40th Symposium on Biotechnology for Fuels and Chemicals

Saturday, April 28

7:00 AM - 8:00 AM TEA/LCA Workshop Registration and Breakfast

8:00 AM - 3:00 PM SIMB Board of Directors

Starfish, Lobby Level

8:00 AM - 5:00 PM Full Day Workshop: TEA/LCA Workshop

Water's Edge, Lobby Level

Sunday, April 29

8:00 AM - 5:00 PM Registration

Grand Ballroom Foyer, Lobby Level

10:00 AM - 4:00 PM Exhibits Setup

Grand Ballroom Foyer, Lobby Level

11:00 AM - 5:00 PM Poster Setup

Grand Ballroom A-E Lobby Level

1:00 PM - 3:30 PM Session: SO: Student Oral Session

Conveners: **Ninad Kothari**, Bourns College of Engineering, University of California Riverside, and BioEnergy Science Center, Oak Ridge National Laboratory, Riverside, CA, USA;**Sandra Notonier**, National Renewable Energy Laboratory, Golden, CO, USA and **Dr. Mario Murakami**, Brazilian Bioethanol Science and Technology Laboratory from the National Center for Research in Energy and Materials, Campinas, Brazil

Grand Ballroom, F-G Lobby Level

1:00 PM SO-1: Full utilization of biomass for fuels and chemicals: a biorefinery concept

Y. Xu, M. Zhang and D. Wang, Kansas State University, Manhattan, KS, USA*

The major challenges to commercialize cellulosic biofuels are low fermentation efficiency, low ethanol titer, and lack of technology to fully utilize the byproduct from bioconversion process such as lignin which has been underutilized. To overcome these technical barriers, we have proposed a novel design to fully utilize each component of lignocellulosic biomass for biofuels and bio-chemicals production, which involves green technologies such as hydrothermal and organosolv pretreatments to produce a cellulose-rich solid with good recovery of clean lignin after solvent recycling for improvement of plant protein-based adhesives as well as xylose remained in the aqueous phase for furfural upgradation. The focus of this study, as a part of the whole biorefinery concept, is to develop modified simultaneous saccharification and fermentation (mSSF) to enhance ethanol titers and yields, which combines the advantages of both separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF) via unique decantation process. The mSSF achieved higher ethanol concentration of 58.5 g/L and ethanol yield of 83.5% as compared to the traditional SSF process (49.9 g/L and 71.1%) at the biomass loadings of 20% (w/v). The mSSF also enabled higher ethanol titers of 72.3 g/L at higher loadings of 30% (w/v) with yields of 70.0%. As compared to published high-gravity fermentation, ethanol concentration of 72.3 g/L achieved in this study was the highest one in the lab-scale process, which proved that the proposed mSSF was an effective process to increase ethanol titers without sacrificing ethanol yields.

1:15 PM SO-2: Selecting best winter wheat variety for cellulosic ethanol production in Pacific Northwest

F. Fitria, H. Ruan and B. Yang, Washington State University, Richland, WA, USA; S. Fransen, Washington State University-Irrigated Agriculture Research and Extension Center, Prosser, WA, USA; H. Tao, Washington State University, Pullman, WA, USA*

Utilizing abundantly available and low cost feedstock for cellulosic ethanol production is preferable to produce a competitive fuel to anticipate the diminishing petroleum-based energy. In the United States, wheat ranks third as the most produced field crop. Winter wheat counts for 74% of the projected 47 MMT of the U.S. wheat production in 2017 while the Pacific Northwest (Washington, Oregon, and Idaho) contributes 17 %. Potential cellulosic ethanol production from wheat residues, i.e. wheat straw, is huge, even with 60% left for ground cover. Winter wheat variety is among the factors that can affect the sugar yield of the straw. To help the farmers choose the most profitable wheat variety for both grain and straw yields, information on sugar yield of different straws is needed to confirm their difference, which might be used by wheat breeders to improve the quality of the straws while still at least maintaining the current grain yield. In this study, both dilute acid pretreatment at 160° C using 0.5% (w/w) sulfuric acid and water-only pretreatment at 190° C in batch tube reactors followed by enzymatic hydrolysis identified two varieties out of 30 different varieties that achieved significantly higher sugar yield than others. The differences in performance were then related to wheat straw compositions. Based on bioprocessing results along with grain yield and popularity among farmers, the most promising winter wheat variety for cellulosic ethanol production in Pacific Northwest was selected.

1:30 PM SO-3: A novel single-step bioethanol production system from cellobiose by *Saccharomyces cerevisiae* RP2-BGL

L.F. Castro Eddy, H.M. Poggi-Varaldo and M.T. Ponce Noyola, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional CINVESTAV, Mexico City, Mexico; E. Cristiani Urbina, Escuela Nacional de Ciencias Biológicas, Mexico City, Mexico*

Saccharomyces cerevisiae RP2-BGL is a yeast strain that expresses an extracellular codon-optimized β-glucosidase from *Cellulomonas flavigena* PR-22 and it is capable of fermenting cellobiose to ethanol in a single-step process. However to be used at industrial level it is necessary to improve the volumetric ethanol production and productivity. In a previous work, the operation conditions in a batch mode regime, were assessed by a surface response methodology, improving the original ethanol yields by 20% (0.50 $g_{EtOH}g_{cellobiose}^{-1}$) reaching a final product concentration of 12.71 gL^{-1}. In the present work we evaluate a semi-continuous regime with the objective of increasing the volumetric production and yields. Different fed conditions were assessed including feed flow rates, cellobiose feed concentrations, and initial sugar concentration. All the experiments were carried out in 0.5 L bioreactors. Semi-continuous regime showed better results, providing a highest ethanol concentration of 18.15 gL^{-1} with a yield of 0.52 $g_{EtOH}g_{cellobiose}^{-1}$, including a consumption of 83% of the cellobiose supplied, which meant 40% more ethanol compared with batch fermentation of cellobiose.

Acknowledgments: This work was supported by Grant CB/14-236895 (CONACyT, Mexico) and Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV, Mexico)

1:45 PM SO-4: Fed-batch strategy of enzymatic saccharification of lignocellulosic biomass maximizing sugar concentrations at high solid loadings

J.A. Gonzalez R., A.U. Valle P. and A. Sanchez C., CINVESTAV - Unidad Guadalajara, Zapopan, Mexico; L. Amaya-Delgado, Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco A.C., Guadalajara, Jalisco, Mexico*

Enzymatic saccharification is a very important stage in the biorefining of lignocellulosic biomass for producing biofuels or high value products. High costs of enzymatic loads (15-20% of total production costs) and low productivities are currently considered the two main aspects to be solved in order to improve the economics of this stage. Increases in high solid loadings improve sugar concentrations, but result in low yields, therefore has a negative impact in process economy. These low yields are, to a great extent, generated by heat and mass transfer problems caused by a combination of the biomass mechanical-rheological properties and reactor characteristics. A common way to solve these problems is working with a fed-batch strategy, improving sugar release, but requiring long reaction times. In this work, a strategy of increases in solid loadings (20%) of wheat straw is using with Box Benkhen design where the variables are Pre-treatment time, number of additions during fed-batch strategy, and time between additions. After testing different feeding strategies at the beginning of the reaction, we saw that Residence time and number of feeding has a beneficial effect in sugar release, increasing concentration in xylose and glucose released (83.53 gGlc/l and 48.11 gXyl/l) in comparison with batch strategy (68.89 gGlc/l and 37.26 gXyl/l). These parameters allow to reach high solid loadings quickly, control the liquefaction in biomass, and reduce rheological problems, shortening reaction time.

2:00 PM Break

2:15 PM SO-5: Understanding solvent tolerance in *Yarrowia lipolytica*

C. Walker, S. Ryu and C.T. Trinh, University of Tennessee, Knoxville, TN, USA*

Microbial biocatalysis in organic solvents such as ionic liquids (ILs) is attractive for making high-value chemicals. However, IL toxicity at a level of 0.5% ~1% (v/v) can drastically reduce microbial activity. In this study, we engineered a mutant *Yarrowia lipolytica* YICW001 that can thrive in up to 18% (v/v) 1-ethyl-3-methylimidazolium acetate ([EMIM][OAc]), lethal to almost all microorganisms. Remarkably, YICW001 also exhibits universal tolerance in most commonly used ILs beyond [EMIM][OAc]. Scanning electron microscopy revealed that ILs significantly damage cell wall and/or membrane of wildtype *Y. lipolytica* while YICW001 maintains healthy cellular morphology even in high concentration of ILs up to 18% (v/v). Through comprehensive metabolomics, lipidomics, and transcriptomics study, we discovered that both wildtype *Y. lipolytica* and YICW001 prominently exhibit upregulation of most glycerophospholipids (GPs), sphingolipids, and sterols under IL-stressful environment. However, the mutant reconfigured membrane composition and structure by increasing the content of GPs and sterols more than the wildtype. This study provides a fundamental understanding of exceptional robustness of *Y. lipolytica* and helps guide future metabolic engineering of *Y. lipolytica* as a microbial manufacturing platform for production of high-value chemicals in organic solvents.

2:30 PM SO-6: Enhancing Terpene Carbon Sink by Downstream Modification

C. Zhao, X. WANG, C. Hu, C. Gorman and J.S. Yuan, Synthetic and Systems Biology Innovation Hub, Texas A&M university, College Station, TX, USA*

Terpenoids provide a diverse range of applications in fuels, chemicals, specialty materials, nutraceuticals and pharmaceuticals. However, it is highly challenging to increase terpene production in higher plants, partially due to downstream consumption and low carbon partition. Several strategies were developed to systemically improve terpene production *in planta* with squalene as a model compound. First, we re-balanced terpene biosynthesis and degradation via partially inactivation of terpene oxidases. Instability of plastidic squalene was predicted by mathematic modeling and verified by experiments in darkness. In order to address the instability, artificial microRNA was designed to target squalene epoxidases to reduce the downstream consumption of squalene. At the meantime, key enzymes in squalene biosynthesis pathway were over-expressed in chloroplast in *Nicotiana tabacum*. The squalene yield in engineered plants has achieved above 3 mg/g fresh weight. Second, we synergized “co-compartmentation” and “C2 redirection” strategies to increase carbon partition toward terpene biosynthesis. A hydrophobic protein was overexpressed in chloroplast to generate terpene storage droplet, co-compartmented with key enzymes of squalene biosynthesis pathway. Simultaneously, a C2 redirection pathway was introduced into tobacco chloroplast to channel photorespiratory glycolate toward terpene biosynthesis pathway. The integration of carbon redirection and storage strategies further enhanced squalene yield to 3.8 mg/g FW without decreasing in photosynthesis rate. Overall, downstream inhibition was effective strategy to increase photosynthetic terpene yield. The integration of carbon redirection with enhanced storage can further enhance the terpene based carbon sink. The technology advances provided new strategies for partitioning photosynthetic carbon into high value bioproducts.

2:45 PM SO-7: Development of antibacterial lignin-based materials guided by lignin's antibacterial activity againstÂ *Staphylococcus aureus*

A. Grossman, K. Rice and W. Vermerris, University of Florida, Gainesville, FL, USA*

Biorefineries that convert lignocellulosic feedstocks to renewable fuels and chemicals generate large volumes of lignin-rich residues that can be burned to generate steam and electricity, or that can serve as the basis for value-added products. Lignin is known to have antibacterial activity, although the responsible mechanism(s) have not been elucidated. The increasing prevalence of antibiotic-resistant pathogenic bacteria necessitates the development of novel approaches to minimize infections.

A particular challenge are pathogenic bacteria that can form biofilms, characterized as cells embedded in an extracellular polymeric substance matrix, because of their tolerance to antibiotics, antiseptics, and the host immune system. In particular, *Staphylococcus aureus* biofilms are major contributors to hospital- and community-acquired infections and are often difficult to treat.

Solutions containing lignin extracted from biorefinery residues were mixed with culture media and inoculated with wild-type clinical strain *S. aureus* UAMS-1 for planktonic (free-floating) and biofilm growth. Compared to standard medium, both planktonic and biofilm growth was significantly inhibited. Microscopy images from lignin-treated cultures displayed large cell clumps, possibly due to an effect on autolysins, enzymes necessary to separate newly formed cells. Moreover, lignin-treated cells had a lower intracellular pH and higher concentrations of staphyloxanthin, an antioxidant produced in response to oxidative stress.

Based on these experiments, we hypothesize that lignin increases oxidative stress and inhibits cell division in *S. aureus*. We are using these results for the development of antibacterial lignin-based materials that can serve as effective inhibitors of *S. aureus* biofilms. This work is supported by an NSF Graduate Research Fellowship.

3:00 PM SO-8: CELF-CBP Integration Enhances Total Biomass Utilization

P. Singh, C. Cai, R. Kumar and C.E. Wyman, Center for Environmental Research and Technology, Bourns College of Engineering,University of California Riverside, Riverside, CA, USA*

Co-solvent Enhanced Lignocellosic Fractionation (CELF) pretreatment of cellulosic biomass produces a hydrolysate that is rich in hemicellulose sugars and lignin and leaves solids containing mostly glucan. Consolidated bioprocessing (CBP) can then very effectively deconstruct the glucan in these solids without the need to add enzymes. An optimized integrated CELF-CBP process therefore offers the potential to achieve low cost bioconversion of lignocellulosic biomass through effective utilization of the three

major biomass fractions. In particular, efficient lignin fractionation by CELF pretreatment reduces lignin toxicity of *C. thermocellum* and provides a clean lignin stream for further upgrading in addition to production of fermentable sugars with high yields. Accordingly, our goal in this project is to maximize total sugar release from hemicellulose and cellulose via the CBP-CELF combination and CELF lignin recovery for valorization from both switchgrass and poplar. The project includes developing and applying lignin fractionation and characterization techniques to define promising options for conversion of CELF lignin into value added products. Understanding sugar release by the CELF-CBP combination and lignin features that are amenable to conversion to valuable products over a wide range of pretreatment conditions can provide valuable insights into paths that can realize maximum value generation from biomass conversion. This presentation will include preliminary results from co-optimization of CELF-CBP coupled with lignin fractionation and characterization.

3:15 PM SO-9: Optimizing conditions for metal adsorption onto lignin and subsequent recovery via microbial combustion

S. Fatemi[*] and A. Engelberth, Purdue University, West Lafayette, IN, USA

The potential valorization of lignin and recovery of toxic though industrially valuable metals is studied through an environmental application. The objectives of this work are to study the adsorption characteristics to optimize removal of lead (Pb) and cadmium (Cd) from water and to investigate the potential of metal recovery through fungal-mediated degradation, called microbial combustion, of lignin which has been contaminated. Previous work indicated successful adsorption of Pb and Cd. Lignin was obtained from corn stover via acid hydrolysis. Metal solutions were prepared from Pb and Cd chloride salts. Solutions had initial concentrations ranging from 50 to 200 ppm. Batch adsorption with lignin was performed at 30°C, 40°C, and 50°C, with pH controlled at 6, 7, and 8 to select optimal conditions. The initial and final concentrations of the samples were analyzed via atomic absorption spectroscopy (AAS). The white-rot fungus *Pleurotus ostreatus* was cultivated in rich medium, with and without the presence of contaminated lignin. Langmuir isotherms were fitted for adsorption of Pb and Cd onto lignin. Further work will investigate the interactions between lignin and non-metal substrates that may typically be found in water systems and its effect on the adsorption mechanism of lignin.

4:00 PM - 4:45 PM Rapid Fire

Grand Ballroom, F-G Lobby Level

5:00 PM - 6:00 PM Keynote

Conveners: Lee R. Lynd, Dartmouth College, Hanover, NH, USA

Grand Ballroom, F-G Lobby Level

6:00 PM - 8:00 PM Exhibits Open

Grand Ballroom Foyer, Lobby Level

6:00 PM - 8:00 PM Session: PS1: Poster Session 1/Reception

Grand Ballroom A-E Lobby Level

S1 Can a multi-step conversion improve the production and selectivity of levulinic acid from lignocellulosic biomass?

E.S. Lopes[*], J.F. Leal Silva and R. Maciel Filho, Chemical Processes, School of Chemical Engineering, State University of Campinas, Campinas, Brazil; M. Savioli Lopes, University of Alfenas, Poços de Caldas, Brazil, Brazil; K.M.C. Dominices, Federal Institute of Tocantis, Paraiso, Brazil; L. Plazas Tovar, University of Santa Maria, Santa Maria, Brazil, Brazil

The use of lignocellulosic biomass as a future substitute for fossil fuels depends on the successful development of biorefinery strategies for the production of renewable derived-building blocks. The goal of this research was to investigate the production of levulinic acid via a multi-step treatment conversion, using sugarcane bagasse (SCB) as feedstock. Pretreatment (PT), delignification (DL), and acid hydrolysis (AH) were integrated under different scenarios. In the first scenario, SCB was submitted to a single-step AH (at 180°C, 75 min, 12% w/v solids loading, and 7.0% w/v H_2SO_4). In the second scenario, SCB was submitted to a coupled PT (at 120°C, 80 min, 20% w/v solids loading, and 1.0% w/v H_2SO_4) and AH (at 180°C, 75 min, 12% w/v solids loading, and 7.0% w/v H_2SO_4). In the third scenario, the three-step conversion, a delignification step (DL) was added between steps PT and AH. DL was performed at 80°C, 90 min, 20% solids loading, and 0.5% w/v NaOH. Other three scenarios were assessed by replacing the acid hydrolysis step by a non-catalyzed equivalent. The six scenarios were compared based on yield and selectivity, and the three-step acid-catalyzed process presented the best results, with a yield of 47 mol% and a

selectivity of 77%. The success of this strategy is attributed to the high accessibility of cellulose in biomass after the removal of hemicelluloses and lignin by steps PT and DL, respectively. This study lays the grounds for further optimization to produce levulinic acid from cellulose by homogeneous acid catalysis with improved selectivity.

