyrate, bacterial cellulose, and poly(γ-glutamic acid) (γ-PGA). It is necessary for pretreatment to separate cells, because the viscosity of γ-PGA culture broth is very high. Therefore, to separate the γ-PGA from culture broth, the cells should be separated from culture broth prior to γ-PGA purification.

We studied influence of various pHs and dilutions on viscosity of culture broth, and studied the effect of solvent for separation of γ-PGA from cell free-culture broth. Furthermore, the more effective and economical purification procedure of γ-PGA from cell free-culture broth was investigated with ultra filtration system. The completed removal of cell was achieved with dilution of 7.5, 5, and 2.5 fold at pH 6, 4, 2, respectively. In the study about proportion of culture broth and ethanol for separation of γ-PGA from broth, the maximal recovery was accomplished in the proportion of 1 to 5 at pH 6. In experiment of UF system, the γ-PGA with less 100 kDa of molecular weight was separated with water, so that γ-PGA with more 100 kDa of molecular weight was concentrated.

Options for obtaining value from proteins during ethanol production from distiller’s grains

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As the ethanol industry begins to transition from solely starch-based ethanol to including cellulosic biomass, distiller’s grains (DGs), the byproduct produced in a conventional dry mill ethanol plant, make an attractive feedstock. Distiller’s grains are already present at an ethanol facility, thus eliminating the costs and logistics of transporting the material, and can be integrated into the existing dry mill plant with a modest capital investment. Furthermore, the cellulose in DGs is relatively easy to hydrolyze, allowing for mild pretreatment conditions and high ethanol yields. Currently, DGs are sold primarily as a low-cost protein meal produced through aqueous extraction and subsequent concentration, and amino acids through protease extraction for use as precursors for bioplastics production. These options will be integrated with ethanol production from enzymatic hydrolysis and fermentation of the fiber in DGs pretreated using Ammonia Fiber Expansion (AFEX), as well as the possibility of a concentrated lipid stream for use as a biodiesel precursor.
Poster 9-25
Fermentative production of lactic acid from sugar cane: the first step to the acrylic acid biotechnological production

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Lactic acid was firstly discovered in 1780 and has two active forms called D- lactic acid and L-lactic acid. It is used in the food, cosmetic, pharmaceutical, chemical, and polymer biodegradable production. Lactic acid can be produced by fermentative or chemical synthesis. The chemical synthesis is mainly based on the hydrolysis of laconitrile by strong acid, where a racemic mixture of the two forms (D- and L-) lactic acid is produced. The biotechnological production of lactic acid has received a significant interest, since it is an attractive process in terms of environmental viewpoint as well as economic, due the combination of the low cost of production from sugar cane fermentation, reduction of dependency of fossil based feedstock and biocatalyst use. This process offers several advantages compared to chemical synthesis, for example the low cost of raw material, low temperature, avoidable pollution, reduced CO2 emission, and high specificity of the product. Bearing this in mind, this work presents descriptions and evaluations of suitable processes for lactic acid production by fermentative process and its application, with a particular focus on the lactic acid production from sugar cane, its esterification for lactate ester production and subsequent dehydration for acrylic acid ester production.

Poster 9-26
Synergistic interactions between commercial cellulolytic-hemicellulolytic enzymes and their purified fractions on AFEX treated corn stover for varying pretreatment severities

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With increasing attention directed towards lower severity pretreatments (e.g. Ammonia Fiber Expansion or AFEX), the identification of a suitable cocktail of cellulases and hemicellulases to completely hydrolyze the residual glucan and xylan becomes pertinent. A fundamental understanding of enzyme synergy in the hydrolysis of pretreated biomass requires a high-throughput combinatorial enzyme screening for lignocellulosic biomass and a rapid compositional assay for crude protein mixtures (e.g. quantitative proteomics).

Standard enzymatic hydrolysis protocols for cellulolytic enzyme systems have several inherent disadvantages including long analysis times, excessive reagent and substrate usage, high labor inputs and non-realistic substrates (e.g., filter paper, purified xylans, and chromogenic substrates). The choice of an optimum enzyme cocktail depends largely on the substrate characteristics rather than standard enzyme-activities that are currently measured. Screening multi-enzyme systems directly on pretreated lignocellulossics would be a better way of identifying optimum synergistic cocktails of enzymes. The automated 96-well BCRL microplate method is a rapid hydrolytic assay technique (essentially a scaled down version of the NREL LAP 009 protocol) that is currently employed in our lab.

Commercially available cellulases and hemicellulases were partially purified based on their molecular weight and ionic properties. Quantification of the individual protein components for each crude mixture was performed using a high-throughput LC-MS/MS procedure. The goal of the current project was to develop an enzyme cocktail specifically tailored for AFEX treated corn stover to help reduce total protein loading employed during enzymatic hydrolysis.

Poster 9-27
Heating Strategy and Power Consumption of a Pilot Plant Anaerobic Digester

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Mixing plays an important role in anaerobic digester performance. A homogeneous medium in terms of chemical composition and temperature enables steady state conditions for biogas production. The pilot plant studied here achieves mixing by pumping digestate and recycling biogas. Temperature control allows a narrow span between the target and the tank temperatures, which is controlled by pumping digestate through an external heat exchanger. Considering excessive mixing by pumping could compromise the consortia performance for methane production and also modify the power consumption, heating strategy has been evaluated by comparing performance when modifying the span for triggering the temperature control. The results showed that under the experimental conditions, increments on mechanical and heating power consumption were observed as the span for control temperature was increased. Global heat transfer coefficients were evaluated for the different experimental conditions. Biogas production and composition were not influenced extraordinary by changes in temperature control span.