S2 Synthesis and characterization of water-soluble anionic carboxylic acid-terminated lignin dendrimer for metal absorption

S. Meng, University of Florida, Gainesville, FL, USA*

There exist numerous interests on the production of high-value materials from renewable and low-cost lignin that can be reclaimed from biorefinery waster stream. The water-soluble anionic dendrimer is a high value functional material, which can act as mimics of anionic micelles or proteins, Ethylenediaminetetraacetic acid (EDTA) similarities, and chelating agents for a spectrum of applications in catalyst, drug delivery, gene transfer, wastewater treatment and micronutrients. Herein, a water-soluble carboxylic acid-terminated lignin dendrimer structure (LDCA) was successfully synthesized from lignin dendrimer via butyl ester termination approach. The water-soluble lignin-based dendrimeric structure was prepared from the commercial lignosulfonate (LS) through firstly grafting with the epoxy group followed by the reaction with amine chemical. The proton nuclear magnetic resonance (^{1}H-NMR) and Fourier-transform infrared spectroscopy (FTIR) characterization confirms the successful grafting of functional groups and the synthesis of lignin-based dendrimeric structure and LDCA. Furthermore, the absorption of Cu(II) ion on the anionic LDCA complex was under investigation and the results from the ultraviolet visible spectrophotometers (UV-vis), Energy-dispersive X-ray spectroscopy (EDX), and FTIR demonstrate the binding of Cu^{2+}on the complex. It was also observed that the uptake of Cu^{2+} could be varied by initial pH and metal ion concentration.

S3 An integrated approach to explore the sugarcane straw potential in a biorefinery context

L. B. Brenelli and T. T. Franco, Interdisciplinary Center of Energy Planning (NIPE), Campinas, Brazil; V.M. Nascimento and S.C. Rabelo, Brazilian Bioethanol Science and Technology Laboratory (CTBE), Campinas, Brazil

The amount of sugarcane straw available in Brazil allows a wide range of feasible applications besides being left on the ground to improve the soil quality or generate bioelectricity. Depending on the fractionation approach or biochemical processes, this material can be converted into sugars for second generation ethanol (E2G) production and others value-added products e.g., xylitol and xylooligosaccharides (XOS). It is known that the pretreatment stage represents approximately 40% from the total investment in an E2G plant; therefore, modifications in process conditions and characteristics of the desired products can bring technological advantages and economic benefits to the technology. In this work, an integrated approach to explore the sugarcane straw potential to make feasible its incorporation in a biorefinery is presented. A combination of low severity pretreatment conditions (mild alkaline step prior to hydrothermal pretreatment) was evaluated as a strategy to remove acetyl groups and maximize xylan conversion into XOS with tailored degree of polymerization. The identified tailored-XOS, present in the hemicellulosic hydrolysate, will be uptake by newly designed GM-yeasts after specific enzymatic depuration while the cellulignin (solid fraction) will be converted into celluloligosaccharides by enzymatic routes. Furthermore, all residues containing acetic acid will be addressed to anaerobic digestion to produce methane. The impact of the deacetylation and hydrothermal pretreatment on hemicellulosic hydrolysate composition derived from sugarcane straw will be reported by kinetics studies. The results demonstrate that the sugarcane straw economic profitability can be supported by integrated production of low and high-value products.

S4 Low-molecularization of kraft lignin by peracetic acid treatment and separation of lignin-derived compounds

S.Y. Park, S.M. Cho, J.H. Choi, S. Yeon and I.G. Choi, Seoul National University, Seoul, Korea, Republic of (South); H. Jeong, National Institute of Forest Science, Seoul, Korea, Republic of (South)*

Conversion processes of the fragmentation of macromolecular lignin have been applied to upgrade lignin properties by changing functional groups or reducing structural heterogeneity. In particular, depolymerization by chemical or biological process has been studied as a main technique for producing value-added chemicals from lignin. Meanwhile, researches on lignin biorefinery have been generally focused on the selective production of low molecular lignin fragments such as monomers. However, not only monomers but also various oligomers are simultaneously generated by depolymerization. Therefore, the total utilization of lignin fragments should be considered to enhance lignin applicability.

Our study was investigated for two purposes. First purpose is low-molecularization of kraft lignin. Specifically, low-molecularization can be involved in reducing structural heterogeneity of lignin. For the purpose, oxidative depolymerization using peracetic acid (PAA) was conducted, producing low molecular lignin fragments. After PAA treatment, lignin were totally dissolved. Solvent extraction using ethyl acetate was performed to isolate the lignin compounds after PAA treatment. Depolymerized fragments were comprised of oligomers, dimers, and monomers, where oligomers and dimers showed lower polydispersity compared to initial kraft lignin.

Second purpose of this study is to separate the lignin fragments depending on their structural characteristics. Solutes (oligomers, dimers, and monomers) extracted with ethyl acetate were separated using silica-gel by changing organic solvent mixtures (chloroform-ethyl acetate, methanol-water), successively. Detailed structure of the separated compounds was analyzed

using GPC, LC/MS-NMR instruments.

S5 Lewis-acid catalyzed condensation between monoterpene hydrocarbons and n-butyraldehyde for drop-in fuel synthesis

S.M. Cho, J.H. Kim, S.Y. Park, J.H. Choi, S.K. Jang and I.G. Choi, Seoul National University, Seoul, Korea, Republic of (South); B. Koo, Korea Institute of Industrial Technology, Cheonan-si, Chungcheongnam-do, Korea, Republic of (South)*

Monoterpene hydrocarbons, one of the widely biosynthesized natural products, attract interest as candidates for drop-in fuel production. Because their functional group which can be utilized in chemical reaction is only olefin, many studies on the synthesis of transport fuel by the chemical upgrading of monoterpenes have been focused on Diels-alder reaction and olefin metathesis.

Lewis-acid catalyzed carbonyl-ene reaction, a new attempt in this study, is one possible strategy for controlling the carbon number of monoterpene hydrocarbons. In the preliminary experiment, condensation between n-butyraldehyde and turpentine oil which was mainly composed of α-pinene was conducted with $ZnCl_2$ and $SnCl_4 \cdot 5H_2O$ as a Lewis-acid catalyst. Reaction conditions were varied with temperature and catalyst dosage. The major obstacles to synthesize carbon controlled product from this system were (a) dimerization or trimerization of monoterpene hydrocarbons, (b) self-aldol condensation of n-butyraldehyde, (c) isomerization which accumulated unreactive monoterpenes towards carbonyl-ene reaction, and (d) condensation of one molecule of monoterpene hydrocarbons with more than two n-butyraldehyde.
To exclude the unwanted condensation, zeolite catalysts (NH_4-ZSM5 and NH_4-BEA) which had a possibility for carrying out selective reaction by size exclusion were introduced. Because there was lacking in Lewis-acid site for carbonyl-ene reaction, modified zeolite catalysts were prepared by zinc ion exchange method. Among zeolite catalysts, only Zn-BEA gave the reasonable yield of expected products.

S6 High lipid production by heterotrophic growth of the green algae *Auxenochlorella protothecoides* using hydrolysates from organosolv pretreated birch and spruce biomass

L. Matsakas, A. Patel, U. Rova and P. Christakopoulos, Biochemical Process Engineering, Division of Chemical Engineering, Department of Civil, Environmental and Natural Resources Engineering,Luleå University of Technology, Luleå, Sweden*

Aim of this work was to establish a process for the heterotrophic growth of green microalgae using forest biomass hydrolysates. Two tree species, namely Norway spruce (*Picea abies L.*) and silver birch (*Betula pendula L.*), were used, due to their abundance and financial importance in Sweden. To provide a carbon source for the growth of the green microalgae, forest biomass was pretreated with a hybrid solvent organosolv-steam explosion pretreated, resulting in inhibitor free pretreated solids with high cellulose content of 77.9 % (birch) and 72% (spruce). Pretreated solids were hydrolyzed with the use of commercial cellulolytic enzymes in order to produce the hydrolysate for the algae cultivation. The heterotrophic growth of *A. protothecoides* was assessed using synthetic media with glucose as carbon source, where the effect of sugar concentration and carbon-to-nitrogen ratio were optimized resulting in the accumulation of 5.42 g/L lipids (63.9% lipid content) after 6 days of cultivation on 20 g/L of glucose. Finally, the optimal conditions were used for the cultivation of the algae on birch and spruce hydrolysates. Use of hydrolysates was favorable for the growth and lipid accumulation of the algae, resulting in a lipid production of 5.65 g/L (66.3% lipid content) and 5.28 g/L (63.1% lipid content) when grown on birch and spruce respectively after only 5 days of cultivation.

S7 Formation of nanoparticles from organosolv isolated lignin from birch biomass

L. Matsakas, A. Karnaouri, U. Rova and P. Christakopoulos, Biochemical Process Engineering, Division of Chemical Engineering, Department of Civil, Environmental and Natural Resources Engineering,Luleå University of Technology, Luleå, Sweden*

Lignin is a very abundant raw material that is generally under-utilized. Several application of lignin for the production of high-added value products have been proposed, with the production of lignin nanoparticles to be among them. Lignin nanoparticles play an important role in fabricating engineered nanocomposites for high-value applications in material fields. Formation of lignin nanoparticles has been reported in the literature, mostly through mechanical or chemical mechanisms. These mechanisms require the use of special equipment (such as sonication) or the use of time-demanding processes with solvent modifications. In our work, we examined the formation of lignin nanoparticles directly after the pretreatment of birch biomass. More specifically, a hybrid organosolv-steam explosion pretreatment was applied, which combined the traditional organosolv cooking with the rapid explosive discharge at the end of the pretreatment. This has a result the formation of lignin nano- and micro-particles with varied size distribution. Pretreatment conditions, such as addition of acid catalyst and the ethanol content affected the formation and size distribution of the nanoparticles. Moreover, the effect of the presence of the explosive discharge was also evaluated in the formation and size of the lignin particles. Finally, in an attempt to further decrease the size of the nanoparticles and also improve their size homogeneity, the lignin samples underwent a post treatment in a homogenizer with ethanol solutions of varied ethanol contents. The secondary treatment resulted in better homogeneity and lower particle size, as it converted the lignin microparticles to nanoparticles.

S8 Ethanol production through syngas fermentation: technical evaluation of a commercial-scale syngas fermentor at different process conditions and feed compositions

E. Almeida Benalcázar[*] and R. Maciel Filho, State University of Campinas, Campinas, Brazil

Uncertainty exists on how syngas H_2/CO ratio, temperature, pressure, gas dilution and broth enrichment with fructose influence ethanol production by fermentation with acetogenic bacteria. The present study evaluates the technical performance of fermentation from the view of ethanol concentration and its volumetric productivity and, energy requirements in a theoretical large bubble column that works at a fixed gas hold-up and is fed with stoichiometric excess of syngas. The effects of variations in temperature (37 - 70 °C), pressure (0.5 - 3 atm), gas dilution with an inert gas (0 to 50 %mol.) and heterotrophy (1 – 20 % ethanol produced from fructose), were assessed for two compositions of syngas i.e. pure CO and a 2:1 mixture between H_2 and CO_2. Monod kinetic equations with no product inhibition were used to simulate the consumption of both sources of electrons, CO and H_2, while Herbert-Pirt equations were used to simulate bacterial growth. The temperature-dependent parameters for both types of equations were estimated from thermodynamics at cellular physiological conditions. In general, ethanol productivities are 30 % higher when H_2/CO_2 are fermented compared to CO; ethanol concentrations fall around 100 g/L. Productivity increases with temperature, pressure and broth fructose enrichment. Product concentration has a direct relation with productivity except when temperature is varied. However, energy requirements only decrease when broth is enriched with fructose. Overall, the highest ethanol productivities are reached at the highest temperatures but also the lowest product concentrations and highest energy requirements.

S9 Evaluation of biobutanol production from eucalyptus sawdust within a biorefinery approach

F. Cebreiros, M.D. Ferrari and C. Lareo[*], Universidad de la República, Montevideo, Uruguay

Butanol is considered an important chemical with wide industrial applications which also presents good properties to be used as biofuel. It can be produced by acetone–butanol–ethanol (ABE) fermentation from different types of substrate. Lignocellulosic biomass, used for bioenergy production because of its abundance, low cost and no competence with food, has also been employed for the production of biobutanol. In this work, eucalyptus sawdust from a local pulp industry was evaluated as a raw material for the production of biobutanol using a biorefinery approach.

Lignocellulosic biomass needs to be hydrolyzed prior to fermentation using an adequate pretreatment and subsequent enzymatic hydrolysis. In this study, autohydrolysis pretreatment was selected under mild conditions in order to enhance the hemicellulose recovery and preserve the xylan fractions for further processing. The sawdust was pretreated using hot water at 180ºC for 45 min in a batch type reactor. The pretreated solids obtained were subjected to enzymatic hydrolysis with cellulases Cellic CTec2 at 50ºC and pH 4.8, solids and enzyme loadings 16% w/v and 25 FPU/g_{glucan}, respectively. Under these conditions, total sugars concentration (mainly glucose) up to 90 g/L was released after 72 h, achieving a cellulose hydrolysis of 92%. Fermentation of this hydrolysate was then evaluated using *C. acetobutylicum* DSM 792 for ABE production. Assays were done in 100 mL bottles at 37ºC, pH 6 and 150 rpm, inoculated with a 10% (v/v) highly active cell culture. Results obtained were evaluated in terms of sugar conversion, butanol yield and productivity.

S10 Production of isopropanol butanol ethanol (IBE) by repeated batch fermentation of industrial sugarcane and sweet sorghum juices

E. Rochón, I. Cabot, G. Cortizo, M.D. Ferrari and C. Lareo[*], Universidad de la República, Montevideo, Uruguay

Butanol is an important bulk chemical with a large market which also presents good properties to be used as biofuel. It can be produced by isopropanol-butanol-ethanol (IBE) fermentation in which the mixture of solvents produced can be used as fuel. The ability of *Clostridium beijerinckii* DSM 6423 to be reused in consecutive IBE fermentations of a mixture of industrial juices of sugarcane (75%) and sweet sorghum (25%) was evaluated. Fermentations were carried out in bottles of 250 mL with 100 mL of medium. The medium with a total sugar concentration of 60 g/L was supplemented with yeast extract (1 g/L), buffer and mineral (P2) solutions and a commercial vitamin complex (1% (v/v)). Fermentation conditions were 35ºC, 150 rpm, and initial pH 6.0. Each fermentation cycle was started by adding 8% (v/v) of fermented medium to fresh medium. An initial batch fermentation showed that the process finished at 48 h, when total solvent production was 11.8 g/L and sugar conversion 72%. Solvent yield and productivity were 0.21 g/g and 0.21 g/Lh, respectively. Therefore, repeated batch fermentations were done every 48 h. It was observed a total solvent production in the range 7.4-16.7 g/L until the seventh fermentation cycle. Productivities were in the range 0.20-0.32 g/Lh. Butyric and acetic acids were also produced (0.4-0.5 and 1.6-2.2 g/L respectively). From the eighth cycle, solvent production decreased significantly. Results showed *C. beijerinckii* DSM 6423 could be used in a repeated batch fermentation, saving operational costs due to inoculum development.

S11 Bioethanol production from eucalyptus wood sawdust using different pretreatments (autohydrolysis, alkaline pulping) in a biorefinery approach

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Sawdust is a residue from the pulp and paper industry that can be used as a raw material to obtain fuel bioethanol and other

marketable products. The ethanol production by Presaccharification and Simultaneous Saccharification and Fermentation (PSSF) of eucalyptus wood sawdust was evaluated. Different strategies of pretreatments were performed in order to enhance cellulose enzymatic hydrolysis, preserving both fractions, xylan and lignin, for further processing into valuable products: autohydrolysis (AH, 170°C and 40 min), autohydrolysis-kraft pulping (AHKP, 155°C, 140 min, alkali charge 10.5%), and autohydrolysis-soda pulping (AHSP, 155°C, 90 min, NaOH 12%). PSSF tests were carried in 250 mL Erlenmeyer flasks using presacarification of 24 h, liquid to solid ratio 7.5 g/g, cellulases Cellic Ctec2 25 FPU/g of glucan, and an industrial yeast strain (*Saccharomyces cerevisiae* PE-2). Ethanol concentrations of 9, 57 and 58 g/L, productivities of 0.2, 1.2 and 1.2 g/Lh, and global efficiencies based on original glucan content of 12, 68, 80% (after 48 h of PSSF) were obtained for sawdust pretreated by AH, AHKP and AHSP, respectively. The best results were reached for pretreated sawdust by AHSP where an ethanol yield of 254 L/t (dry basis) was obtained, and 114 kg of lignin and 46 kg of xylose per tonne of dry sawdust were respectively recovered. PSSF of AHSP pretreated sawdust was also conducted in a laboratory bioreactor (Labfors 5 BioEtOH reactor, Infors-HT, Switzerland, working volume 3.6 L), showing similar results.

S12 Conversion of pretreated hardwood to medium-chain fatty acids for production of biolubricants.

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Medium-chain fatty acids (MCFA, e.g., caproic, heptanoic, caprylic acid) are versatile chemical compounds that are suitable precursors for production of high-carbon-number, value-added chemicals such as bio-based lubricants. Short-chain fatty acids (SCFA, e.g., acetic, propionic, butyric, valeric acid) and methane are the natural fermentation products of anaerobic digestion, however, they have lower value compered to MCFAs. Our current investigation seeks to integrate production of MCFAs through chain elongation fermentation of pretreated biomass with catalytic conversion of MCFAs to lubricant molecules.

By adding reducing agents such as ethanol and lactic acid to the anaerobic digestion, and inhibiting methanogenesis, microorganisms can elongate SCFA to MCFA. At low ethanol concentration the elongation reaction does not reach a high concentration of MCFA, whereas very high ethanol concentration has an inhibitory effect, and the fermentation ends before consuming all the ethanol. To maximize MCFA production, different ethanol concentrations were investigated in the mixed-culture fermentation of pretreated hardwood, inoculated with a mixture of manure, beach sediments and corn steep liquor. Addition of ethanol at the initiation of fermentation resulted in increased accumulation of MCFAs. Incremental addition of ethanol during the fermentation resulted in significantly higher titers of MCFAs. Results on production of MCFA from addition of lactic acid will also be presented.

S13 Design and Development ofÂ Catalysts for Lignin Based Jet Fuel Production

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The synthesis of high-efficiency and low-cost catalysts for hydrodeoxygenation (HDO) of waste lignin into advanced biofuels is crucial for enhancing current biorefinery processes. The choice of catalysts for these processes is restricted to high stability in aqueous phase as well as the best compromise between performance and price. In this study, Super Lewis acids containing the triflate anion [e.g., $Hf(OTf)_4$, $Ln(OTf)_3$, $In(OTf)_3$ $Al(OTf)_3$] and noble metal catalysts (e.g., Ru/C, Ru/Al_2O_3) formed efficient catalytic systems to generate saturated hydrocarbons from lignin in high yields. In such catalytic systems, the metal triflates mediated rapid ether bond cleavage through selective bonding to etheric oxygens while the noble metal catalyzed subsequent hydrodeoxygenation (HDO) reactions. Super Lewis acids plausibly interact with lignin substrates by protonating hydroxyl groups and ether linkages, forming intermediate species that enhance hydrogenation catalysis by supported noble metal catalysts. Meanwhile, the hydrogenation of aromatic rings by the noble metal catalysts can promote deoxygenation reactions catalyzed by super Lewis acids. In addition, the combination of a 3d transition metal (Fe, Ni, Cu, Zn) with Ru can modulate the hydrogenolysis activity of Ru and help prevent the hydrocarbon products from forming gaseous products through over-hydrogenolysis. Among these catalysts, Ru-Cu/HY showed the best HDO performance, giving the highest selectivity to hydrocarbon products due to (1) high number of strong acid sites, (2) good dispersion of metal species and limited segregation, (3) high adsorption capacity for polar fractions, including hydroxyl groups and ether bonds.

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S14 Simultaneous wet storage and pretreatment of rice straw via RT-CaCCO process for cascade recoveries of multiple bioproducts

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Agricultural residues, such as rice straw (RS) and corn stover, are often produced in a wet form due to bad weather in the crop harvesting seasons, and they are readily deteriorated into a bad quality for bioconversion to fuels and chemicals. For utilization of these feedstocks, wet storage is expected as a low energy-input process to keep feedstock quality high. We developed a simple process for a simultaneous wet storage and pretreatment of biomass, termed “the Room Temperature-Calcium Capturing by Carbonation (RT-CaCCO)” process: lime pretreatment at ambient temperature for longer than a week and the subsequent neutralization by carbonation. Herein, RT-CaCCO treated RS (RTRS) was used for cascade recoveries of mushroom and

fermentable sugars. The mixture of RS, lime and water was stored at room temperature for 120 days and neutralized by carbonation, and wheat bran was added to the neutralized sample prior to autoclaving. *Pleurotus ostreatus*, was inoculated to it and incubated for 70 days. Xylan degradation and production of acid soluble lignin in the RTRS were more significant than those in the control RS in the absence of lime. The average yield of fruit body of RTRS samples was better than that of the control samples. Fermentable sugar recovery was performed by enzymatic hydrolysis of solid part after removal of fruit bodies, and a significant effect of lime pretreatment on the enzymatic digestibility was found. Thus, RT-CaCCO process could give us a new chance for efficient conversion of wet biomass to multiple bioproducts.

S15 High Yield Fructose Production from CELF Pretreated Corn Stover Using Fungal Enzymes and Immobilized Glucose Isomerase

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Production of platform chemicals such as 5-HMF (hydroxymethylfurfural) from cellulosic biomass has garnered attention due to their potential for facile catalytic conversion into drop-in fuels and chemicals. However, the severe biomass pretreatment conditions required to directly produce 5-HMF results in high operating costs and sugars loss to humin formation. To address these limitations, thermocatalytic pathways to convert sugars derived from biological conversion of pretreated solids into 5-HMF have been developed. However, an isomerization step is required to convert glucose into fructose that can be converted into HMF with much higher yields than the glucose. Therefore, the objective of this research was to achieve high yields of fructose by isomerization of the glucose released from lignocellulosic biomass at low cellulase loadings (5 mg enzyme protein/g glucan) applied to glucan-rich solids produced by low severity co-solvent enhanced lignocellulosic fractionation (CELF) pretreatment of corn stover. Potential advantages include: 1) more mild pretreatment conditions, 2) low cellulase loadings, 3) high sugar yields, and 4) minimal humin formation. For this research, milled corn stovnr was soaked overnight in a 1:1 water: THF (w/w) solvent mixture with 0.5 wt% acid in the water. The impregnated corn stover was then pretreated at 150°C for 25 minutes at a 5 % (w/w) solids loading. The pretreated solids then were subjected to enzymatic hydrolysis and glucose isomerization to produce fructose. Fructose yields from these solids were determined versus time over a range of temperatures, pH values, and buffer systems.

S16 Pretreatment system of liquid anaerobic digestate for microalgae cultivation

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Using liquid anaerobic digestate for microalgal cultures has been considered as a Win-Win paradigm for sustainable biogas and microalgae industry. However, the high turbidity caused by suspended materials and the ammonium-nitrogen levels in liquid digestate have the potential to inhibit the microalgae growth. The traditional pretreatments such as separation and dilution usually consume a large quantity of energy and freshwater. In this study, two pretreatment methods are established. The firstly is struvite precipitation. To obtain struvite-precipitated supernatant that has an ideal transmittance, NH_4^+-N concentration, salinity, and N:P ratio for microalgal growth, the NH_4^+: Mg^{2+}: PO_4^{3-} molar ratio in the liquid digestate should be adjusted to 1:1.2:1.2 with KH_2PO_4 and $MgCl_2$ under a continuous stirring, which should subsequently be stopped as soon as the pH reaches 8.5, following addition of NaOH. Grey relational analysis (GRA) was applied to obtain a relative grey scale for the evaluation of multiple outputs. *Chlorella* and *Dictyosphaerium* were optimal microalgae species. The second method is SBR. The optimal conditions were determined to be 25°C, a 7.0 g/L seed sludge concentration, a 5 mg/L DO concentration, and a 6h HRT. After SBR, *Chlorella* could grew well in the liquid after SBR. These two methods can be separately for pretreatment of different concentrations of liquid anaerobic digestate.

S17 A Novel Platform for Bioupgrading of Lignin to Valuable Nutraceuticals and Pharmaceuticals

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To enhance the economics of lignocellulosic biomass utilization, lignin has been extensively studied as a promising feedstock for biofuels and bioproducts. This study is the first demonstration of lignin bioupgrading to high-value nutraceuticals/pharmaceuticals by using fungoid marine protists, e.g., *Thraustochytride* (patent in pending). It was found that *Thraustochytrium striatum* is able to transform various types of lignin compounds into polyunsaturated long-chain fatty acids (EPA and DHA) and carotenoids (e.g., β-carotene and astaxanthin). In screening of fourteen lignin model compounds as carbon sources, *T. striatum* can grow in most compounds at different concentrations, while achieving the best growth in 3, 4-dihydroxybenzoic acid (3, 4-DHBA) and 4-hydroxybenzoic acid (4-HBA). The maximum biomass yield reached 2-3 g/L under 5 g/L of 3, 4-DHBA or 2 g/L of 4-HBA even without optimization. In both batch and fed-batch cultivation modes, *T. striatum* can accumulate decent amount of long-chain fatty acids (15% cell dry weight) and carotenoids (0.1 mg/g biomass). The capability of *T. striatum* on utilizing real lignin was also evaluated using Kraft pine lignin, with chemical or enzymatic depolymerization prior to fermentation. Another interesting finding was that *T. striatum* could directly use black liquor from alkaline pretreatment of corn stover with slight pH adjustment. Our preliminary study indicated that *T. striatum* is able to digest a wide range of lignin materials. By investigating gene sequence, critical enzyme and metabolites, we are now interested in the mechanisms behind the behavior of this microorganism on lignin utilization given its marine growth environment where little lignin exists.

S18 Bioprospection of yeasts for production of xylitol from sugar cane biomass hydrolysates

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Xylitol is a five-carbon polyol that is being widely studied because of its applications in the food and pharmaceutical industries. This compound is industrially produced by the catalytic hydrogenation of xylose. However, more economic and environmental friendly production processes have been considered. In this context, conversion of xylose, which is present in biomass hydrolysates, to xylitol employing microorganisms appears to be a good opportunity. However, the chosen microorganism should be able to keep high production yield and productivity even in presence of lignocellulosic derived inhibitors. This work aimed to select yeasts able to produce xylitol from sugarcane bagasse derived sugars. For this, 960 yeast strains were screened by the ability to grow in minimal medium supplemented with xylose (40g/L) as sole carbon source. Then, the 42 strains that showed the highest growth rate were cultivated on xylose-supplemented medium and the metabolite production profile analyzed by HPLC. Based on these results, the six strains that most consumed xylose were chosen for fermentative kinetic comparisons on defined medium and on sugarcane bagasse hydrolysate. Yeasts consumed more than 80% of the xylose provided, with yields higher than 0,6 g/g. Finally, effects of acetic acid and aeration on xylitol production will be discussed.

S19 The Application of Stochastic Optimization in Lignin Depolymerization Process

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Biochemical refinery problems often involve uncertainty such as randomness in materials, reactions, and operations. In this work we establish a multistage stochastic programming model for the optimization and control of chemical processes under this type of uncertainty. We discuss the implementation of a stochastic dual dynamic programming (SDDP) algorithm to compute an optimal solution for this problem. We then applied the algorithm on a simulated lignin depolymerization process to test the efficiency under several conditions.

S20 Biological Pest Control Agents as Lignocellulose-based Biorefinery Products

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There is growing evidence that broad use of the azole class of compounds to control fungal diseases and other problems in agriculture has led to azole resistance of plant pathogens and medically important fungi. In the potato industry, several fungal diseases have been controlled by thiabendazole (TBZ), which is no longer effective against its target diseases due to the occurrence of widespread resistance. Over 80% of *Fusarium sambucinum*, causative of potato dry rot, are now resistant to TBZ, and chemical substitutes are limited, especially in postharvest potatoes destined for food use. There is growing pressure to move away from azole use in agriculture necessitating the development of non-azole alternatives. ARS (Peoria, IL) has had a long term effort to develop *Pseudomonas* spp as biological control agents (BCAs) to substitute for TBZ to control potato dry rot and other maladies, including sprouting, late-blight (incited by *Phytophthora infestans*) and pink rot (incited by *Phytophthora erythroseptica*). It is proposed that such *Pseudomonas* spp., which utilize diverse sugars (including pentoses) and other organic compounds to yield antifungal BCAs, can be developed as valuable coproducts for the lignocellulose biorefining industry, where they can be grown using either waste or dilute sugar streams. Our recent results suggest that sugars, acetic acid, and furanaldehydes generated from lignocellulose can be used for the production of a broad spectrum antifungal microbial alternative to azole compounds. Data will be presented showing BCA production on various sugar streams from hydrolyzed switchgrass and their impact on BCA productivity, yield and performance.

S21 A molecular modeling approach to using high-oleic soybean oil to sweeten sour gas

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Predictive methods for sweetening natural gas using bio-based solvents provide an opportunity to add value to the soybean industry and develop an economical method for cleaning sour gas. Improvements in fracking technology have increased availability of natural gas, a valuable fuel source that releases less CO_2 than coal and fewer greenhouse gases than gasoline. However, natural gas often contains high concentrations of hydrogen sulfide, a corrosive compound that damages processing equipment and is harmful to humans and the environment. Soybean oil is a readily available bioresource, and the high degree of saturation in high-oleic soybean oil offers several potential binding sites for sulfur. While laboratory experiments can be costly and time-consuming, predictive models that examine interactions at a molecular level can be efficient tools for solvent screening and choosing process parameters. An approach based on statistical thermodynamics known as the Conductor-like Screening Model for Real Solvents (COSMO-RS) simulated the partitioning of hydrogen sulfide between liquid soybean oil and methane gas phases. Predicted *K* values ranged from 0.16 – 0.20 at temperatures of 25 – 100°C; *K* values between 0 – 1 are indicative of near-full extraction of the hydrogen sulfide. Theoretical and experimental results are compared after mixing soybean oil with hydrogen sulfide and analyzing phase concentrations of the H_2S using chromatography. Economic analyses examine the cost of using soybean oil in an H_2S extraction process. This study provides fundamental, proof-of-concept for using high-oleic soybean oil as a bio-based solvent in a new method for cleaning sour gas.

S22 From waste to high-value products: Impact of galactoglucomannan purity on selected hydrogel properties

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Hydrogels are a hydrophilic network of polymers (usually cross-linked polysaccharides) that are natural or synthetic. Applications of hydrogels are many in the biomedical field. Because of the high water absorption (10 to 200 grams of water per gram of hydrogel), hydrogels have a high degree of flexibility similar to natural human tissue. Human cells can be incorporated into the hydrogel, which in turn can be used to repair damaged tissue. An option is to incorporate drugs in the gels to treat a damaged area by a sustained-release drug-delivery system. Other uses have been in the production of bio-sensors, as absorbent in, e.g. diapers or in the production of contact lenses.

Hemicelluloses (in this work galactoglucomannan (GGM)) are a promising renewable raw material for the production of hydrogels. Given their high abundance (constituting up to 25% of the wood cell walls) and current lack of use (usually incinerated together with other biopolymers in the pulp and paper industry), makes this work valuable from an economical and industrial point-of-view.
In this work, we examine the possibility to produce hydrogels from GGM extracted from sodium-based spent-sulfite-liquor using a combination of membrane filtration and anti-solvent precipitation. The impact of GGM purity or the addition of lignosulfonates to the cross-linking reaction mixture (in-direct effect of the downstream processing, which affect the overall process economy) on the mechanical, thermal and chemical properties of the hydrogel have been examined and evaluated.

S23 Ghost of *Chitinibacter tainanensis*

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Chitin is the major component of fungal cell walls, insect exoskeletons and the shells of crustaceans. Besides cellulose, chitin is the most abundant polysaccharide on the earth. Derivatives of chitin, inclusive of deacetylation polysaccharides, oligosaccharides and monosaccharides, have been applied for adsorption, enzyme-immobilization and biomedical materials because of the unique characteristics of being biodegradable, histo-compatible and nontoxic. The monomer of chitin, N-acetylglucosamine (NAG) has specific roles in physiology and cell biology as well as for its therapeutic uses in treating arthritis, pediatric enteritis and Crohn's disease. It also promotes the synthesis of hyaluronic acid to anti-wrinkle. In the market, the NAG is high industrial output value in raw material of pharmaceutics, food and agriculture.

Recently, a new species microorganism, *Chitinibacter tainanensis*, was isolated from the soil of southern Taiwan. The bacteria can produce NAG by degrading chitin, and the yield reaches almost 90%. The high purity (99.9%) of NAGcan be conventionally obtained by recrystallization. The transformation of chitin is subjected on the surfave of membrane in *C. tainanensis*. For the elucidation of the mechanism for the production of NAG. A plasmid with autolysis will be introduced for ghost production. The efficiency of NAG production is also determined.

S24 Evaluation of butyric acid addition on the isopropanol-butanol-ethanol (IBE) production from sugarcane and sweet sorghum juices by *Clostridium beijerinckii* DSM 6423

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Biobutanol is considered an attractive biofuel. It can be produced by isopropanol-butanol-ethanol (IBE) fermentation. The microorganism produces butyric and acetic acids in a first stage (acidogenic phase) and then these acids are re-assimilated for solvent production (solventogenic phase). The swift from acidogenic phase to solventogenic phase is not fully understood. Butyric acid has been reported as a triggering substance for the solventogenesis and could be used as a co-substrate added from an external source or produced by co-culture fermentation. The effect of butyric acid addition on IBE fermentation of a mixture of industrial juices of sugarcane (75%) and sweet sorghum (25%) by *Clostridium beijerinckii* DSM 6423 was studied. Fermentation assays were carried out in 250-mL bottles at 150 rpm, 35°C, initial sugar concentration ~65 g/L. Butyric acid additions were in the range 0 - 6 g/L. The assay without adding butyric acid, but with an initial concentration of 0.5 g/L, from the inoculum, reached a butanol concentration of 6.5 g/L and a sugar conversion of 41 %, after 32 h. Butanol yield and productivity were 0.23 g/g and 0.20 g/Lh respectively. Similar results were found when initial butyric acid concentration was 1.5 g/L. However, in this assay sugar consumption started after 24 h showing a prolonged lag phase. When initial butyric acid was 2.2 g/L or higher no solvent production was observed. No improvement on IBE production was observed by butyric acid addition under the conditions evaluated.

S25 Production of acetone butanol ethanol (ABE) from *Physaria fendleri* press cake using *Clostridium beijerinckii*

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Physaria fendleri (PF) is a member of the mustard family that is of current interest for commercial production as an oil crop because its oil has industrial uses. Oil production costs are highly sensitive to finding a use for the press cake left over following oil extraction. In this study, PF press cake is evaluated as a feedstock for butanol production because of its high carbohydrate content. To extract the carbohydates, 60 gL^{-1} PF was pretreated with 0.5% (v/v) sulfuric acid at 121 ^{o}C for 1 h followed by cooling to 25 ^{o}C and agitating the mixture at 200 rpm using a magnetic stir bar for 72 h. The resultant hydrolyzate contained 15.83 gL^{-1} total sugars. Then the hydrolyzate was fermented using *Clostridium beijerinckii* P260 and the culture produced 6.39 gL^{-1} ABE with a productivity of 0.18 $gL^{-1}h^{-1}$. These experiments suggested that enzymatic hydrolysis of PF after pretreatment was essential to release more sugars in the fermentation medium. Upon hydrolysis of 60 gL^{-1} PF with enzymes followed by fermentation provided with13.45 gL^{-1} ABE in the fermentation broth. Increasing PF concentration to 100 gL^{-1} followed by enzymatic hydrolysis resulted in the production of 19.22 gL^{-1} ABE with a productivity of 0.40 $gL^{-1}h^{-1}$. These results are comparable to ABE/butanol production observed from a control fermentation using glucose. On a routine basis not more than 18-20 gL^{-1} ABE is produced in batch reactors from glucose based medium. These results suggest that PF press cake is a good substrate for biobutanol production.