Poster 9-28
Environmental oscillations occurring in small and large-scale bioreactors during an Escherichia coli BL21 fed-batch culture

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Escherichia coli BL21 is widely used as a recombinant microorganism for the production of several foreign proteins. The metabolic characteristics of the strain impose a strict control of the environmental conditions of the cultivation reactor. A lot of previous works have dealt with the glucose and dissolved oxygen feeding strategies to apply in order to avoid undesired metabolic states, such as overflow metabolism and mixed acid fermentation. In the present work, two strategies for the glucose addition have been investigated: exponential feed control and dissolved oxygen feed control. The results show that, even at small scale, the controller induces a lot of oscillations at the level of the environmental factors, i.e. the substrate level and the dissolved oxygen concentration, which can affect the microbial growth and further the recombinant protein synthesis. However, little is known about the evolution of these environmental oscillations at larger scale. The scale-up effect on the oscillating behavior of the process has been first studied by using a structured hydrodynamic model. This model has been elaborated to take into account the homogeneity efficiency of the bioreactor. In a second time, the large-scale environmental oscillations have been reproduced in a scale-down reactor. The results suggest that the scale-up process induces some variability at the level of the characteristic frequencies of the glucose and dissolved oxygen oscillations. In front of these results, a stochastic model has been proposed in order to capture this variability and to predict the efficiency of the E. coli BL21 fed-batch process at large-scale.
This work has the purpose of studying the microbial growth and xanthan gum production in fermentations using the specific strain of Xanthomonas campestris pv. campestris NRRL B-1459, and diluted sugar cane broth. The production medium contained sucrose (15.0, 25.0 or 35.0 g.L⁻¹), yeast extract (3.0 g.L⁻¹), NH₄NO₃ (0.86 g.L⁻¹), Na₂HPO₄ (2.2 g.L⁻¹) and KH₂PO₄ (2.2 g.L⁻¹) were fermented in bioreactor and rotary incubator shaker. In shaker, the fermentations were carried out for 60 h at a constant agitation rate (150 rpm) and temperature (25 ± 1°C) with the initial pH adjusted to 7.0. In bioreactor, the fermentations were done for 24h at 28 ± 1°C, pH 7.2, 800 rpm and 0.5vvm. The unstructured kinetic model of Weiss and Ollis (1980) was used to determine the yields (Y), the maximum growth specific rate (μmax), and the saturation cellular concentration (X'). The parameters values of the model (μmax, x', m, λ, α and β) were obtained by non-linear regression technique using a multiresponse algorithm and the integration of the set of differential equations forming the model was carried out using a fourth-order Runge-Kutta algorithm. While μmax assumed values of 0.142, 0.133 and 0.199 h⁻¹ and the yields obtained were of 0.352, 0.333 and 0.263 g g⁻¹ in the rotary incubator shaker for sucrose concentrations of 15.0, 25.0 and 35.0 g.L⁻¹, respectively, those in bioreactor were 0.436, 0.458 and 0.488 h⁻¹ (μmax) and 0.280, 0.580 and 0.430 g.g⁻¹ (Y'). The results presented show good correlation with the predictions supplied by the model.

**Presentation 9-30**

Extraction and Identification of Julibroside Saponins from the bark of Albizia julibrissin

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Value-added products may be found in a number of biomass feedstocks used in the production of energy. One such product, saponins, can be found in the bark of Albizia julibrissin (mimos). A leguminous species that has a forage yield of 4.5 tons/acre-yr. Saponins are a natural detergent and can be found in a variety of plants, ranging from Yucca schidigera in Mexico to Panax ginseng in China. Saponins contain both a water-soluble and a fat-soluble component. Saponins have been shown to have antiprotozoal activity in ruminals and to reduce blood cholesterol levels in mammals. Tripterpenoidal saponins are known to be present in Albizia julibrissin, aptly called julibrosides, and have previously been extracted from the plant material with methanol and other organic solvents. They were then separated by RP-HPLC and finally analyzed by mass spectrometry. Unfortunately, julibrosides are not available commercially as reference compounds. Hence, the objective of this paper is to present results from the extraction of saponins from mimos bark in order to secure reference material. Centrifugal partition chromatography (CPC), a unique separations tool based on the principles of countercurrent chromatography, is used to separate and purify the extracted saponins. In the future, pressurized hot water will be used to extract saponins, in a green manner, from Albizia biomass prior to conversion to a liquid fuel.
**Poster 9-33**  
Biological conversion of hemicellulose extracted from hardwood  
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Extraction of hemicellulose from hardwood chips prior to pulping is a proposed method for producing ethanol and acetic acid. The hemicellulose is currently dissolved during pulping and burned to generate energy. Because hemicellulose has a low heating value, more would be generated by producing commodity chemicals. In an integrated bio-refinery, hemicellulose could provide the sugars for fermentation into ethanol, and also generate acetic acid.

Hemicellulose was extracted from hardwood chips using green liquor, a solution of NaOH, Na2CO3, and NaS produced as an intermediate in the pulping process. This treatment was done in a 10L rocking digester at 160°C for 110 minutes. Extracts mainly contain xylo-oligosaccharides and acetic acid. Following hydrolysis and neutralization, approximately 5g/L of total monosugars were present. Fermenting such low sugar concentrations into ethanol is economically unfavorable due to the high energy cost of distillation; therefore the hemicellulose extracts were concentrated. Extracts were removed from the evaporator at 3, 6 and 10% solids. Higher sugar content was thus achieved, but also higher concentrations of inhibitors such as acetic acid and sodium salts.