S26 Alkaline Fractionation ofÂ LignocellulosicÂ BiomassÂ for Delignification and Subsequent Formic Acid Recovery from the Hydrolysate through Esterification and Distillation

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The objectives to this research were to optimally alkaline fractionation of pinecone for delignification and formic acid recovery through esterification and distillation process. The pinecone contains relatively higher lignin content 35.80% compared to other lignocellulosic biomass on a dry basis. Also, a large amount of formic acid is present in the hydrolysate derived from pinecone. The pinecone of 100 g was reacted on various alkaline solution (1-8 % w/w) at 170 ^{o}C for 53 min in a 1L Parr reactor. The hydrolysate was conducted esterification with ethanol in water bath under various parameters for formic acid recovery with respect to the mixed ratio (1:1-1:4 v/v), temperature (30-45 ^{o}C), and reaction time (60-100 min). Subsequent, the ethanol mixture (ethanol and ethyl formate) was recovered through distillation. The highest recovery yield of AIL obtained from the pinecone was 79.20% with 8% w/w NaOH. Also, the highest content of formic acid has appeared in the equal conditions. The formic acid in hydrolysate was recovered approximately more than 85% at mixed ratio 1:2 condition. The sodium sulfate that is additionally obtained through this experiment was recovered through filtration.

S27 Effect of oxygen availability on xylose fermentation in the presence of glucose by yeast *Spathaspora passalidarum* NRRL Y-27907

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Yeast *Spathaspora passalidarum* is a promising microorganism for industrial application in the production of ethanol from lignocellulosic biomass (second-generation bioethanol) due to its great capability to ferment the pentose sugars that are obtained from hydrolysis of biomasses. In a large-scale fermentation process, controlling the oxygen concentration in the media could be a hard task, rendering the process technically difficult and expensive. Unlikely other pentose-fermenting yeasts that need specific microaerophilic conditions for fermentation, it's reported in the literature that *S. passalidarum* is capable to produce ethanol from pentose under strict anaerobic conditions. In this work, *S. passalidarum* was evaluated under different oxygen concentrations with the aim to investigate its capacity to ferment xylose under anaerobiosis and to find the best oxygen condition to optimize ethanol production. For that, fermentations were executed under k_La (oxygen transfer coefficient) values of 0.0 (anaerobiosis), 4.9, 8.0 and 15.0 h^{-1}, using bioreactor BioFlo® 115 3.0 (New Brunswick Scientific Co., Inc., Edison, NJ). Initial cell concentration was 20 g L^{-1}. The media was composed by a mixture of xylose and glucose in the concentrations of 63 and 27 g L^{-1}, respectively, and fermentations were carried out for 48 h. For all the conditions, glucose was completely consumed in the first 12 h. However, under anaerobiosis, there was a significant residual concentration of xylose (47 g L^{-1}). The higher the k_La value the faster xylose was consumed, achieving an ethanol concentration about 40 g L^{-1} at the highest k_La.

S28 Life Cycle Environmental, Economic, and Employment Evaluation of Renewable Lubricants

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Co-producing value-added bio-based products such as lubricants along with biofuels can greatly improve the economics of a biorefinery. Moreover, such products offer many environmental, economic, and social benefits, including reduced toxicity and disposal costs[1] relative to petroleum-based products. We evaluate the life cycle environmental, techno-economic, and employment prospective of using forest residues and biomass sorghum to produce premium lubricants in a 500 ton per day demonstration-scale facility. Specifically, we apply life cycle assessment (LCA) to evaluate the greenhouse gas (GHG)

emissions for lubricants co-produced with jet fuel and renewable diesel; techno economic analysis (TEA) to estimate the minimum selling price (MSP); and the jobs and economic development impact (JEDI) tool to estimate job creation for such a biorefinery. We found that lubricant produced from forest residue has the lowest GHG emissions, -1.7 kg CO2/kg lubricant, compared to lubricant produced from biomass sorghum, which were higher than petroleum based-lubricant due to the GHG-intensity of agricultural operations. Such a biorefinery can support 60 direct (on-site) jobs during its annual operation. If using forest residue as the feedstock, the biorefinery can yield 81 and 77 indirect (supply-chain related) jobs if sited in Texas and Pennsylvania, respectively. If biomass sorghum is used as the feedstock, the biorefinery could generate 190 and 181 indirect jobs in Texas and Pennsylvania, respectively, due to the higher labor requirements for feedstock cultivation. Finally, the MSP of a forest residue-based lubricant was found to be $5.5/kg ($15.73/gal), which would be competitive with petroleum-based premium lubricants sold on the market.

S29 Synthesis of lignin-based absorbance to prevent nitrate leaching in soil

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The lignin is a naturally abundant, low-cost, environmentally friendly aromatic polymer, as well as one of main by-products from the biorefinery industries. Currently, there is a lot of interest to convert lignin waste into valuable products instead of burning as a low-cost energy source such as soil amendment, plant nutrient, slow release of pesticides and etc. In this study, we attempt to develop two types of functionalized lignin products, which can effectively precipitate nitrate ion in the soil. The motivation of this work is to reduce the nitrate loss and leaching. Water-soluble nitrate ions (NO_{3-}) have been largely utilized for most species of plants and leached into nearby waterbody, which reduce the nutrient use efficiency and lead to environmental pollution problems. In this study, two types of newly synthesized lignin-based materials were compared for their retention capability to nitrate ions. The cationic lignin was synthesized through reaction of phenolic hydroxyl group with an epoxy chemical. The grafted lignins with different numbers of secondary and tertiary amines were synthesized through the Mannich reaction. The grafting reactions were confirmed by H-NMR and FTIR. The precipitation of nitrate was quantitatively measured by the UV spectra. The leaching experiment in the sandy soil has also been conducted as well. The results demonstrate that both types of grafted lignins have strong precipitation capability of nitrate ions, which could significantly reduce the nutrient loss and benefit the soil-water ecosystem.

S30 Thermal Properties of Isolated Lignin Preparations

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There is a need to develop value-added products from lignin to improve the economic feasibility of the biorefinery. One option is its use as a component of polymer systems in a variety of applications and markets. These types of materials are often thermally processed; however, information regarding chemical structure effects on key polymer parameters is very limited for isolated lignin preparations. The glass transition temperature (T_g) is one example where considerable knowledge is needed to better design crops, fractionation technologies, downstream processes, and products. In a limited study of structure-property relationships impacting utilization, yellow poplar (*Liriodendron tulipifera*) biomass was fractionated using a mixture of methyl isobutyl ketone, ethanol, and water with sulfuric acid as catalyst at 140 °C over a two-hour period. Small portions of the reaction liquor were collected by sampling every 15 minutes during the fractionation process to generate a series of lignins. The results showed that lignin purity improved from 90.3 to 94.6% and the glass transition temperature increased from 117 to 137 °C with increased fractionation time. Aliphatic hydroxyl groups decreased and phenolic hydroxyl functionality increased with reaction time, leading to a more condensed lignin structure and increased polydispersity at times greater than 90 min. Overall, this study demonstrates that thermal and chemical properties of lignin change with the organosolv fractionation time. The relationships between structural features and important thermal properties, particularly T_g, will be discussed in this presentation.

S31 Antimicrobial activity of parietal hemicelluloses of *Retama raetam*Â roots

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The present work aims to evaluate the antibacterial effect of cell wall hemicellulose one of the fibers of a medicinal plant *Retama raetam.* The antibacterial activties of hemicellulose were tested using a solid medium technique. Hemicellulose of brut fraction (brut wall) showed a very important inhibitory effect against six bacterial strains tested with maximal inhibition zone of 19 mm for *Escherichia coli* (ATCC25922) *and Staphylococcus aureus* (ATCC25923)*; but no activity against Klebsiella pneumonia (ATCC 4352).* hemicellulose of brut fraction (lignified fraction) reveals an important antibacterial effect that hemicelluloses of delignified fraction*.* The minimum inhibitory concentration (MIC) allowed us to classify bacterial strains tested of such a sensitive strains *Staphylococcus aureus and Escherichia coli with 8.33, 11.33 mg/ml respectively as for Bacillus cereus is resistant strain with MIC 25 mg/ml. this is a first report on antibacterial activity of hemicellulose cell wall extracted from roots Retama raetam*. However no antifungal activity was observed against *Candida albican.*

S32 Metabolic design for production of various metal-binding porphyrin derivatives in engineered *Corynebacterium glutamicum*

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A porphyrin derivative is an important organic compound in a variety of fields of study such as biochemistry, solar cell and biomedical, due to the ability to be used as an essential cofactor in organism and absorb light to generate the electric energy. Most of porphyrin derivatives used in applications are commonly dependent on the synthesis by chemical reactions or the extraction in animal blood. In this study, *Corynebacterium glutamicum* was engineered to biologically synthesize the porphyrin derivative. The first rate determining step in the porphyrin synthesis pathway was activated by the overexpression of genes related to the precursor synthesis. Under the control of the gene expression by different promoters, the precursor production was enhanced in the engineered strain BEPP02 harboring the expression vector containing the strong promoter, compared to the engineered strain BEPP01. For the enhanced production of metal-free porphyrin building block, the porphyrin synthesis pathway was regulated by the transcriptional regulator-mediated reaction. Metabolically engineered strain, called the engineered strain BEPP03, successfully showed the increased metal-free porphyrin production, but wild-type strain didn't produce in the flask cultivation. And then, the final engineered strain BEPP04 harboring the medium-copy-number recombinant plasmid, showed the highest porphyrin production among the engineered strains. In the cultivation condition adding different metal ions, each metal ion-bound porphyrin derivative was successfully biosynthesized in the engineered strain BEPP04. Thus, the engineered *C. glutamicum* can be used as bio-based producer of the porphyrin derivative.

S33 Using starch-rich byproducts from a sweet sorghum biorefinery for butanol production

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Butanol, together with acetone and ethanol, can be produced by bacteria in an anaerobic fermentation. Many of these organisms are also capable of using starch as a carbons source. Therefore, we collected starch-rich sludge from the clarification of sweet sorghum juice and investigated if this material could be used as starting material for butanol production. The results show that it was possible to produce the solvents at titers similar to those obtained when dextrose was used as a substrate.

S34 Lignin-based adsorbents for wastewater treatment

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Various traditional and emerging technical lignin (i.e., alkaline lignin, kraft lignin, organosolv lignin, lignin recovered from deep eutectic solvent (DES) pretreatment) was test for its suitability for hydrogel fabrication. Such lignin-based hydrogel was shown to be an effective adsorbent for the treatment of dye-containing wastewater. However, the performance varied among types of lignin. The test using a model dye methylene blue showed nearly 100% dye removal by lignin-based hydrogel. The properties of lignin-based adsorbents were also characterized using a series of techniques. Our study proved that lignin could be a good building block for the fabrication of gel adsorbents toward industrial wastewater treatment.

S35 Enhancement of cellulose accessibility by the combined process of hemicellulose removal and delignification

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Lignocellulosic biomass based biorefinery, so-called 'intergrated biorefinery', has attracted a lot of interests because it has high potential of economic value as a process to produce useful chemicals for various fields of industry. Among the useful products, glucose, which is a precursor of organic acids and furan derivative, has been known as a key-intermediate for biorefinery. However, there is a difficulty to apply the ligncellulosic biomass based biorefinery due to its recalcitrance, and it is mainly caused by complex structure of biomass components in the cell wall, e.g. cellulose, hemicellulose, and lignin. Therefore, the recalcitrant structure needs to be broken down to improve the competitiveness of the biorefinery.

In this study, liquid hot water pretreatment was conducted for speration of hemicellulose to create additional economic value. After liquid hot water pretreatment, delignification processes were performed for investigating to increase cellulose accessibility in the mild reaction temperature. Using the solid fraction obtained after delignification process, enzymatic hydrolysis was conducted to convert cellulose into glucose. And then, the improvement of the glucose yield was interpreted in terms of cell wall morphology.

S36 Commercially feasible fermentation of gaseous feedstocks using bacterial strains developed at elevated pressure

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The commercial feasibility of many bio-processes can depend on how fast gas transfer takes place. This is especially true if gas solubility is poor, for example when working with gases such as hydrogen and methane in the context of gas fermentation for the production of fuels and chemicals from waste gas. The engineering solution to poor gas transfer is limited to kLa increase through changes in sparger and stirring arrangement. This offers very limited scope for improvement and therefore many

potentially interesting processes can be rendered uneconomic. A much more effective alternative is to operate the bio-reactor at elevated pressure as this can in principle increase gas transfer rate several-fold without any changes to sparging or agitation.

This presentation will discuss data from a mini-bioreactor platform used to screen and then develop bacterial strains at elevated pressure, allowing process economics to be directly improved through higher production rate and better yield. Substantial increases is solubility and mass flux will be demonstrated while at the same time achieving fine control of dissolved oxygen profile to suit different bacterial strains. Through operating at under 100ml in each bio reactor, successful scale up to multi-litre volume will also be demonstrated to show that scale-up from laboratory scale is possible and reliable.

S37 The use of radicals' formation in hydrogen peroxide activated persulfate for biomass pretreatment and process optimization

M. Davaritouchaee, Washington state university, Pullman, WA, USA; M. Gagaa and S. Chen, Washington State University, Pullman, WA, USA*

Currently, much effort is directed at moving away from a fossil fuel based economy towards renewable feedstocks and biorefineries development. The major challenge for refining the complex chemical structure of biomass is lignin separation from cellulose and hemicellulose. Wood-eating termites provide an excellent model for the degradation of biomass. Recent studies of the termite's digestive system suggest the presence of radical species and hydroxyl radical intermediates as the main components in this process. In the present investigation, persulfate oxidizing system was selected to generate the important radicals including hydroxyl radical, sulfate radical, and superoxide radical anion. Sodium persulfate activated by hydrogen peroxide (SPHP) system was studied to pretreat wheat straw. The production of hydroxyl radical, sulfate radical, and superoxide radical anion were evaluated using nitrobenzene, hexachloroethane, and anisole respectively, as reaction specific probe compounds. Statistical experimental design was used to optimize pretreatment variables affecting the yield of sugar such as persulfate concentration, activator concentration, and reaction time by using response surface methodology (RSM) based on central composite design (CCD). Enzymatic hydrolysis and compositional analysis were performed to quantify biomass content changes, lignin removal, and glucan yield. Fourier transmission infrared spectroscopic and scanning electron microscopy were used to study the pretreatment effect on biomass structure. The results indicated the maximum sugar yield of 41.7% (w/w) was obtained in SPHP using 0.425 M $Na_2S_2O_8$, 2 M H_2O_2 for 180 minutes, while untreated material showed only 3.7 % (w/w) glucan yield. The radical analysis confirmed the production and existence of radicals.

S38 Optimization of wheat straw oxidation pretreatment using response surface methodology

M. Davaritouchaee, Washington state university, Pullman, WA, USA; M. Gagaa and S. Chen, Washington State University, Pullman, WA, USA*

Persulfate system as a new stable oxidation system generated various and powerful radicals that cause lignin modification and degradation. In this study response surface analysis was used to estimate the best combination of parameters in persulfate oxidation process for better glucan yield. Design Expert 7 was applied to investigate the effect of three parameters in five levels and conduct regression and graphical analysis of the data. The highest sugar yield of 49.4% (w/w) was obtained using 0.3 M $Na_2S_2O_8$, at 100 C^0 for 130 minutes while untreated material showed only 3.7 % (w/w) glucan yield. For optimum condition, chemical probes degradation illustrated that persulfate under the heat generated a higher amount of sulfate radical (0.4 mM) and hydroxyl radical (0.19 mM) which attacked the biomass structure and removed a portion of lignin and hemicellulose. Compositional analysis and FTIR study revealed oxidative pretreatment degraded lignin and part of hemicellulose. SEM showed an increase in porosity of the biomass structure. The glucan yield showed a quadratic model response in this pretreatment method and the interaction of process variables on hydrolysis yield were analyzed by 3D contour plots.

S39 Development of an integrated process for biogas production from sugarcane trash

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The use of fossil fuels in sugarcane production, transport and milling is a major cost burden to Australian sugarcane industry. Meanwhile, the sugarcane industry produces large amounts of fibre residues like sugarcane trash and bagasse. Production of biofuels from sugarcane residues and integration of biofuels in the industry has the potential to reduce the industry's fossil fuel cost as well as greenhouse emission. This project aims to develop an integrated process for biogas production from sugarcane trash with maximisation of the trash value as fuel source and reduction of the waste quantity. This poster presents the latest progress of this biogas production project. Firstly, low-cost biomass pretreatment technologies were screened and compared in terms of biomass digestibility, levels of inhibitor and biomethane potential. Secondly, anaerobic digestion of trash pretreated with the selected methods was conducted to produce biogas. Thirdly, the digestates from the anaerobic digestion processes were collected and liquefied for the production of biocrude, which can be further upgraded to transportation fuel. In addition, the effects of pretreatment on the production of both biogas and biocrude were preliminary investigated. Finally, a preliminary techno-economic assessment was conducted to identify the key factors limiting the process economics. Further research activities towards commercial application of this integrated biogas production process are discussed in this poster.

S40 Effective pretreatment of corn stover using extremely low-liquid ammonia (ELLA) method for improved enzymatic saccharification

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Corn stover is a low-cost lignocellulosic material, which can be used for biological conversion to biofuel and chemicals. Pretreatment method using aqueous ammonia was investigated to enhance the enzymatic saccharification of corn stover for production of fermentable sugar. A low-liquid pretreatment method of corn stover using aqueous ammonia, designated the extremely low-liquid ammonia (ELLA) method, was developed, which was intended to reduce the water and chemical consumptions for effective and environmentally-friendly pretreatment.

In this method, corn stover is mixed with aqueous ammonia solution in the form of mist (ammoniation step), then followed by pretreatment at the elevated temperature (90~150°C) for an extended time (24~120 h) with various solid/liquid (S/L) ratio in the pretreatment step. After pretreatment step, the excess ammonia is removed by evaporation, which can be consecutively saccharified by enzyme without washing step.
In our paper, various processing factors including chemical and water loadings, pretreatment temperature, pretreatment time which determine the effectiveness of pretreatment were evaluated by the compositional analysis and enzyme digestibility test.

S41 Effects of preheating on briquetting and subsequent hydrothermal pretreatment for enzymatic hydrolysis of wheat straw

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Densification of biomass enables more efficient logistics in a biobased society. When pretreating densified material prior to enzymatic conversion showing positive, neutral, and negative effects of densification have been reported without a clear understanding of the underlying reasons. This study investigates the effects of preheating prior to briquetting of wheat straw on subsequent hydrothermal pretreatment for enzymatic conversion to fermentable sugars. Wheat straw was densified to make briquettes applying; no preheating, preheating at 75°C and 125°C for 5 or 10 min, respectively. Subsequent hydrothermal pretreatment was done for un-briquetted wheat straw and wheat straw briquettes. Enzymatic hydrolysis was performed for all unpretreated and pretreated samples. The results indicated that briquetting itself had no direct effect on enzymatic sugar release from wheat straw (without pretreatment), while preheating at 125 °C for 5 or 10 min before briquetting promoted sugar yield. When combined with pretreatment, briquetting showed neutral effect (no preheating or preheating at 75°C for 5 min), or slight negative effects (preheating at 125°C for 5 min), or significant negative effects (preheating at 75, 125°C for 10 min) on sugar yield. Preheating for 10 min at 75 and 125°C before briquetting may result in irreversible hornification-like effects, which hindered the removing of hemicellulose during pretreatment, and decreased the accessibility of cellulose in enzymatic hydrolysis. Hydrothermal pretreatment played a more important role than briquetting in the structure modification and sugar release of biomass. The hornification-like effects played the main negative role in hydrothermal pretreatment and sugar conversion of biomass.

S42 Hydrothermal pretreatment of herbaceous energy crops to enhance the sugar release

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Biomass recalcitrance to sugar release is a major limitation for cost-effective industrial conversion of lignocellulosic biomass to biofuels. Fourteen herbaceous energy crops were selected to investigate their recalcitrance to cellulosic enzyme and hydrothermal conditions. The results about enzymatic hydrolysis of energy sorghum and Pennisetum hybrids indicated that both hemicellulose and lignin content had a negative influence on enzymatic digestibility (ED), especially for the latter. Moreover, we found a negative correlation between the lignin level and the total xylose yield from energy sorghum. It was indicated that the ratio of syringyl and guaiacyl units of lignin played an important role on the hemicellulose hydrolysis in hydrothermal (liquid hot water) pretreatment than branch degree, but latter contributed more on the characterization of xylooligomers degree of polymerization. The analysis of multi-scale changes of substrate suggested that cellulose crystallinity index and degree of polymerization seemed no direct relationships for increase of enzymatic digestibility. While lignin barrier was the main factor limiting efficiency of sugar release, and Pennisetum hybrid with low lignin content and high sugar recovery was proved to be a prospective plant feedstock for cellulosic ethanol production.

S43 Steam explosion pretreatment for the efficient conversion of animal bedding into ethanol

M. Sanchis-Sebastiá, B. Erdei, M. Galbe and O. Wallberg, Lund University, Lund, Sweden*

Feedstock is one of the biggest contributions to production costs in 2nd generation bioethanol processes and therefore the use of low-grade biomass could be beneficial for the economic viability of these processes. This work looks particularly into ethanol production from animal bedding, a mixture of straw and manure common in some areas of Denmark.

Animal bedding was washed with water and subsequently pretreated through dilute-acid steam pretreatment. A design of experiments was used to determine the operating conditions (temperature, residence time and pH) in the pretreatment reactor. Fermentability of the pretreated slurry in an SSF configuration was selected to evaluate the pretreatment.
The result of the design of experiments is an empirical model that quantifies the influence of the operating conditions in the pretreatment reactor on the overall ethanol yield of the process. Additionally, preliminary results show that steam explosion pretreatment allows obtaining high ethanol yields, proving the relevance of this feedstock for the biofuel industry.

S44 Application of mass transfer modeling to improve predictability of scale-down industrial fermentation processes

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Oxygen mass transfer is one of the primary constraints in scaling up aerobic fermentation processes. New fermentation conditions or strains that demonstrate elevated respiratory demands or broth viscosities often lead to oxygen limitations, thereby preventing implementation at large scale. Understanding which conditions will ultimately lead to oxygen limitation at industrial scale is of critical importance for R&D programs that rely on scale-down models for process development. This presentation will describe a novel approach for utilizing volumetric oxygen mass transfer coefficient (k_La) modeling to emulate industrial scale conditions in real-time. The construction of the k_La model will be reviewed and its value illustrated through a case study on a new strain for secondary metabolite production.

S45 Recovery of fermentable sugar from corn stover using hot-water and calcium chloride treatment

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Corn stover, renewable source of fermentable sugar production, is one of the most abundant lignocellulosic feedstock in the world.

In our study, corn stover was treated by the two-stage fractionation process for consecutive recovery of free sugar and hemicellulose. This fractionation process consists of two stages; for example, in the first stage, hot-water at moderate temperature was used for effective recovery of extractive including nonstructural sugar, which was followed by the fractionation using $CaCl_2$ at higher temperature. The first stage treatment using hot-water can recover considerable amount of the sucrose (nonstructural sugar), which produces the sugar and extractives-rich liquid stream. Then, in the second stage of treatment, $CaCl_2$ treatment hydrolyzes the hemicellulose, which produces another liquid stream with xylooligomer. The optimum conditions for the recovery of xylan were established by the response surface methodology (RSM) based on the central composite design (CCD) method in the secondary treatment. The enzyme digestibility was measured and the feasibility of $CaCl_2$ as a pretreatment catalyst will be presented.

S46 2-Naphthol as a carbocation scavenger in the pretreatment of lignocellulosic biomass

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Steam-explosion pretreatment for the biochemical conversion of biomass is currently the most common pretreatment method in commercial biorefineries (S. Brethauer and M. Studer, *Chimia*, 2015, 69) and has been examined in many studies. Due to the higher recalcitrance compared to hardwoods, hydrothermal/autohydrolysis pretreatments work poorly for softwoods (M. Galbe and G. Zacchi, *Appl Microbiol Biotechnol.*, 2002, 59, 618-628) .

In previous works we could show that lignin repolymerization reactions during the pretreatment have a negative influence on enzymatic hydrolysis. This is due to unproductive binding of enzymes on these structures (Pielhop et al., *Green Chem.,* 2015, **17**, 3521). Adding 2-naphthol as a carbocation scavenger can increase the enzymatic cellulose digestibility by up to 179% (Pielhop et al., *Biotechnol Biofuels*, 2017, 10:130). Further effects on the subsequent fermentation are not examined yet. Based on studies with Avicel and 2-naphthol we think that it might act as an inhibitor resulting in a reduced ethanol yield.
Furthermore, 2-naphthol has a very low influence on the enzymatic cellulose digestibility from other biomass types. Since we could also show a strong positive effect of 2-naphthol in the dilute acid pretreatment of spruce wood, it might also improve the enzymatic cellulose digestibility of other biomass types like beech or corn stover in dilute acid pretreatments.
Current research focuses on the effect of 2-naphthol on the fermentation of hydrothermal pretreated spruce wood and on the effect of 2-naphthol in dilute acid pretreatments of corn stover and beech wood. Results from these studies will be presented.

S47 Techno-economic and environmental analysis of fuel bioethanol production from liquid hot water pretreated switchgrass: Effect of total solids loading in enzymatic hydrolysis

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Environmental and economic metrics for the production of ethanol and energy on a facility that processes 250 dry t/day of

switchgrass were evaluated through life cycle assessment (LCA) and techno-economic analysis (TEA). The stages considered were: feedstock production and supply, pretreatment (LHW, liquid hot water), enzymatic cellulose hydrolysis, fermentation, purification, co-product generation, utilities and wastewater treatment. A process model in AspenPlus®, based on the NREL model for bioethanol from corn stover, was developed applying experimental and public data. SimaPro was used as tool for LCA. The use of high solids content could lead to high ethanol concentrations and reduce energy consumption of the distillation process, but can also inhibit hydrolysis and fermentation. Sensitivity analysis were performed to estimate the effect of solids concentration in bioreactors on minimum selling price, life cycle greenhouse gas (GHG) emissions and non-renewable energy. Solids content was varied between 12.5-22.5%, which led to a descent on minimum selling price from 1.07 to 0.67 US dollars per liter, values consistent with those obtained currently for advanced fuel alcohols. Net GHG emissions were also reduced from 60 g CO_2 eq/L to -3 g CO_2 eq/L and the energy return on investment (EROI, ethanol+electricity/fossil energy consumed) from 14.5 to 8.9 MJ/MJ respectively. The environmental parameters showed low values due to minor requirements of soil nutrients in Uruguay and chemicals for LHW pretreatment, respectively. Energy consumed and generated for each solids concentration was the main parameter affecting the GHG emissions and EROI values.

S48 The effect of multiple hydrotropic treatments on the delignification of hardwood

J. Olsson, M. Galbe, K. Kovacs and O. Wallberg, Lund University, Lund, Sweden*

Hydrotropic extraction (HEX) is a method for the delignification of lignocellulose that has been given increasing amounts of attention during the last couple of years. A hydrotrope is an amphiphilic substance, which, in high enough concentrations, can improve solubility of otherwise poorly soluble substances in water. The most commonly used substance for HEX-delignification is sodium xylenesulfonate (SXS), a compound commonly found in personal-care products such as shampoos and cosmetics. Above a certain concentration the substance will create a hydrophobic local environment where the lignin molecule can be solubilized. By adding water, and lowering the concentration of SXS, the lignin will precipitate and can then be filtered off. One advantage of using SXS for delignification is that the activity of the hydrotropic solution increases with the number of cycles (to a certain limit) using the same liquid and that it can be reused up to six times without losing efficiency.

A hardwood mixture consisting of 80% birch and 20% beech was submitted to pretreatment with steam explosion as a first stage in order to recover the hemicelluloses i.e. xylan. Results have shown that approximately 80% of the xylan can be recovered in the liquid phase during this treatment. The remaining solid phase was then subjected to the HEX treatment using SXS as hydrotrope. In this study results of repetitive extractions where the hydrotrope was reused will be presented.

S49 Hydrothermal pretreatment of sugarcane bagasse with and without sulfuric acid -New insights on effects of pretreatment severity

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Sugarcane bagasse has great potential for production of bio-based commodities, such as cellulosic ethanol. Pretreatment is essential for efficient processing of lignocellulosic feedstocks by biochemical conversion. Pretreatment of sugarcane bagasse was investigated using dilute-sulfuric acid (DAP) and uncatalysed hydrothermal pretreatment (HTP). The temperature (175-205°C) and the time (4-51 min) were varied in such a way that allowed holding constant the severity factor for a set of pretreatment runs. The concentrations of sugars and by-products were determined, and the effects of different pretreatment conditions on the susceptibility of pretreated bagasse to enzymatic saccharification were investigated. Glucose was the main sugar in DAP liquors, while xylose was predominant in HTP liquors. Glucose concentration always increased proportionally with temperature. Xylose concentration increased with temperature for HTP, but decreased for DAP. DAP and HTP showed different trends with respect to the effect of severity on furfural concentration. However, for both DAP and HTP the concentration of furan aldehydes was directly proportional to the temperature in experiments with similar severity. The enzymatic digestibility of pretreated solids was directly proportional to the severity for HTP, but it was inversely proportional to the severity for DAP. For HTP, increasing temperature enhanced the enzymatic digestibility, but for DAP it resulted in poorer digestibility. The inhibition of cellulases by the pretreatment liquid increased with the severity, and was more significant for DAP than for HTP. The investigation gives better understanding of fundamental aspects of hydrothermal pretreatment of sugarcane bagasse, and provides guidance for the development of efficient industrial processes.

S50 Effect of integrated 1st and 2nd generation bioethanol production on DDGS

M. Persson, B. Erdei, K. Kovacs, M. Galbe and O. Wallberg, Lund University, Lund, Sweden*

Integration of 1st and 2nd generation bioethanol production has been suggested as a way to facilitate the commercialization of 2nd generation ethanol production. It has been shown that integrating these processes can potentially confer various benefits to the overall process such as decreasing the need for external energy inputs and dilution of the inhibitor concentration in the fermentation. To maximize the benefits of shared equipment the mass flows from the respective processes should be mixed before fermentation. Integrating the processes at this stage may result in components from the 2nd generation process being mixed in with the residues from the 1st generation process that are traditionally sold as animal feed. This could potentially have a negative impact on the value of the animal feed product since the 2nd generation material contains components with no nutritional value, such as lignin and byproducts from pretreatment that might have adverse effects in high concentrations.

In this study different integration strategies have been designed and will be evaluated with respect to the ethanol production as well as effect on the composition and nutritional value of the resulting residues intended to be used as animal feed. Varying ratios of 1st and 2nd generation materials are going to be assessed through simultaneous saccharification and fermentation. The results from this on-going study will be presented.

S51 Redox potential control in anaerobic *Clostridium beijerinckii*Â fermentation using single-use bioreactors

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Redox potential is an important physiochemical factor which measures the tendency of the medium to acquire electrons. In *Clostridium beijerinckii* fermentation, redox potential indicates the status of the $NAD(P)^+$ pool regeneration which directs the electron flow leading to solvent production including butanol. In this study, anaerobic *C. beijerinckii* (ATCC 6014) fermentation was conducted in the Eppendorf BioBLU® 3f Single-Use Vessel controlled by the BioFlo® 120 bioprocess control station. The parameters being monitored throughout the fermentation were redox potential and pH using the ISM® redox/pH sensors. The objectives of this study are (1) to investigate the effects of redox control on the growth and butanol production of *C. beijerinckii*; and (2) to validate the feasibility of using the BioFlo 120 and BioBLU 3f Single-Use Vessel for anaerobic fermentation applications. When *C. beijerinckii* was grown without redox control, a continuous and tremendous change of redox potential between -600 and 0 mV was observed in the broth. When fermentation ended at 124 h, OD_{600} was 0.8, glucose consumption was 33% and butanol production was limited. When redox potential was controlled at -500 mV by redox sensor guided addition of $Na_2S{\cdot}9H_2O$ solution, OD_{600} was 1.6, glucose consumption was 51%, and butanol production showed a 2-fold increase. In summary, with the combination of ISM Redox sensors and BioBLU Single-Use Vessels, the high variability of redox potential during *C. beijerinckii* fermentation can be actively controlled to increase biomass growth and solvent production.

S52 Kinetic modeling of the cell treatment stage in fed-batch ethanol production with cell recycling

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The Brazilian ethanol industry mostly uses fed-batch fermentation with cell recycling; 70- 80% of distilleries utilize this technology to produce ethanol. In this configuration, 99.5% of the cells are reused in sequential fermentations. The high cell density in the bioreactors contributes to reducing the fermentation time to 6-11 h and consequently increasing the ethanol productivity. The ethanol titer varies between 8 and 11 °GL. One method to improve the current ethanol fermentation profitability is through process intensification. Very-high-gravity (VHG) technology is one type of improvement based on the process optimization aimed at doubling current productivity while maintaining a high ethanol titer. In a recent study is determined that the cell reactivation composed by an acid treatment followed by cell reactivation is a fundamental step in VHG fermentation. Understanding the kinetic mechanisms and developing mathematical models of the ethanol fermentation process are important aspects to improve the implementation of adequate operational strategies for optimization and control to achieve high operational performance. This work aimed to study the kinetic behavior of cell reactivation in ethanol fermentation. A mechanistic model is proposed to predict the kinetic rates of the concentrations of cell, substrate and ethanol. Experimental data from several cycles of ethanol fermentation from sugarcane juice and molasses are used to calibrate the model. An advanced parameter estimation procedure is used to guarantee the accuracy of the proposed model. The model predictions are particularly precise, as determined by the residual standard deviation.

S53 Mechanical refining of lignocellulosic biomass for biosugar production

D. Corbett, R. Venditti, H. Jameel and S. Park, North Carolina State University, Raleigh, NC, USA*

Mechanical refining of lignocellulosic biomass has emerged as a promising industrial technology for the improvement of enzymatic hydrolysis yields during biosugar production. Mechanical refining is known to result in fiber deconstruction and modification that enhances enzyme accessibility to carbohydrates. However, further understanding of the morphological changes occurring to biomass during mechanical refining and the impacts of these changes on enzymatic digestibility is necessary to maximize yields and reduce energy consumption. In this work, the mechanical refining process was investigated with a focus on the impact of refining conditions (gap-width and consistency) on fiber morphology and resulting enzymatic digestibility. Mildly pretreated mixed southern hardwood pulp was disk refined for two passes at various intensities (specific edge load). Pulps were subjected to various analyses including enzymatic hydrolysis, water retention value, fiber quality analysis, and microscopy. Refined and unrefined fibers were further classified based on particle size using a Bauer-McNett fiber classifier. Fiber fractions were analyzed for the presence of fibrillation/delamination structures and were subjected to enzymatic hydrolysis. Results showed that enzymatic carbohydrate conversion is a function of specific refining energy independent of refining intensity. At lower enzyme loadings, gap-width adjustments were observed to be most effective at improving enzymatic digestibility. After classification, refined fibers showed clear signs of mechanical deconstruction leading to enhanced enzymatic digestibility.