Fermentation experiments were conducted with E. coli K011. The initial 3% solids extract generated 1.2g/L of ethanol in the presence of 10g/L acetic acid. The 6% solids extract was also fermentable, producing 2.1g/L ethanol in the presence of 15g/L acetic acid. The 10% solids extract wasn’t initially fermentable due to the high concentration of inhibitors, including 25g/L acetic acid. Strain adaptation is underway to select for organisms capable of withstanding this level of toxins.

**Poster 9-34**  
Continuous Enzymatic Saccharification of Paper  
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The cost of enzyme such as cellulase is still a barrier to practical bioconversion of cellulosic biomass today. To minimize the enzyme consumption in a long term, we constructed a continuous enzyme-recycling saccharification process using copy paper as the substrate. This process was composed of a ceramic filter to keep residue paper remaining in the reactor and an ultrafilter to recycle enzyme back to the reactor; as a result it could be operated for a long interval without new enzyme addition and at the same time the product sugar could be carried outside from the process continuously.

In order to achieve a high enzyme recovery rate and a high sugar yield, enzyme/Multitex (CX10L) concentration in the reactor, paper supply flow rate and retention time were optimized. We found that enzyme could be recovered efficiently and show a high reducing sugar yield of 90% stably for over 40 days by increasing enzyme concentration to have a ratio of substrate to enzyme in the reactor to be 2 under the condition of retention time as 12 hrs.

**Poster 9-35**  
Liquid-liquid extraction using Triton X-114 for nisin and green fluorescent protein (GFPuv) extraction with electrolytes  
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Green fluorescent protein (GFP), expressed by E.coli DHS-α, is widely applied as a biosensor and can be detected by spectrofluorometry or using hand UV lamp, it has become a versatile tool for a variety of biotechnological uses and as a potential biological indicator, for preservation of manufactured and processed products. Nisin, antimicrobial peptide, produced by L.lactis ATCC 11454, used as a natural agent of biopreservation; and it has been accepted as a safe and natural preservative in different areas of food and pharmaceutical industry. This study aimed to evaluate the aqueous two-phase system (ATPS) composed by Triton X-114 (TX), in presence or absence of electrolytes, to separate nisin and GFP. The system was composed by 2%TX with and without MgSO4 or NH4SO4. Nisin activity was determined by agar diffusion with L.sakei as bioindicator. GFP concentration was determined by fluorimetry. The phase diagram of the TX in buffer was obtained by the cloud-point method. Each solution was placed in a thermo-regulated device set at a temperature of 36.2°C for 2h to attain partitioning equilibrium. The coexisting phases formed were withdrawn and the biomolecules concentration was determined in each phase, in TX system without electrolytes. Results indicated that nisin partitions preferentially to the micelle-rich phase, with significant antimicrobial activity increase (up to 10-fold). GFP partitioned evenly between the phases in TX system without electrolytes. After partitioning in the presence of salts, nisin was more strongly recovered relative to the micelle-rich phase, while GFP was driven to the micelle-poor phase.

**Poster 9-36**  
Synthesis and Purification of Monoglycerides through Lipase-Catalyzed Glycerolysis and Molecular Distillation  
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Monoglycerides (MG) and diglycerides (DG) present great importance in the food, cosmetic and pharmaceutical industries. Industrially, the production of MG consists on the glycerolysis reaction, in the presence of inorganic catalysts at high temperatures (above 200°C). Lipase-catalyzed glycerolysis of oils and fats at atmospheric pressure and lower temperature is believed to be a practical alternative method in the production of commercial MG. This methodology avoids several drawbacks in the products, e.g., low yield, dark color and burnt taste. To separate the products of the reaction in order to obtain essentially MG, is necessary a distillation process. But ordinary distillation is difficult because the low vapor pressure and thermal instability of the acylglycerols. Instead, molecular distillation is adequate for obtaining a product with high MG concentration.

In this work, MG and DG are produced through lipase-catalyzed glycerolysis of soybean oil using Candida Antartica B in a solvent-free system. The determination of the composition of the triglycerides (TG), DG, MG, free fatty acids (FFA) and GL were performed using a high-performance size-exclusion chromatography (HPSEC). After 24 hours of reaction, the mixture of acylglycerols and fatty acids (FFA) was distilled into a centrifugal molecular distiller. Starting from a material with 22.23% of TG, 49.21% of DG, 22.1% of MG, 4.86% of FFA and 1.53% of GL, the maximum MG purity in the distillate stream (MGD) was 65 % at evaporator temperature (TEV) equal to 230°C and (feed flow rate) Q equal to 5 mL/min. At these conditions, the MG recovery was 70.63%.
Pressurized hot water (or subcritical water) has received attention as a green extraction solvent, but little attention has been paid to the thermal degradation products formed during pressurized hot water extraction (PHWE). This study investigates the generation, identification, and quantification of thermal degradation products formed during PHWE of *Silybum marianum* fruits. To generate thermal degradation products from the *S. marianum* flavonolignans, each of the individual flavonolignans (silichristin, silidianin, silibinin, and isosilibinin) were dissolved in methanol and exposed to pressurized hot water at 500 kPa and 413 K for 0.5 hr. LC/MS/MS characterization of the extracts showed the presence of multiple degradation products. PHWE of silibinin A and B resulted in the formation of two degradation compounds. PHWE of silichristin (which occurs in three forms, A, B, and C) resulted in a complete loss of form A, and the formation of one degradation compound. PHWE of isosilibin A and B created six degradation products. The extracts, before and after subcritical water extraction, were evaluated for their athero-protective effects by the thiobarbituric acid-reacting substances (TBARS) assay. The TBARS assay evaluates the ability of the flavonolignans to inhibit the copper-induced formation of oxidized low-density lipoproteins (oxLDL). Comparable athero-protective effects were observed for both the pure flavonolignan preparations and the extracts, indicating that degradation products formed during extraction do not diminish the potency of the flavonolignans, and likely do not display pro-oxidant effects. 300 μM silibinin standard decreased oxLDL formation by 63.6%, and 300 μM silibinin PHW extract decreased oxLDL formation by 55.9%.