S54 Liquid- liquid extraction as an alternative detoxification step of hemicellulosic hydrolysate from

sugarcane bagasse for 2G ethanol production

L. Roque, V. Nascimento, R. Boni, J. Ienczak and S.C. Rabelo, Brazilian Bioethanol Science and Technology Laboratory (CTBE), Campinas, Brazil*

Lignocellulosic biomass is a renewable source of energy production on a scale sufficient to play a significant role in developing vital programs for renewable energy. Among the lignocellulosic biomasses, bagasse and straw from sugarcane in Brazil stand out as being economically viable for the production of "environmentally friendly" fuel.

In the process of second generation ethanol, a pretreatment step is required, mainly to reduce the recalcitrance of the material and maximizing the subsequent hydrolysis and fermentation of sugars. During this step, in addition to the sugars, aliphatic acids (acetic, formic and levulinic acid), furan derivatives (furfural and 5-hydroxymethylfurfural) and phenolic compounds are formed and represents inhibitions factors for subsequent bioconversion of the solubilized sugars into ethanol.

An alternative to minimize inhibitory effects of the fermentation medium is the removal of these constituents prior to fermentation. Thus, the aim of this work was studied the removal of the inhibitors by liquid-liquid extraction and the evaluation of the process proposed through the results obtained after the fermentation step by *Sheffersomyces stipitis* NRRL Y-7124 yeast.

The commercially known solvents such as ethyl acetate, n-butyl acetate, n-heptyl acetate and isobutyl acetate were evaluated in terms liquid-liquid extraction, distribution coefficients, environment concerns and a simulation with the most used thermodynamic models NRTL and UNIQUAC, and group contribution method, UNIFAC were employed.

In summary, the use of solvents in liquid-liquid extraction showed that it is possible to reduce inhibitors effect in the hemicellulose hydrolysate to maximize the fermentation of the sugars to produce ethanol 2G.

S55 Design and Construction of a Microfluidic Reactor for Real-Time Imaging of Pretreatment and Enzymatic Hydrolysis

R. Ong and M. Chandrasekar, Michigan Technological University, Houghton, MI, USA*

Lignocellulosic biomass has complex cell wall chemistries and structures that differ based on the species, tissue and cell types. A greater understanding of the influence of tissue and cell types on biomass deconstruction is needed, which is best facilitated through imaging. In particular, real-time imaging of biomass deconstruction (pretreatment and enzymatic hydrolysis) can be extremely useful to understand the fundamental characteristics of the feedstock that hinders its deconstruction. However, this can be challenging given the fragility of plant materials and the time-consuming nature of the experiments. In order to address these issues and increase the potential throughput, we designed a PDMS microfluidic, imaging reactor. The reactor consists of six chambers (20 mm diameter each) on a 75 x 50 mm microscope cover slide, with ports for reagent addition and sampling. A prototype was constructed using standard photolithographic techniques by casting PDMS on an SU-8 mold. We 3D printed a custom-designed holder for the microfluidic reactor and a frame that can accommodate up to six microfluidic reactors at a time to be anchored to a shaking incubator, to facilitate both temperature control and mixing requirements. To evaluate the feasibility of the system, we performed separate acid and alkali pretreatment on the corn stover stems sectioned using a cryomicrotome, followed by enzymatic hydrolysis in the microfluidic reactor. To test the real-time imaging performance, we localized hemicellulose before and after pretreatment and enzymatic hydrolysis using specific antibodies and carried out fluorescence microscopy. Sugar yields from the samples were evaluated using ion chromatography.

S56 Evaluating the impact of a high-temperature alkaline pre-extraction step on copper-catalyzed pretreatment of hybrid poplar

A. Bhalla, P. Fasahati, N. Bansal, R. Semaan, T. Phongpreecha, C. Saffron, D. Hodge and E. Hegg, Michigan State University, East Lansing, MI, USA*

Biomass pretreatment is an essential step that enables enzymatic hydrolysis of lignocellulosic biomass through breaking down its recalcitrant structure. Maximizing the sugar yields, however, can cause the process to become cost-prohibitive. Previously we demonstrated that addition of copper coordinated by 2,2´-bipyridine during alkaline hydrogen peroxide (Cu-AHP) pretreatment results in a substantial improvement in sugar yields following enzymatic hydrolysis compared to AHP-only pretreatment. Moreover, the sugar yields could be significantly improved by ~20% by incorporating an alkaline pre-extraction step prior to Cu-AHP pretreatment. However, the process still utilized prohibitively high chemical inputs (catalyst loadings, H_2O_2, and enzyme dosing). To decrease chemical inputs and residence time, we investigated the effects of changing the severity of the alkali pre-extraction step by (1) increasing the pre-extraction temperature and (2) using either water or ethanol solvents. Alkaline aqueous pre-extraction resulted in slightly better results than ethanol in all criteria, resulting a reduction of H_2O_2, enzymes, and bi-pyridine catalyst loadings by 40%, 33%, and 55%, respectively, while still maintaining >90% glucose and xylose yields. Using techno-economic analysis (TEA) coupled with the experimental results, we identified scenarios that resulted in significant reduction in capital and raw material costs. For the identified best-case scenario, the modification of the alkali pre-extraction step would reduce the capital and raw material costs of the pretreatment process by 21% and 35%, respectively, relative to the benchmark base case.

S57 A study on the effect of cotreatment on anaerobic digestion process and microbiome development

A. Bharadwaj[*] and T.L. Richard, The Pennsylvania State University, University Park, PA, USA; J.M. Whitham, Oak Ridge National Laboratory, Oak Ridge, TN, USA; E. Holwerda and L.R. Lynd, Dartmouth College, Hanover, NH, USA
Mechanical disruption of lignocellulosic biomass during fermentation, or cotreatment, has recently been introduced as an alternative to thermochemical pretreatment for enhancing biologically-mediated solubilization of cellulosic biomass. Cotreatment has been shown to be effective in increasing biomass digestibility in pure culture fermentation systems using *Clostridium thermocellum* (Paye *et al*., 2016, Balch *et al*., 2017). Here we explore the application of cotreatment to a mixed, anaerobic methanogenic cultures. Replicate anaerobic digesters were operated in semi-continuous mode with senescent switchgrass as the feedstock. Once the reactors reached steady-state, partially digested material from these reactors was extracted and subjected to cotreatment using a ball mill. Following milling, another round of fermentation was performed on the partially digested and milled material. Biomass solubilization was determined by measuring methane production rates and consumption of sugars. The microbiome composition was followed via 16s RNA analysis during establishment of the culture, at steady-state prior to milling, and after milling. Reported results include the extent to which cotreatment enhances methane production in mixed culture systems, the rate of solubilization of biomass, the effect of milling technologies on the anaerobic microbiome populations.

S58 Integrated Wastewater Treatment in Lignocellulosic Biorefineries

T. Tobin, R. Gustafson[*], R. Bura and H. Gough, University of Washington, Seattle, WA, USA
Production and use of bio-based products offer a number of advantages over conventional petrochemicals, yet the relatively high cost of production has restricted their mainstream adoption. Optimization of waste treatment processes could reduce capital expenditures, lowering the barrier to market entry for lignocellulosic biorefineries. This paper characterizes waste production from lignocellulosic ethanol production and analyzes potential wastewater treatment operations. It is found that organic material is intrinsic to bioconversion wastes, supplying up to 260 kilograms of biological oxygen demand per tonne of feedstock. Inorganic material, however, is largely added to waste streams throughout the bioconversion process as a result of pretreatment and pH adjustment operations which increase the inorganic loading by 44 kilograms per tonne of feedstock. Adjusting unit operations to limit addition of inorganic material can reduce the demands and therefore cost of waste treatment. Various waste treatment technologies – including those that take advantage of ecosystem services provided by feedstock production – are evaluated in terms of capital and operating costs, as well as technical feasibility. It is concluded that waste treatment technologies may be better integrated with conversion processes and even feedstock production. In general there should be an effort to recycle resources throughout the bioenergy supply chain through application of ecosystem services provided by adjacent feedstock plantations and recovery of resources from the waste stream to reduce overall capital and operating costs of bioconversion facilities.

S59 Techno-economic evaluation for the process optimization of galactoglucomannan and lignin recovery by ultrafiltration

B. Al-Rudainy[*], M. Galbe and O. Wallberg, Lund University, Lund, Sweden
Galactoglucomannans (GGM) can be used as a precursor for the production of surfactants, plastics, hydrogels etc. However, separation of galactoglucomannan (GGM) from lignin and lignin-carbohydrate-complexes using membrane filtration is difficult. Precipitation of GGM with anti-solvents is an approach that has been previously studied. A membrane filtration step prior to precipitation is economically beneficial to decrease anti-solvent requirements with increasing concentration of GGM. However, previous studies have shown that membrane fouling is a problem that can have a large impact on the life-time of the membranes but also the overall yield of the products.

The raw material used in this study was a sodium-based spent-sulfite-liquor (SSL) provided by Domsjö Fabriker (Örnsköldsvik, Sweden) and is the outtake after the first pulping step of softwood (60 % *Picea abies* and 40 % *Pinus sylvestris*). The SSL was concentrated with a 50 kDa polysulfone membrane (hydrophobic) and four regenerative cellulose membranes (30, 20, 10 and 5 kDa) (hydrophilic) to a volume reduction of 90 %. The resulting retentate was analyzed for the composition and the product yields were calculated. Membrane data, such as, flux, trans-membrane pressure, cross-flow velocity and degree of fouling were used together with the GGM yields in a techno-economic evaluation to find a cost-efficient process for the separation and purification of GGM and lignin from SSL.

S60 Impact of alkali pretreatment and torrefaction on glucose production from wheat straw

B. Memis[*], Pennsylvania State University, University Park, PA, USA and D. Ciolkosz, The Pennsylvania State University, University Park, PA, USA
Producing biofuel from lignocellulosic feedstock is a multi-step process which includes pretreatment, and hydrolysis to glucose, followed by fermentation and distillation. Wheat straw is an abundant agricultural residue that can be used as a lignocellulosic feedstock. This study investigated two pretreatment methods on the glucose yield for producing bioethanol from wheat straw: torrefaction, alkaline pretreatment, and their combination. To determine the individual and combined effect of these pretreatments on the glucose yield the ground wheat straw samples are torrefied and/or exposed to sodium hydroxide solution, followed by enzymatic hydrolysis, as per the NREL protocol. Results indicate that glucose yield from mildly torrefied biomass are comparable to those from raw feedstock, but severe torrefaction dramatically reduced glucose yield.

S61 Pulping processes as pretreatment methods: Alkali-oxygen impregnation and mechanical refining of corn stover

F. Nielsen, J. Wu and R.P. Chandra, University of British Columbia, Vancouver, BC, Canada; J. Saddler, The University of British Columbia, Vancouver, BC, Canada*

A decreasing demand for newsprint has made considerable mechanical pulping capacity redundant and has put pressure on the newsprint sector to create revenue streams from new products by building on existing assets. A promising possibility is to reposition newsprint mills as biorefineries and produce value-added products from various types of biomass through the biochemical route. Mechanical pulping as a pretreatment method has the potential to generate a sugar platform with high yield of carbohydrates and low concentration of inhibitors and a sulphur-free and reactive lignin platform, both suitable for further valorization. Further, the use of existing facilities, equipment, and technology reduces the economical risk and capital costs of the investment.

In the current study the pretreatment of corn stover by alkali-oxygen impregnation and mechanical refining was investigated. Extended impregnation with molecular oxygen under various alkaline conditions, varying sodium carbonate concentrations and temperatures, provided deacetylation of the hemicellulose and selective modification and solubilisation of lignin. Mechanical pulping provided fibrillation and fiber cutting by shearing and compression forces. The combination improved the ease of enzymatic hydrolysis of the cellulosic fractions, and reduce the required enzyme load for efficient saccharification. The pretreated materials were characterized and evaluated with regard to hydrocarbon recovery, material properties, and ease of enzymatic hydrolysis. The results were benchmarked against the predominant pretreatment technologies.

S62 Lignin Value Prior to Pulping

T.T. Kwok, C.O. Luettgen, M.J. Realff and A.S. Bommarius, Georgia Institute of Technology, Atlanta, GA, USA*

Organic solvent treatments of biomass may facilitate the production of chemicals, fuels, nanocellulose, and pulp. These treatments are characterized by their effects on the two main components of biomass: lignin and cellulose. This work leverages our understanding of the pulp and paper industry, the biggest consumer of lignin and cellulose. We describe a process that employs an organic solvent to remove lignin from biomass while maintaining cellulosic integrity for pulp production. Our treatment of actual wood chips provides insight into the challenges and implications of operating within this size regime. This next-generation pulping system, called lignin value prior to pulping (LVPP), provides an un-sulfonated lignin stream and improves the operating economics of a pulp mill. Through a techno-economic analysis, we describe the LVPP implementation of organic solvents like alkylene carbonates and alcohols. This analysis provides a minimum lignin-selling price (MLSP) and highlights the feasibility of an LVPP process.

S63 Overcoming Difficulties in *Trichoderma reesei* Fermentation and Increasing Production with Near-Starvation Fed-Batch Regimes

T.A. Vander Wall, A. Amore and J.M. Yarbrough, National Renewable Energy Laboratory, Biosciences Center, Golden, CO, USA; S.R. Decker and M.E. Himmel, National Renewable Energy Laboratory, Golden, CO, USA*

The mesophilic ascomycetes fungus, *Trichoderma reesei*, secretes large amounts of celluloytic enzymes and is thus considered one of the highly industrially-relevant workhorses of biofuels and biomaterials production. However, it's filamentous morphology and extremely high cell density presents laboratory-scale researchers with a plethora of unique challenges. From proper agitation, to oxygen mass-transfer, to inline reactor data acquisition; few standard practices and instrumentation find usefulness in a *T. reesei* bioreactor. Even protein production deviates from the adage of more cells equals more protein. Therefore, a fed-batch protocol was developed for maintaining the culture in near-starvation conditions resulting in substantially higher productivity.

S64 Comparison of base-catalyzed depolymerization of lignin sourced from multiple biochemical conversion processes, biomass sources, and feedstock processing scenarios

R. Katahira, X. Chen, E.M. Kuhn, M.P. Tucker, G.T. Beckham and N. Nagle, National Renewable Energy Laboratory, Golden, CO, USA; A.E. Ray, Idaho National Laboratory, Idaho Falls, ID, USA*

Lignin valorization is a critical factor to achieve feasible industrial-scale lignocellulosic biorefineries. Lignin content and features depend on the type of feedstocks and pretreatment processes. In this work, two corn stover-switchgrass 50:50 blends (pelleted and non-pelleted) were subjected to two bioconversion deconstruction processes, dilute-acid pretreatment (DAP) and deacetylation and mechanical refining (DMR). The two feedstock blends from DMR and DAP were then enzymatically hydrolyzed (EH) and the resulting liquor was separated from the residual solids. We determined the carbohydrate yields resulting from deconstruction and the solid, aqueous, and gas yields from base-catalyzed depolymerization (BCD) of the DAP and DMR-EH residues to assess lignin susceptibility to BCD.

DMR xylose yields improved upon pelletization, but the same trend was not observed in DAP. DMR-EH feedstock blends from the low severity deacetylation condition resulted in the greatest aqueous fraction yields at approximately 81%, which was higher

than the non pelleted corn stover-only DMR-EH sample, even though the initial total sugar and lignin contents in the blend were higher and lower, respectively, than CS/DMR-EH. This suggests that sugar content affects the yield of aqueous fraction more than lignin content. There was no significant difference in yields of the three fractions between the pelleted and non-pelleted samples. DMR-EH residues have lower solids fraction yields (15%-20%) from BCD than DAP-EH residues (25%-27%) due to a greater condensed lignin structure in the DAP-EH residue. In this work, detailed effects of biochemical conversion processes, biomass sources, and feedstock processing scenarios on BCD results are discussed.

S65 The impact of pretreatment on the productive cellulase binding capacity of lignocellulosic biomass

W. Wu, University of California, Davis, Davis, CA, USA, N. Karuna, Silpakorn University, Nakhon Pathom, Thailand and T. Jeoh, University of California - Davis, Davis, CA, USA*

Cellulosic biofuel is an attractive renewable alternative to fossil fuel because it can be generated using agricultural waste, i.e. lignocellulosic biomass. The recalcitrance of lignocellulosic biomass is a major bottleneck in the bioconversion process that is in part overcome by a thermochemical pretreatment step to improve the enzymatic digestibility of the substrate. The goal of pretreatment is to improve the accessibility of cellulose to cellulases, yet a reliable and relevant metric for success is still elusive. Here we present a novel way of quantifying the accessibility of cellulose in pretreated biomass by measuring its productive *Trichoderma reesei* Cel7A (*Tr*Cel7A) binding capacity. The productive cellulase binding capacity (µmoles/g) is a measure of the maximum number of cellulase enzymes per mass of biomass that can initiate cellulose hydrolysis on the pretreated substrate. Real time hydrolysis captured at varying enzyme/substrate loading with a Cellobiose dehydrogenase (CDH) functionalized electrochemical biosensor allows accurate determination of maximum hydrolysis rates at the start of the reaction. The concentration of productively bound *Tr*Cel7A (µmoles/g) is obtained from the maximum hydrolysis rates by a first order relationship, and the productive binding capacity of the biomass is determined from the saturation concentration of productively bound *Tr*Cel7A. Preliminary results with alkali pretreated rice straw and ionic-liquid pretreated tomato pomace suggest that the extent of glucan conversion can be correlated to productive *Tr*Cel7A binding capacity. We also present productive *Tr*Cel7A binding capacities of sulfur dioxide pretreated spruce and discuss the predictability of the enzymatic digestibility of the softwood substrate.