**Poster 9-38**

Analysis of Fermentation Yield Coefficients from Autonomously Oscillating Open Yeast Cultures

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Previous work has established the fact that self-sustained, autonomous oscillations exist within open yeast fermentations under a variety of operational parameters. These oscillations may have a variety of impacts on substrate conversion to biomass and subsequent products. This analysis assumes that the fermentor cell mass follows Monod kinetics. Substrate and cell concentration data are fitted to mathematical models, permitting direct determination of fermentor yield. The functional form of the fermentor yield is then analyzed under a variety of conditions. Our analysis will elucidate the effects of these oscillations to potentially allow engineers to utilize these oscillations as a tool, rather than an obstruction. The analysis could also be used to increase fermentor productivity in the biomass production and conversion industry.
**Posters**

**Poster 9-41**

**Minimizing Mass Transport Effects in a Continuous Process for Production of Ethanol from Starch**

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In a previous work, a continuous SSF process to produce ethanol from cassava starch was studied, using a set of fixed-bed reactors. The biocatalyst consisted of glucoamylase immobilized in silica particles and co-immobilized with S. cerevisiae in pectin gel. Using 3.77 U/mL reactor and 0.05 g wet yeast/mL reactor, starch hydrolysis was the rate-limiting step and the maximum ethanol productivity was 5.8 g ethanol/L/h, with 94% conversion, and 83% of the theoretical yield. In this work, the molar mass of the substrate and the biocatalysts’ size were reduced, aiming at increasing mass transfer rates. The conditions of the pre-hydrolysis with a-amylase were changed: longer reaction times and higher enzyme concentrations were tested, until obtaining limit dextrin. The diameters of silica and pectin gel particles, in their turn, were reduced: from 100 mm and 3-4 mm, respectively, in the previous work, to 60 mm and 1-1.5 mm. The performance of the packed-bed reactors was then studied, using immobilized glucoamylase concentrations of 2.09, 2.76 and 3.77 U/mL reactor, keeping constant the initial cell concentration as 0.05 g wet yeast/mL reactor. Each run was carried on for 11 days at least. In the present work, with a feed concentration of 154 g/L (TRS), and using 3.77 U/mL reactor, fermentation became the rate-limiting step. Productivity reached 11.7 g/L/h, with 97% of conversion and 92% of the theoretical yield.

**Index Entries:** ethanol, cassava starch, Saccharomyces cerevisiae, glucoamylase, packed-bed reactor, simultaneous saccharification and fermentation, mass transport effects

**Poster 9-42**

**Evaluation of kinetics and nutritional parameters on Rhodotorula glutinis growth and pigmentations**

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Several Rhodotorula yeast strains are used for industrial production of carotenoids, important pigments responsible for coloration of some vegetables and microorganisms. Its industrial production using microorganisms is highly efficient. *Rhodotorula glutinis* is extensively used in fermentation, being able to produce β-carotene (Vitamin A precursor). An important aspect regarding fermentation process is the development of a culture medium that maximizes the production of a product of interest, using a cheap raw material. Due to the increasing interest in biofuels, like biodiesel for example, one can observe an increase in the amount of available glycerol, what has caused pronounced price decrease. Thus, new applications for large amounts of this product are needed. In this context, carotenoid production, among other fermentative processes, has interested different research groups. As β-carotene has intracellular nature, a highest biomass production is directly related to the obtaining of a highest concentration, being an important parameter to be considered in the optimization of the desired product. In this work, the influence of glycerol as a carbon source in the biomass production, through submerse fermentation using the *Rhodotorula glutinis* microorganism, was studied. For the evaluation of the kinetic parameters such as temperature and agitation, the culture media were prepared using the dosages of 20g/L glycerol, 5g/L yeast extract and 5g/L peptone. Once obtained the best results for the mentioned parameters, different nitrogen sources were tested, as yeast extract, peptone and ammonium sulfate.

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**Poster 10-07**

**Synergistic interactions between poplar and endophytic bacteria to improve plant establishment and sustainable feedstock production on marginal soils**

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Producing biomass that is tailored toward energy production, but that does not negatively impact food supply is one of the critical social-economical issues of the proposed US biofuel program. Poplar is considered as the model tree species for bioenergy feedstock production. Plants, however, live in close association with symbiotic microorganisms. We showed that specific strains of endophytic bacteria had a beneficial effect on the development and growth of poplar on marginal soils, resulting in a 30% increase in biomass production. To better understand the complex interactions between endophytes and poplar, including production of phytohormones, antibiotics to inhibit the growth of pathogens or ACC de-aminase activity to counteract the plant’s stress-ethylene response, the genomes of four plant growth promoting endophytic bacteria were sequenced. Genome annotation, analysis of metabolic properties, and comparisons with closely related non-endophytic bacteria resulted in the identification of several unique pathways by which endophytic bacteria can promote plant growth and health. Using directed mutagenesis we are presently examining the role of these pathways in plants growth and development.

In addition, the sequenced genome of *Populus trichocarpa* provides us with the unprecedented tools to study the interactions between poplar and its endophytic partners on the ‘omics’ level, and to combine these data with results on plant growth, physiology, and biomass composition. This should result in a better understanding of the synergistic interactions between poplar and its growth promoting endophytes, which can be exploited to improve plant establishment and sustainable bioenergy feedstock production on marginal, non-agricultural land.