Monday, April 30

7:00 AM - 8:00 AM Speaker Breakfast

Starfish, Lobby Level

7:00 AM - 5:00 PM Registration

Grand Ballroom Foyer, Lobby Level

8:00 AM - 11:25 AM Session: 1: Next-Gen Feedstocks- Breeding, Engineering, Handling I

Conveners: Tim Rials, UTK, USA;**Robert Henry**, University of Queensland, Australia and **Tim Volk**, SUNY-ESF, USA

8:00 AM 1-1: Engineering and environmental resilience of plants with improved biomass composition

A. Brandon, J. Yan, A. Aznar, D. Birdseye, M.Y. Lee, K. Vuu, A. Eudes, D. Loque and H.V. Scheller, Joint BioEnergy Institue, Lawrence Berkeley National Laboratory, Emeryville, CA, USA*

Biomass consists largely of complex polysaccharides and lignin of plant cell walls. The most abundant hemicellulose in biomass is xylan, which is composed of xylose, arabinose, glucuronic acid and acetate esters as the main constituents. Pentoses such as xylose and arabinose are more difficult to convert into biofuels and bioproducts in a cost-efficient manner than hexoses such as the glucose originating from cellulose. Furthermore, acetate is an inhibitor of yeast. Lignin is a main cause of biomass recalcitrance and is difficult to convert into valuable intermediates. Therefore, we have devised strategies to develop plants with a higher ratio of C6 to C5 sugars, a low content of acetate, and a low content of lignin in their cell walls. An efficient way of reducing xylan relies on dominant negative mutations in the xylan synthase that apparently inhibits a xylan synthase complex. Because the inhibition is dominant, it can be easily transferred to a variety of bioenergy crops. Likewise, we have used several methods based on dominant gene constructs to reduce or alter lignin composition. The resulting plants are indistinguishable from the wild type under normal growth conditions. Changing the cell walls of plants may lead to altered environmental resilience, and we have therefore tested the drought tolerance of the engineered plants. Surprisingly, many of the plants with reduced xylan and lignin show increased drought tolerance. Some plants show an abscisic acid (ABA) dependent drought tolerance, while other employ an ABA-independent mechanism.

8:25 AM 1-2: Significance of lignin characteristics in biomass recalcitrance of *Populus* for biomass valorization

C.G. Yoo, A. Dumitrache, Y. Yang, W. Muchero, T.J. Tschaplinski, S.D. Brown, B. Davison, G. Tuskan, J.G. Chen and Y. Pu, Oak Ridge National Laboratory, Oak Ridge, TN, USA; X. Meng, The University of Tennessee, Knoxville, Knoxville, TN, USA; A. Ragauskas, The University of Tennessee - Knoxville, and Oak Ridge National Laboratory, Knoxville, TN, USA*

Biomass recalcitrance to chemical or biological conversion is a factor to overcome for the effective valorization of biomass. The natural characteristics of biomass arising from its structural heterogeneity and complexity of cell wall constitutions are directly and/or indirectly related to biomass recalcitrance. Among many, physicochemical characteristics of biomass feedstock including chemical composition, cellulose and lignin's molecular weights/degree of polymerization, cellulose crystallinity, lignin composition, and cellulose accessibility not only elucidate the biomass recalcitrance, but also provide insights to overcome its physical and chemical barriers for biomass conversion. In particular, lignin characteristics has been investigated as an important factor in biomass recalcitrance to bioethanol production.

In this study, 11 *Populus trichocarpa* natural variants grown under the same conditions with similar total lignin content were selected to minimize environmental effects. Ethanol production and physicochemical characteristics of *Populus* natural variants were measured by diverse analytical methods such as HPLC, GPC, 2D ^{13}C-^{1}H HSQC NMR, and Py-MBMS. The lignin S/G ratio of the selected *P. trichocarpa* natural variants showed negative correlations with *p*-hydroxybenzoate (PB) and β-β linkage contents, while it had positive ones with β-*O*-4 linkage, lignin molecular weight, and ethanol production. This study showed the importance of lignin S/G ratio as an independent recalcitrance factor that may aid future energy crop engineering and biomass conversion strategies.

8:50 AM 1-3: Pretreatment and fermentation "killers" from biomass: the removal of non-structural components from short rotation coppice poplar as an economically feasible preprocess in biorefinery

R. Bura and R. Gustafson, University of Washington, Seattle, WA, USA*

Depending on the lignocellulosic species and the growing conditions, the non-structural organic and inorganic components (NSCs) can contribute more than 10% of the short rotation coppice poplar. However, the influence of NSCs of biomass on the production of fuels and chemicals is not well known. In this study, we assessed the impact of NSCs removal on the overall sugar recovery and fermentation yield of short rotation coppice poplar after pretreatment and enzymatic hydrolysis. In addition, we focused on evaluating the economics of preprocessing as a new unit process in the biorefinery. Coppice poplar was preprocessed by neutral or acidic washing before steam pretreatment, enzymatic hydrolysis, and fermentation. Preprocessing of poplar significantly reduced ash and extractives content as much as 70% and 50%, respectively. The overall sugar yield was 18-22% higher when the biomass had been preprocessed, which was explained by higher sugar yield in liquid fraction and more efficient enzymatic hydrolysis of solid fraction. The ethanol yield was 36-50% higher for the preprocessed biomass during fermentation of liquid fraction. It appears that preprocessing methods changed the buffering capacity of the biomass via ash removal and thereby improved the enzymatic hydrolysis. Meanwhile, removal of extractives during preprocessing improved the fermentation yield. The economic modelling shows that introduction of one preprocessing unit in the biorefinery could bring in $43.1 MM additional gross ethanol revenue and greatly benefit the economics. Based on the results from this study, there is great technical and economic potential for preprocessing of coppice poplar in biochemical conversion.

9:15 AM Break

9:45 AM 1-4: Efficient conversion of municipal solid waste to biofuels and bioproducts

L. Liang, J. Yan, Q. He, C. Gutierrez, T. Pray and N. Sun, Lawrence Berkeley National Laboratory, Berkeley, CA, USA; C.H. Chu, Recology Inc., San Carlos, CA, USA*

This study provides methods of converting currently non-recyclable and non-compostable food and paper waste streams through bioprocessing to biofuels, biochemicals, and/or intermediates to bioproducts. An integrated process for co-production of sugars and lactic acid (LA) is developed and discussed. The sugars can be further fermented to either LA or other chemicals. The food- and paper-rich waste streams collected from residential and/or commercial municipal solid waste (MSW) are homogenized to increase the surface area. Addition of the enzyme cocktails to the waste substrate results in LA productions while C6/C5 sugars accumulate in the hydrolysate, due to the presence of LA bacteria carried by the waste substrate. An homogenization step greatly increased the substrate accessibility resulting in greater than 80% sugar yields. Sugars in the hydrolysate are proven readily fermentable to ethanol (with > 70% conversion based on the theoretical maximum) without solid/liquid separation or detoxification steps. Both sugar and ethanol yields are comparable to that derived from the control substrate (Avicel and Avicel hydrolysate) indicating minimal inhibition on enzymes or microbes. It is the further object of the present study to provide a method for reducing existing landfills by converting the food and paper fractions as well as to provide an alternative feedstock for biorefineries.

10:10 AM 1-5: Expectation vs. Reality: The Unexpected Abrasive Nature of Biomass Feedstocks

J.A. Lacey, J.E. Aston and V. Thompson, Idaho National Laboratory, Idaho Falls, ID, USA*
Early reports from corn stover-fed biorefineries have indicated that equipment is requiring extensive maintenance and replacement due to wear caused by the feedstock. It is suspected that biomass ash, both physiological and introduced, is responsible for the observed accelerated wear rates as it contains minerals of varying hardness. To address this problem, wear caused by biomass needs to be characterized. To simulate biomass wear on material handling equipment, a sandblaster was modified to shoot ground biomass at a metal coupon. The angle of impingement was set at 90°, 65.5°, or 45° by rotating the coupon. The coupon was weighed before and after shooting a known quantity of biomass to determine mass loss, and the surface characteristics of the blasted coupon were analyzed using laser microscopy. Coupon mass loss was positively correlated with ash content. Coupons blasted with loblolly pine (0.58% ash) lost 2.2±0.3mg per kg biomass shot, while high-ash stover (12.56% ash) coupons lost 104.7±2.1mg per kg biomass shot. Coupons blasted with NaOH-leached low-ash stover (4.01% ash) lost 10.1±2.7mg per kg biomass shot, showing that feedstocks can be modified to improve wear properties. Decreasing the angle of impingement (90° to 45°) increased mass loss by about 17%. The concentrations of soil elements (silicon, aluminum, iron) were positively correlated with coupon mass loss, indicating that introduced ash is likely the primary source of wear to equipment. Future studies will identify mineral forms of ash present in biomass to further characterize biomass wear properties and to identify strategies for quality improvement.

10:35 AM 1-6: Designing corn stover bale storage yards to reduce potential fire growth and spread

E. Webb, R. Chambers, M. Shedden and T. Theiss, Oak Ridge National Laboratory, Oak Ridge, TN, USA; D. Steppan, UL LLC, Northbrook, IL, USA; K. Webster, Iowa State University, Ames, IA, USA; R. Clark, QMT Group, Oak Ridge, TN, USA; F. DuPont and J. Pieper, DuPont Industrial Biosciences, Nevada, IA, USA; R. Reynolds, City of Nevada, Nevada, IA, USA*
Unavoidable fires in corn stover bale storage yards caused by arson and lightning have become a significant risk factor for cellulosic biorefineries and feedstock suppliers. Stover fires ignite and spread quickly and are, in many cases, impossible to extinguish. Stover bale fires pose danger to firefighting personnel and neighboring properties. Smoke from these fires, which can burn or smolder for weeks to months, is an irritant and health hazard to local communities. In this research, we conducted a series of controlled fire tests to better understand the dynamics of fire growth and spread within a stack of large rectangular corn stover bales. Recognizing the importance of vertical air gaps between bales within the stack on fire growth and spread, we developed a cross-stacking strategy to block fire growth and spread. This bale stack design proved to be successful in indoor and outdoor fire tests in slowing fire growth and reducing the amount of water required to extinguish the fire. Based on these experimental results of fire growth within the stack, an empirical model is being developed to predict the rate of fire spread from stack-to-stack via radiant heat and ember transmission.

11:00 AM 1-7: Metabolic engineering and genome editing to improve sugarcane as a biofuel feedstock

F. Altpeter, B. Kannan, J.H. Jung, S. Parajuli, R. Karan and T. Oz, University of Florida, Gainesville, FL, USA; H. Liu and J. Shanklin, Brookhaven National Laboratory, Upton, NY, USA; E. Garcia-Ruiz, H. Zhao and S. Long, University of Illinois at Urbana Champaign, Urbana, IL, USA*
Metabolic engineering to divert carbon flux from sucrose to oil in a high biomass crop like sugarcane has been proposed as a strategy to boost lipid yields per acre for biodiesel production. The energy content of plant oils in the form of triacylglycerols (TAGs) is two-fold greater compared to carbohydrates. However, vegetative plant tissues do not accumulate oil to a significant amount since fatty acid synthesis in these tissues serves primarily membrane construction, in addition TAGs undergo rapid turnover. Therefore, our objectives include metabolic engineering to:

1.) increase fatty acid synthesis,
2.) increase TAG synthesis from diacyl-glycerol and acyl-CoA
3.) optimize TAG storage
4.) minimize TAG hydrolysis in vegetative tissues.

Following delivery of single or multiple gene expression/suppression cassettes stably transformed plants were regenerated and analyzed for presence and expression of target constructs by PCR and RT-PCR, respectively. Plants were analyzed for TAG content by gas-chromatography and mass spectrometry (GC-MS). Accumulation of TAG to 8 % of leaf dry weight and total lipids to 13% of leaf dry weight were recorded. This is equivalent to 400-fold increase of TAG compared with non-transgenic sugarcane. Lines with low TAG accumulation co-expressed either fewer transgenes, or expressed the transgenes at lower levels. This research outcome will add value to the abundant sugarcane post-harvest residues for production of advanced biofuels.

A genome editing approach that resulted in modified cell walls and improved saccharification efficiency for biofuel production from lignocellulosic sugarcane biomass will also be presented

8:00 AM - 11:25 AM Session: 2: Engineering Operations for Biomass Processing I

Conveners: Charlie Wyman, UC-Riverside, USA;**Jack Saddler**, UBC, Canada and **Maria Cuellar Soares**, TU Delft, The

Netherlands

8:00 AM 2-1: Rapid and simultaneous production of furfural and cellulose-rich residue from sugarcane bagasse using a pressurized phosphoric acid-acetone-water system

Q. Wang, W. Wang, Q. Yu and Q. Wei, Guangzhou Institute of Energy Conversion, Chinese Academy of Sciences, Guangzhou, China*

In order to minimize hemicellulose (or cellulose) waste upon the simultaneous production of furfural and 5-hydroxymethylfurfural (or furfural and levulinic acid) from the hydrolysis of lignocellulose in one-pot acid/organic solvent systems, we have developed a novel pressurized phosphoric acid-acetone-water system (PPAWS) to convert hemicellulose into furfural with a high retention of cellulose. In the PPAWS (acetone/water = 7:3, *v/v*) at 150 °C under 1.5 MPa nitrogen, furfural production (yield 45.8%), delignification (lignin removal rate 89.8 wt%), and cellulose-rich residue extraction (retention rate 72.9 wt%, purity 92.5 wt%) from sugarcane bagasse were achieved simultaneously in just 5 min. Gas chromatography-mass spectrometry analysis of the liquid products revealed that pressure significantly inhibits the generation of aldol condensation products, i.e., acetone self-condensation products and 4-(2-furyl)-3-buten-2-one from the condensation of acetone and furfural, and acetone was retained in large quantities. In addition, kinetic analysis revealed that the “xylose → furfural → degradation products” pentose-hydrolysis path occurs in the PPAWS and that the rate constant of the “xylose → furfural” step is significantly increased, which is a clear departure from the reaction conducted without additional pressure. Furfural degradation experiments confirmed that adding pressure also inhibits the degradation of furfural in the initial stage of the reaction. The PPAWS exploits the benefits of acetone for lignin removal and the promotion of furfural production, and can be easily integrated into advanced jet fuel production from the aldol condensation of furans and ketones.

8:25 AM 2-2: Why pulping processes are best suited to be the pretreatment front ends for biorefineries.

R.P. Chandra, J. Wu, F. Nielsen and M. Takada, University of British Columbia, Vancouver, BC, Canada; J. Saddler, The University of British Columbia, Vancouver, BC, Canada*

The production of substrates that are susceptible to enzymatic hydrolysis at low enzyme loadings while separating the cellulose, hemicellulose and lignin in a usable form remains a major challenge for biomass pretreatments aiming to process woody biomass. Although several pretreatments including steam and organosolv can process wood, the pulp and paper industry has existing infrastructure, supply chains, chemical recovery, effluent treatment etc. which would enable the ready transition of a pulp mill towards the production of fibre and value added lignin and hemicelluloses. Although the price of chemical pulps precludes their use for biochemical conversion, mechanical pulping has experienced an unprecedented decline in the demand for their newsprint products. The potential availability of mechanical pulping infrastructure has shifted pretreatment research towards the development of technologies that include SPORL and Deacetylation Mechanical Refining. The main challenge when using mechanical pulping is the inability to separate lignin, which decreases hydrolysis yields. Therefore, we will describe our work developing mechanical pulping based treatments that retain most of the cell wall components in the substrate while increasing enzymatic hydrolysis yields. We detail that the key to this approach is the fortification of acid groups within the lignin component either through sulfonation or oxidation. We also show that the hemicellulose component can be pre-extracted prior to an oxygen reinforced mechanical pulping pretreatment to cleanly fractionate >65% of the hemicellulose while producing a lignin fraction and a cellulose component that can be readily hydrolyzed at enzyme loadings of 15 mg/gram cellulose in 48 hours.

8:50 AM 2-3: The AFEX™ Pretreatment Process: A 38 Year Journey from Laboratory Technique to (Near) Commercial Application

B. Dale, Michigan State University, Lansing, MI, USA*

Biological conversion of cellulosic biomass to fuels and chemicals at high rates and yields requires pretreatment. But pretreatment is problematic. As Dr. Charlie Wyman has correctly observed, “The only thing more expensive than pretreatment is no pretreatment.” Pretreatments become even more expensive when they use costly or difficult-to-recover chemicals, require severe processing conditions, generate inhibitors, degrade sugars and/or produce waste streams that must be treated. The AFEX™ process was invented to minimize or eliminate these costs.

Cellulosic biorefineries must be very large scale. Thus biomass pelleting is required for low cost transportation, storage and handling. Biomass is AFEX-treated and then easily pelleted in distributed processing centers (called “depots”). These pellets are stored and handled with conventional grain-handling equipment. AFEX is thus the only thermochemical pretreatment that effectively addresses the critical biomass transport and logistics issues with untreated biomass.

AFEX-treated biomass is also a good cattle feed, thereby promoting farmer participation in the biofuel system, while eliminating the “food vs. fuel” argument and the attendant indirect land use change (ILUC) controversy. Furthermore, AFEX pellets enable high solids fermentation and inexpensive cell/enzyme recycle, significantly reducing biorefinery capital and operating costs.

AFEX appears to be the only new pretreatment in decades that has proceeded from the laboratory to the pilot plant scale. AFEX is now nearing its first commercial scale application. This presentation will summarize the scientific and engineering knowledge we have gained on the AFEX process during this thirty-eight year journey.