**Poster 10-08**

**MicroDrive – A research program on sustainable bioethanol and biogas systems**

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MicroDrive – Microbially Derived Energy – is a thematic research program on sustainable biofuel production at the Faculty for Natural Resources and Agriculture (NL), Swedish University of Agricultural Sciences (SLU). The program has the following long term goals: To maximise the energy yield of ethanol and biogas processes, improve overall process economy through development of novel co-products, and to minimise environmental impact. The program strategy is: To integrate research projects on biopreservation, enzymatic pretreatment of plant feed stock, ethanol fermentation, bioprocessing and fermentation of ethanol process by-products, biogas fermentation, and the re-circulation of plant nutrients in biogas digestates. Initially, the focus will be on using cereal grains and sugar beets as feed stock, later straw and other cellulosic biomass sources will be explored. MicroDrive presently involves 16 scientists, post-docs and PhD students with specialist competence in microbiology, molecular biology/evymology and natural products chemistry. The scientists supervise MSc thesis students doing 20 week-projects, commonly in joint projects with our industrial partners (Syngenta seeds, Chematur Engineering, Jästbolaget, Medipharm, Tekniska verken/Svensk Biogas and Sala Heby Energi). MicroDrive is funded by the NL-faculty (50%), our industrial partners, the Swedish Farmers Research Foundation (SLF) and the National Energy Board. The research program started March 1, 2007, and is planned to run until 2013, with an annual budget exceeding 0.9 M USD. MicroDrive welcomes international cooperation!
Increasing Profit Margins in Biofuels Production

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The U.S. Energy Policy Act of 2005 (EPAct) requires that renewable fuels make up 4 billion gallons of the nation’s gasoline market starting in 2006 and 7.5 billion gallons by 2012. To sustain this growth, long-term biofuel feedstocks have to shift from food-stocks grown on high quality farmland to chemical crops grown on marginal land. The biofuels industry has evolved from one where selling the product was the primary challenge to one where finding sufficient quantities of technically and economically viable feedstocks dominate as the key concern obstructing the real potential of these bio-based chemicals toward solving ever-growing global energy concerns.

In addition, although biofuel costs are fairly constant, the cost of raw materials can increase significantly thus affecting the profitability. To reduce business risks, it is not only important to have long-term supply and sales contracts, but it is worthwhile to consider supplementary materials can increase significantly thus affecting the profitability. To reduce business risks, it is not only important to have long-term supply and sales contracts, but it is worthwhile to consider supplementary

This paper will address key issues that affect the overall profitability and manufacturing lifecycle:

a) Chemical crops that are agriculturally good fits for the Southeastern U.S. from both a technical and economic basis of view

b) Processing techniques that can economically convert feedstocks into biofuels of acceptable quality and quantities

c) Manufacturing techniques that may be used to produce secondary value-added products as a means of increasing profitability

Removal of Total Nitrogen in Waste-Water Using Biological Treatment Process (I)

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Biological nitrification and denitrification are the most commonly used process for nitrogen removal from waste-waters. Nitrification is the autotrophic oxidation of ammonium ion, firstly to nitrite, and secondly to nitrate. However, denitrification is the heterotrophic, anoxic conversion of nitrate, firstly to nitrite, and then to gaseous nitrogen. In order to remove total nitrogen ingredients (T-N) of waste-waters discharged from D oil & fat Co. located in G-city of Korea, nitrification and denitrification process were performed using A2O process with waste-butanol as external carbon source. Nitrification and denitrification process were performed to plant of inlet flow of 35 µg/L. The range of inlet concentration of total nitrogen was 220-300 mg/L during tested period. The concentration of T-N in outlet flow after denitrification process was maintained to 10-76 mg/L. The T-N removal yields of process are 67.4%, 73.8%, 96.1%, and 75% at 1, 10, 15, and 20 day of processing period, respectively. Acknowledgements:

This research was financially supported by the Ministry of Commerce, Industry and Energy (MOCIE) and Korea Industrial Technology Foundation (KOTEF) through the Human Resource Training Project for Regional Innovation.

Bioethanol subproducts as a basis of plant biorefinery

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The philosophy of our research is built on the behaviour of non-sugar compounds from biomass during ethanolic fermentation. Saccharomyces cerevisiae industrially used to bioconvert sugar in ethanol, under anaerobic conditions, assimilates some of the compounds present in the fermentable juice (proteins, amino acids).

But others compounds are not used by the yeast. They leave in the vinasse after ethanol distillation and are thus concentrated by the process. We will discuss the case of the beet-sugar roots by following the behaviour of betaine, saponins, raffinose in sugar juices (raw juice, thin juice, thick juice) and in vinasse. An attention will be given to some high added value products (like betaine) and the potentialities to use fermentation as a tool of purification in white chemistry.

Production of value added chemicals from xylan extraction in a Kraft pulp mill and the effect on pulp quality

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In the Kraft process hemicelluloses are lost in the cooking procedure to the black liquor stream, which is subsequently burnt in the recovery boiler to recover cooking chemicals and to produce steam and energy. Hemicelluloses have a low heating value compared to lignin and therefore recovery of hemicelluloses at an earlier stage of the Kraft process followed by biochemical conversion into high value-added products might offer a much better economic opportunity. In collaboration with the research and development department of Smurfit Kappa Kraftliner AB, Piteå, Sweden, alkali and water extractions of birch wood were performed under conditions compatible with the Kraft process, at different times, temperatures and alkali charges. The extraction conditions were set in a range suitable with the current pulp process at Smurfit Kappa Kraftliner. The requirements for process configurations, based on either hot water or alkali extraction were also explored. The xylan yields from different extraction trials were measured and the chips from those extraction trials were cooked, refined and turned into sheets of paper. The effects on paper quality were compared with a reference pulp made from the same wooden chips. Recovered xylans from water extracted birch wood chips were subjected to secondary hydrolysis, enzymatic or sulphuric acid.

Detoxification of the hydrolysate with active carbon and regulation of pH were performed before fermentation. Fermentation of the xylose hydrolysate to succinic acid was demonstrated by the use of the succinic acid producer Escherichia coli AFP184.
Poster 11-09
The IBUS process - Large scale SSF at high dry matter content (>25% WIS)
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In the IBUS process all process steps are carried out at high dry matter content thus minimising water and energy consumption. This gives rise to special challenges, especially in the enzymatic hydrolysis and SSF when working with dry matter contents above 25% (water insoluble solids). This is due to difficulties with agitation of the initially very viscous mash. Earlier published experiments with SSF at high dry matter content have shown that the yield of ethanol decreases as the dry matter content increases (1). This is most likely caused by problems with mass transfer caused by high viscosity.


Poster 12-07
Evaluation of Acremonium cellulolyticus enzyme cocktail on efficient enzymatic hydrolysis of Douglas-fir pretreated by ball milling

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Lignocellulosic biomass, such as wood and agricultural residues, is an attractive material for fuel ethanol production since it contains large amounts of potentially fermentable sugars in the form of cellulose and hemicellulose. The interest of using hemicellulose as a resource for the efficient production of ethanol is currently increasing. Softwood is one of promising feedstock, since its principal hemicellulose component, O-acetyl galactoglucomannan, gives readily fermentable mannose as the major sugar after hydrolysis. This is advantageous over hemicellulose from hardwood or agricultural residues hydrolyzed to unfermentable xylose. In this study, we investigated an efficient enzymatic hydrolysis of both glucan and mannan components in a ball mill (BM)-treated softwood, Douglas-fir. A culture supernatant from Acremonium cellulolyticus CF-2612, which is a powerful cellulase-producing fungus, was used as the cellulase (ACase-CF) for the enzymatic hydrolysis. The glucan digestibility of BM-treated Douglas-fir was estimated to be 75% using 10 FPU/g-substrate ACase-CF, but mannose from the mannan component was hardly detectible in this fir was estimated to be 75% using 10 FPU/g-substrate ACase-CF, but enzymatic hydrolysis. The glucan digestibility of BM-treated Douglas-fir reached 75% and 70%, respectively, using the enzyme cocktail consisting of 10 FPU/g-substrate ACase-CF, 1 U/g-substrate β-mannosidase, and 200 U/g-substrate β-mannanase.

Poster 12-08
Cellulosic ethanol: hydrolytic enzymes produced by wild Aspergillus niger 12 and mutant Aspergillus niger 3T5B8 from tropical environmental

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Cellulosic ethanol can be produced from a wide variety of cellulosic biomass feedstocks from agricultural and industrial plant wastes. They are composed of cellulose, hemicellulose and lignin. Separating of these polymeric structures into fermentable sugars is essential to get efficiency and economical production of cellulosic ethanol. Enzymatic hydrolysis, one alternative method, can be utilized after a physical and chemical pretreatment process. The objective of this study was to select the most appropriate strain for production of cellulases and other hydrolytic enzymes. In advance, there were selected the wild Aspergillus niger 12 and the morphological mutant Aspergillus niger 3T5B8. The enzymes production were evaluated by solid-state fermentation in aerated glass columns during 96h, using wheat bran as support, enriched with 0.91% (m/v) of ammonium sulfate. In the most favorable time fermentation the wild A. niger 12 produced CMCase 143 U/g; FPCase 4 U/g; polygalacturonase 513 U/g; β-glicosidase 422 U/g and xylanase 733U/g, while the A. niger 3T5B8 produced CMCase 230 U/g; FPCase 8,5U/g; polygalacturonase 1169 U/g; β-glicosidase 1356 U/g and xylanase 1259U/g. Both enzymatic extracts will be tested by a Brazilian company in the step of enzyme saccharification of cellulose.

Poster 12-09
Optimization of inductor concentration and fermentation conditions for lipolytic enzyme production by isolated bacterium strain from petroleum contaminated soil

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A large number of microorganisms, including bacteria, yeasts and fungi produce different groups of enzymes. Lipolytic enzymes have been shown to have many applications in detergents, food processing, chemical industry, as well biodiesel production. The objective of this work is to present alternatives for lipolytic enzymes production by an isolated bacterium strain from petroleum contaminated soil under submerged fermentation (SmF). Experiments were performed in Erlenmeyer flasks with 200mL medium containing (% w/v): KH2PO4 (2.4), MgSO4.7H2O (0.05), NaNO 3 (0.3), yeast extract (0.6), peptone (0.13), and starch (2%) as carbon source. Coconut oil was used as lipolytic enzyme inducer and was added at different concentrations after 72h cultivation. Experimental design methodology (DOE) was used to optimize the fermentations conditions as a function of temperature (24, 37 or 50°C), pH (5, 7 or 9) and inducer concentration (1, 2, 3 or 4%, v/v). Maxima activities for lipase were approximately 1,700 U/mL using high concentration level of coconut oil 4% (v/v) at pH 7.0 and 37°C. Validation of the experimental results based on DOE methodology allowed to enhance the enzyme production by this isolated bacterium, indicating the importance of this methodology to evaluate the main variables (pH and temperature) and their interaction effects for process optimization.
Oleic acid delays and modulates the transition from respiratory to fermentative metabolism in Saccharomyces cerevisiae after exposure to glucose excess

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This work aimed to study the transition from respiratory to fermentative metabolism in Saccharomyces cerevisiae CEN.PK 113-7D and more specifically to evaluate the implication of the acetyl-coenzymeA-derived carbon transport from cytosol to mitochondria in the onset of the metabolic shift. The strategy consisted in introducing, during aerobic glucose-limited chemostat (D=0.16 h⁻¹), a local perturbation around the step to be studied by the addition of cosubstrates and in analyzing the consequences of such a perturbation on the metabolic transition. Oleic acid and L-carnitine were among the tested cosubstrates because they were known to stimulate enzymes implicated in the acetyl-coenzymeA transport between the different cell compartments, such as the carnitine acetyl transferases. The metabolic transition was then comparatively quantified in sole glucose and in glucose/oleic acid cosubstrats in presence/absence of L-carnitine after a pulse of glucose. Feeding the culture with oleic acid (Dole=0.0041 and 0.0073 h⁻¹) led to a delay in the onset of the metabolic shift (up to 15 min), a 33% decrease in the ethanol production and a redirection of the carbon flux toward biomass production. The data clearly showed a modulation of the carbon distribution among respiration and fermentation, in favor of a decrease in the “short-term” Crabtree effect by the oleic acid.

**Keywords** Crabtree effect . Saccharomyces . Ethanolic fermentation . Oleic acid . Carnitine . Carnitine acetyl transferase

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Cellulase and hemicellulase are the enzymes that can hydrolyze lignocellulosic biomass to fermentable sugars. Acremonium cellulolyticus secretes sufficient amounts of cellulolytic enzymes to completely convert cellulose materials to glucose. Cellulase from A. cellulolyticus has notably higher β-glucosidase activity than those from Trichoderma species.

To efficiently produce cellulase and hemicellulase, A. cellulolyticus CF-2612 mutant strain was obtained with random mutagenesis. The enzyme activities in the supernatant of the culture as crude enzyme preparations were measured, followed by shake flask culture at 30°C using Solka Floc as the carbon source. The FPase, avicelase, and β-glucosidase activities of crude enzyme from CF-2612 strain were significantly higher than those of the parent strain. FPase activity (U/ml) and FPase-specific activity (U/mg protein) reached 18.0 and 1.0, respectively, using the batch culture with 5% Solka Floc for 5 days in a 2-L jar fermenter. When the crude enzyme from CF-2612 strain was used for saccharification of pretreated rice straw, glucose, xylose, arabinose and mannose were released. This result suggests that not only cellulase but also hemicellulase are secreted by CF-2612 strain.

In conclusion, A. cellulolyticus CF-2612 strain has an ability to efficiently produce cellulase and hemicellulase for hydrolysis of biomass. [This study was supported by a Greenhouse gas mitigation technology development program of the Ministry of Environment of Japan.]
**Poster 12-14**
Assessment the morphological, biochemical and kinetic properties for Candida rugosa lipase immobilized on hydrous niobium oxide to be used in the biodiesel synthesis

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Lipase from Candida rugosa (CRL) was successfully immobilized on niobium oxide. The matrix could effectively attach the enzyme, with high retention of activity and prevent its leakage. Following immobilization, CRL exhibited improved storage stability and performed better at higher incubation temperatures. The enzyme retained most of its catalytic efficiency after successive operational cycles. The immobilized derivative (IE) was fully characterized with respect to their morphological properties: particle size, surface specific area and pore size distribution (B.E.T). Structural integrity and conformational changes, such as surface cavities in the support, were observed by SEM. A comparative study between free and immobilized lipases was provided in terms of pH, temperature and thermal stability. The catalytic performance of the IE was also evaluated for the synthesis of biodiesel employing babassu oil and short chain alcohols (ethanol, propanol and butanol). The alcohol was also evaluated for the synthesis of biodiesel employing babassu oil and short chain alcohols (ethanol, propanol and butanol). The alcohol carbon chain showed a strong influence on the transesterification performance. As the length of the alcohol carbonic chain increased higher yield and productivity were detected. The highest yield (85%) and productivity (7.5g.L⁻¹.h⁻¹) were attained for butanol and the lowest values (36% and 1.2 g.L⁻¹.h⁻¹) for propanol. No ester formation was detected for the system ethanol/oil and this was attributed to the high polarity of the ethanol (log P<0), which leads to an unfavorable partition of water between enzyme and support, thus stripping essential water from enzyme molecules and reducing the activity. The application of this IE was found to be unsuitable for the ethanalysis of babassu oil.

**Poster 12-15**
Production of Lipase by Aspergillus niger 11T53A14 in Semi-solid Fermentation using Aerated Columns

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Lipases hydrolyze triglycerides into di- and monoglycerides, glycerol and fatty acids. The interest in lipases has increased due to its application in detergent and food sectors. Semi-solid fermentation, using agroindustrial residues, presents advantages over submerged fermentation, mainly for fungi growth and its economical potential for countries such as Brazil, with an important biodiversity. In this work, it was investigated the production of lipase by Aspergillus niger 11T53A14 in semi-solid fermentation using aerated columns and testing two lipid sources, commercial olive oil and by-product from corn oil refining codified as soapstock. This strain was previously screened and selected for lipase production. Fifteen grams of sterilized medium (100g of wheat brain was moistened with 60 ml of 0.91% w/v of ammonium sulphate pH 7.0 and 2% w/v of lipid source) was used in each column.

The air rate was of 0.5vvm at the temperature of 32°C. At 12h intervals one column was taken for enzyme extraction with sodium phosphate buffer pH 7.0. Lipase activity was determined by the titrometric method and the best results were obtained after 72h with the activity of 242 U/gds using corn oil by-product. The optimal temperature and pH and the thermostability of enzyme have been determined.

**Poster 12-16**
Ultrasound Enhancement of Enzymatic Hydrolysis of Cellulose Plant Matter

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The work reported here is based on acceleration of enzymatic hydrolysis of plant biomass substrate by introduction of low intensity, uniform ultrasound field into a reaction chamber (bio-reactor).

This method may serve as improvement of rates in the hydrolysis of cellulolic materials to sugars, which in turn may be converted to biofuel ethanol in a fermentation step.

In experiments, corn stover was contacted with a buffered enzyme solution in a reactor capable of delivering a uniform sonication of cellulosic materials. The result showed that the improvement in the saccharification rates was 44 to 61 percent, dependent on the addition of sonication field, but was also dependent on the type of agitation.

A mathematical model was used to define the effectiveness factor.

**Poster 12-17**
Purification and immobilization of lipase from Penicillium simplicissimum by selective adsorption on hydrophobic supports

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Lipases (glycerol ester hydrolases E.C. 3.1.1.3) consist of a class of enzyme that has great importance as biocatalysts applied to traditional organic chemistry. However, it is still necessary to search for new enzymes with special characteristics such as good stability towards high temperatures and organic solvents and high stereoselectivity. The aim of the present work was to purify and simultaneously immobilize the pool of lipases produced by P. simplicissimum (PSL), a filamentous fungi isolated from Pantanal soil in Brazil. PSL was separated into three different fractions using selective adsorption method on different hydrophobic supports (butyl-, phenyl-, octyl-agarose) at low ionic strength. After immobilization, a hyperactivation of these fractions was observed in the range of 131% to 1133%. This phenomenon probably occurs because the immobilization of the lipase is in active open form onto hydrophobic supports. Those fractions, showed different thermal stability, different specificity and enantioselectivity upon some substrates, with enantioselectivity from 1 to 7.9 for the hydrolysis of (R,S) 2-O-butyryl-2-phenylacetic acid. Prochiral diethyl phenylmalonate were partially hydrolyzed to the corresponding chiral monoesters by the different immobilized PSL fractions. Those fractions neither catalyzed the hydrolysis of the monoesters nor produced the final achiral di-carboxylic acid yielding chiral PSL fractions. Those fractions showed different thermal stability, different specificity and enantioselectivity upon some substrates, with enantioselectivity from 11.8 to 16.4 depending on the immobilized PSL fractions used. Partial purification of a microbial crude extract through sequential adsorption methodology demonstrated to be an efficient strategy to obtain new biocatalysts with different degrees of enantioselectivity.
Effect of medium carbon source on the hydrolytic potential of cellulases

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Lignocellulosic biomass is a potential source of biofuels. Conversion of cellulose from lignocellulosics into sugars performed by enzymatic catalysis requires high enzyme loading to obtain high degree of cellulose conversion that increases the cost of the process. Thus, efficiency of the enzymes has to be improved in order to achieve competitive enzyme technology.

The aim of our experiments was to investigate the connection between the carbon source used in cultivation and the efficiency of the produced enzyme mixture in the hydrolysis of the given substrate. Enzymatic conversion of lignocellulosic materials was carried out using “in-house” fermented enzymes produced by Trichoderma reesei, the well studied soft-rot fungus. Two widely available agricultural residues, wheat straw and corn stover were investigated as carbon sources in the fermentation and as substrates in the hydrolysis. Raw materials were pretreated by steam-explosion in the presence of catalytic amount of sulphur dioxide (at Lund University, Sweden and ENEA, Italy).

Cultivation of T. reesei RutC30 strain in shake flasks was performed in spent grain-based medium buffered with 0.1 M Tris-maleate (pH 5.6), at 28°C, 200 rpm. The hydrolysis experiments were carried out using 20 FPU per g cellulose cellulase enzyme dosage at 50°C, 2% dry matter content, in 0.05 M acetate buffer solution (pH 4.8). In order to avoid accumulation of cellobiose, b-glucosidase was supplemented to 20 BGL U per g cellulose with commercial enzyme preparation, Novozym 188 (Novozymes). Hydrolytic process was monitored and evaluated by HPLC analysis of the supernatant for various sugars produced.

Hydrolysis of Steam Explosion Pretreated Sugarcane Bagasse Using Cellulase and Cellobiase

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Currently, Brazil is faced with the prospect of a significant increase in the demand for fuel ethanol. This is a result of three main factors: (i) increasing domestic consumption of hydrated alcohol, (ii) expansion of Brazilian alcohol exports on the basis of a growing worldwide interest in the mixture of gasoline and alcohol, and (iii) the Brazilian option for the production of biodiesel using ethanol for the transesterification of vegetable oils. To meet this increasing demand, without reducing the area dedicated to food production, the alternatives are to increase the alcohol productivity per hectare of sugarcane plantation and to develop an economically viable technology to use the sugarcane bagasse surplus for the production of additional ethanol. The sugarcane bagasse is an abundant, renewable resource in Brazil, and it contains a high level of cellulose, which can be employed for the production of ethanol. This work objective was to evaluate the influence of the ratios of sugarcane bagasse:cellulase enzyme and bagasse:cellobiase enzyme on the bagasse hydrolysis reaction, which produces fermentescible sugars as glucose. The tests showed that the maximum production of glucose was reached when a 100 mL volume of a 3% (w/v) sugarcane bagasse was hydrodized with 1.25 mL cellulase (20.84 U/100 mL) and 0.625 mL cellobiase (0.98 U/100 mL).

Effect of medium carbon source on the hydrolytic potential of cellulases

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