9:15 AM Break

9:45 AM 2-4: Recovery of Ultrapure Low and High Molecular Weight Lignin Fractions via the ALPHA Process

J. Ding, A.S. Klett and M.C. Thies, Clemson University, Clemson, SC, USA*

Lignin is unique among biopolymers in having significant aromatic character, which makes it potentially useful for a wide range of applications. Unfortunately, most of the commercial-grade lignins available today (primarily Kraft lignins) are too polydisperse and have a high metals and ash content, essentially eliminating them for high-value applications.

We have developed a process for simultaneously solvating, fractionating, and purifying the lignin polymer recovered from biomass by-product streams of pulp-and-paper mills and lignocellulosic biorefineries. Aqueous Lignin Purification with Hot Acids (ALPHA) involves combining solid lignin with hot acetic acid–water solvent mixtures to produce two liquid phases: a highly solvated, (lignin) polymer-rich phase and a solvent-rich phase. ALPHA can be operated in two ways: (1) by using increasingly aggressive solvent mixtures to isolate ultrapure, metals-free (i.e., <100 ppm) lignin fractions of medium/high molecular weight (MW), or (2) by “reversing” the process and using solvent mixtures of decreasing strength to isolate metals-free lignin fractions of low molecular weight (MW).

ALPHA was used to generate ultrapure, high MW fractions of lignin that were then converted into carbon fibers; the resulting tensile strengths and moduli were almost 50% greater than any reported to date. Furthermore, “reverse” ALPHA was used to generated ultrapure, low MW fractions of lignin for application in coatings. Also discussed in this work will be how one can tune the parameters of ALPHA (e.g., temperature, acetic acid/water ratio, solvent-to-lignin ratio) to generate lignin fractions with the properties (including phenolic content, molecular weight, and purity) required for specific applications.

10:10 AM 2-5: Rapid and near-complete dissolution of wood lignin at ≤ 80°C using a recyclable acid hydrotrope for sustainable production of high-value building blocks

J. Zhu, U.S. Department of Agriculture Forest Service, Madison, WI, USA*

Here we present the discovery of the hydrotropic properties of a recyclable aromatic acid, *p*-toluenesulfonic acid (*p*-TsOH), for low-cost and efficient fractionation of wood through rapid and near-complete dissolution of lignin. Approximately 90% of poplar wood (NE222) lignin can be dissolved at 80°C for 20 min. Equivalent delignification using known hydrotropes such as aromatic salts can be achieved only at 150°C or higher for over 10 h, or at 170°C for 2 h with alkaline pulping. *p*-TsOH fractionated wood into two fractions: (1) a primarily carbohydrate-rich water-insoluble solid fraction that can be used for sustainable production of high-valuebuilding blocks, such as sugar/biofuels through subsequent enzymatic hydrolysis, pulp fibers and lignocellulosic nanomaterials, and (2) a spent acid liquor stream containing mainly dissolved lignin that can be easily precipitated as lignin nanoparticles simply by diluting the spent acid liquor to below the minimal hydrotrope concentration, and hemicellulose sugars that can be dehydrated into furfural through catalysis directly using using *p*-TsOH. *p*-TsOH has a low water solubility, which facilitates efficient recovery simply using commercially proven crystallization technology by cooling concentrated spent acid solution to ambient temperatures to achieve environmental sustainability. Preliminary laboratory evaluation show a *p*-TsOH recovery of 95% can be achieved in the first cycle. Therefore, a recovery rate of 97% can be achieved after two cycles.

10:35 AM 2-6: Acclimatization of fermentation yeast on pretreatment hydrolysate from Co-solvent Enhanced Lignocellulosic Fractionation (CELF) of switchgrass

A. Patri, University of California Riverside, Riverside, CA, USA; C. Cai, University of California, Riverside, CA, USA; R. Kumar and C.E. Wyman, Center for Environmental Research and Technology, Bourns College of Engineering,University of California Riverside, Riverside, CA, USA*

Lignocellulosic biomass represents the most abundant renewable resource and provides the only known route to sustainably produce liquid fuels on a large scale and low cost. Due to its complex and recalcitrant structure, raw biomass hinders biological conversion of polysaccharides, thus necessitating pretreatment to deconstruct plants by allowing cellulose and hemicellulose to be more accessible to enzymes for release of fermentable sugars. Several pretreatment methods, including hydrothermal and dilute acid approaches, have been applied to improve enzyme accessibility, but each is limited in their ability to remove lignin, a key contributor to biomass recalcitrance. Recently, we developed a novel pretreatment called Co-solvent Enhanced Lignocellulosic Fractionation (CELF) that applies aqueous tetrahydrofuran (THF) in approximately equal proportions with dilute sulfuric acid to remove about 85 to 90% of the lignin, while achieving high yields of five carbon sugars from hemicellulose in solution during pretreatment and subsequently solubilizing most of the glucose from the pretreated solids with very low enzyme loadings. In this study, CELF pretreatment was applied to switchgrass over a range of temperatures and times to establish conditions that maximized total glucose plus xylose yields from CELF combined with subsequent enzymatic hydrolysis at low solids loadings. We then determined ethanol yields from fermentation of the xylose-rich liquid produced by CELF pretreatment after most of the lignin had been removed through THF evaporation. Fermentation of pure sugars showed that the yeast employed performed well as long as THF concentrations were reduced below 5 g/L and yeast was acclimatized to inhibitory dissolved lignin.

11:00 AM 2-7: Effect of mixing on enzymatic hydrolysis of corn stover at high solids loading

A.C. Freitas Dos Santos, E. Ximenes and M. Ladisch, Purdue University, West Lafayette, IN, USA; J. Dooley, Forest Concepts, LLC, Auburn, WA, USA; D.N. Thompson and A.E. Ray, Idaho National Laboratory, Idaho Falls, ID, USA*

Pelletized corn stover was pretreated at 190 °C for 20 min in liquid hot water at initial solids loadings of 360 g/L. The biomass was washed and transferred to a 1 L reactor agitated with a marine impeller at 290 rpm for enzymatic hydrolysis at pH 4.8 and 50°C for 72 hours with Cellic CTEC2 at 3 mg protein/g solids. At initial solids concentrations of 10, 100 and 200 g/L, the extent of hydrolysis was found to be independent of solids concentration, giving 50% conversion of cellulose to glucose for all solids loadings. In contrast, hydrolysis at the same conditions in 250 mL shake flasks gave lower conversions at the higher solids loadings: 40% at 200 g/L vs 51% at 100 g/L. These data show that mixing is an important factor in maintaining the extent of hydrolysis as solids loadings increase and that a combination of static liquid hot water pretreatment at high solids loadings using densified biomass, followed by enzyme hydrolysis in a reactor agitated with a marine impeller, enables significant conversion of the cellulose in pretreated and washed corn stover. The benefit of mixing at high loadings includes improved conversion of cellulose to glucose and higher volumetric productivity.

9:00 AM - 3:00 PM Exhibits Open

Grand Ballroom Foyer, Lobby Level

1:00 PM - 4:25 PM Session: 3: Driving the Bioeconomy I

Conveners: James D. (Jim) McMillan, NREL, Golden, CO, USA;**John Evans**, AB Mauri, St. Louis, MO, USA and **Tae Hyun Kim**, Hanyang University, S. Korea

1:00 PM 3-1: Focused innovation to create sustainable cellulosic biofuel systems: our ten year effort

B. Dale, Michigan State University, Lansing, MI, USA*

In the past decade, huge investments have been made to research and commercialize liquid cellulosic biofuels, particularly ethanol. However, commercialization of cellulosic ethanol has stalled. Why? One reason is that cellulosic biofuels require functioning systems, not just system pieces. This presentation summarizes a decade of effort to overcome commercialization barriers by applying innovative, critical systems level thinking to cellulosic biofuels. Barriers we have addressed include: 1) biomass supply chain problems, including lack of farmer participation, 2) the slow fermentation and hydrolysis of pretreated biomass, 3) the “food vs. fuel” issue, 4) high biorefinery capital costs and 5) the sustainability of corn stover harvesting.

Biorefineries must be very large scale, and that requires biomass pelleting. Pretreatments that generate inhibitors, or that use non-volatile chemicals, produce waste streams and thereby decrease sugar yield. Biomass treated by the ammonia fiber expansion (AFEX) process does not require washing and is easily pelleted. AFEX-treated biomass is also a good cattle feed, thereby promoting farmer participation in the system, while also eliminating the “food vs. fuel” argument. AFEX pellets enable high solids fermentation and inexpensive cell/enzyme recycle, significantly reducing biorefinery capital and operating costs. Co-locating biorefineries with coal-burning power plants enables the plants to meet green portfolio standards while sharing utilities and waste heat. These innovations reduce biorefinery capital costs to about $2.40/annual gallon. Finally, corn stover removal is an environmentally-marginal practice unless it is combined with soil-conserving practices such as double-cropping.

1:25 PM 3-2: A review of the production of second-generation biofuels via thermochemical and biochemical conversion

Y. Sorunmu, B. Riazi and S. Spatari, Drexel University, Philadelphia, PA, USA; P. Billen, University of Antwerp, Antwerp, Belgium; V. Larnaudie, Universidad de la República, Montevideo, Uruguay*

Infrastructure compatible (100% oxygen-free) second-generation biofuels are under development as alternatives to petroleum-based fuels to mitigate greenhouse gas (GHG) emissions, address energy security, and stimulate domestic energy markets. Different technological routes, in particular, thermochemical and biochemical ones can be used to convert biomass to multiple biofuel products (renewable diesel, gasoline and jet fuel) and value added coproducts (electricity, organic and inorganic chemicals).

While the development of second-generation biofuels is promising, commercialization has been slow. Therefore, understanding the current state of technology for multiple biomass conversion processes under development could help identify the technical barriers to be addressed in order to move towards commercialization. Herein, we review the environmental, economic and technological aspects of alternative biofuel production technologies by using life cycle assessment, techno-economic analysis and technological readiness levels to examine specific metrics and evaluate tradeoffs among thermochemical and biochemical routes.

Results show that in spite of their improved environmental performance for metrics like GHG emissions (4.4 to 83.6 g CO_2 eq.

per MJ compared to 91.7 to 116 g CO_2 eq. per MJ), second-generation biofuels remain more costly to produce with minimum fuel selling prices (MFSP) 22 times greater for biochemical and 4 times greater for thermochemical production routes, compared to petroleum-based fuels. The high MFSP of the fuels is due to high feedstock cost in some cases and the complex nature of the biomass conversion processes, which have high capital costs. This poses a challenge in the commercialization of these second-generation biofuels; therefore requiring additional research before commercialization.

1:50 PM 3-3: Gas fermentation: Waste to value at commercial scale

S. Brown, LanzaTech, Skokie, IL, USA*

The production of biofuels and platform chemicals via gas fermentation is a rapidly commercializing technology for high volume, sustainable, production of fuels and chemicals. LanzaTech is commercializing a complete process platform to allow the continuous biological production of fuels and an array of chemicals intermediates from gases at scale. To date, this technology has been successfully demonstrated with such diverse gas streams as by-product gases from steel making, reformed natural gas, syngas produced from gasified biomass and municipal solid waste. At the heart of the process is an acetogenic microbe *Clostridium autoethanogenum* capable of autotrophic growth on a range of low cost C1 substrates such as carbon monoxide (CO) and/or CO2. Around this chassis, the company has developed an advanced strain engineering platform. The process has been successfully scaled up from the laboratory bench through in-lab and in-field pilot plants to fully integrated 100,000-gallon/year pre-commercial demonstration plants. An update on the first full commercial unit will be presented.

2:15 PM Break

2:45 PM 3-4: Biofuels at POET

M. Slupska, POET, Sioux Falls, SD, USA*

Biofuels at POET

POET, the world's largest biofuel producer, is a leader in biorefining through its efficient, vertically integrated approach to production. The 30-year-old company has a network of 27 production facilities with a production capacity of 1.8 billion gallons. POET employs approximately 1,800 team members and in addition to ethanol produces 9 billion pounds of distillers' dry grains and approximately 550 million pounds of corn oil per year. The company is also known for its excellent record of innovation with a "raw starch" process (BPX) being the most widely known.

In addition to starch-ethanol production, POET operates a commercial-scale cellulosic ethanol plant in Emmetsburg, Iowa (with its joint venture partner DSM). Project LIBERTY has a 20-25 million gallon capacity. In 2017 the company solved the critical challenge in pretreatment, overcoming what has been the No. 1 hurdle to commercialization for producers around the world. Project LIBERTY is now running pretreatment at 80 percent uptime, ramping up production and achieving a conversion rate of 70 gallons per ton of biomass. In 2017 POET-DSM announced construction of an on-site enzyme manufacturing facility to directly pipe DSM enzymes into the process. The latest achievements and challenges at Project LIBERTY will be further discussed during the meeting.

3:10 PM 3-5: ICM's BioEconomy Strategy

C. Mitchell, S. Hartig, J.E. Javers and B. Emme, ICM, Inc., St Joseph, MO, USA*

Continued progress towards achieving a sustainable BioEconomy in the United States has proven to require more than just good science and engineering. Political, social, economic and environmental factors have at times imparted dominant pressures on development and commercialization of processes and technologies based on renewable resources. At the heart of a healthy and growing BioEconomy is a need for additional process intensification and product diversification to economically produce more and higher value products. If done correctly, this can increase the availability of agriculture for energy while simultaneously mitigating food vs. fuel concerns, and bring ever more cost competitive biotechnology to the market. ICM played a significant role in the massive buildout of USA's industrial corn and grain fuel ethanol capacity in the 2000's, and since has brought even greater value to agriculture through development of additional corn ethanol coproducts for fuel, feed, lignocellulosics and biotech, with four new advanced processing technologies launched in 2017 and construction of the ELEMENT™, LLC plant in Colwich, Kansas, USA in 2018. Learn how this next phase of process development for the corn ethanol industry will drive opportunities for new biochemicals for crops, animals and people.

3:35 PM 3-6: Integrated Sugarcane-Microalgae Biorefineries for CO_2 Uptake and Product Portfolio Diversification of the Brazilian Sugar-Energy Sector

B.C. Klein and A. Bonomi, Brazilian Bioethanol Science and Technology Laboratory, Campinas, Brazil; R. Maciel Filho, State University of Campinas, Campinas, Brazil

The search for industrial processes with higher sustainability has led to a change towards the utilization of renewable sources for energy generation in substitution of fossil fuels, with the intention of modifying the global energy matrix. Under this scope, the possibility of using microalgae for the production of biofuels and other bioproducts is currently being viewed as an interesting

option for the near future due to the high associated biomass productivities. The present work aims at the evaluation of the techno-economic feasibility and environmental impacts of the integration of large-scale microalgae facilities and sugarcane mills in Brazil, into a true biorefinery concept. Process integration was based on (1) utilization of CO_2 produced during ethanol fermentation and that contained in biogas, from the anaerobic digestion of vinasse, for the photoautotrophic growth of microalgae; (2) employment of vinasse as the carbon source for the heterotrophic growth of microalgae; and (3) use of electric energy and process steam from the sugarcane mill to produce microalgae biomass. The process integration strategies were assessed, via modelling and simulation through the Virtual Sugarcane Biorefinery (VSB) framework, using data obtained from the scientific literature. The results indicate that this type of integration leads to a promising economic performance of sugarcane-microalgae biorefineries, especially when anaerobic digestion of vinasse is considered. Also, significant reductions in climate change impacts of anhydrous ethanol production in integrated biorefineries were between 15-20% lower than in conventional sugarcane mills, thus attesting the environmental benefits of microalgae production.

4:00 PM 3-7: Amyris: Making a Healthier Planet One Molecule at a Time

P. Hill, Amyris Inc., Emeryville, CA, USA*

Amyris is a bioscience company devoted to making No Compromise® products that deliver sustainable, competitively priced ingredients with no compromise on quality. Fuelled by breakthroughs in biology, automation, and computer science, economically replacing chemicals with renewables is not only possible, but essential. Amyris is at the forefront of this revolution, but many challenges remain. Entrenched chemistry mindsets, a fully depreciated petroleum infrastructure, and fear of a new technology all slow entry of renewables into the marketplace. In our 13 year history, we have successfully developed and delivered fourteen ingredients to the marketplace, with more on the way. Building a manufacturing business has required developing a technology platform for strain engineering and fermentative production, creating business relationships with a broad diversity of commercial partners, and making good on our promises of providing No Compromise® products. During this talk I will discuss the technology that drives our business, the challenges of cost-effectively producing small molecules at the kilo-ton scale, and where the future of business lies.

1:00 PM - 4:25 PM Session: 4: Performance-advantaged bioproducts

Conveners: Gregg Beckham, NREL, USA;**Jay Fitzgerald**, DOE-BETO, USA and **Dr. Ken Tokuyasu**, Nat Ag Food Res, Japan

1:00 PM 4-1: Leveraging Bioprivileged Molecules to Generate Performance-Advantaged Bioproducts

B. Shanks, Iowa State University, Ames, IA, USA*

Leveraging Bioprivileged Molecules to Generate Performance-Advantaged Bioproducts

Much of the effort in converting biomass to biobased chemicals has been driven by the retrosynthesis of target molecules. This approach works when the target molecule is known but is problematic when the goal is to generate performance-advantaged molecules. Due to the lack of structure/function understanding for chemical species and their end uses, novel molecules needed to be synthesized and tested for their efficacy in the desired end use application. Therefore, the search for performance-advantaged biobased chemicals can be more efficiently realized if a family of similar molecules can be synthesized and tested. Presented will be a new paradigm in which bioprivileged molecules are produced that can be subsequently diversified into a range of chemical species (analogous to diversity oriented synthesis in pharmaceuticals. The important role of these bioprivileged molecules has been demonstrated as key intermediates in the integration of biology and chemistry by our NSF Engineering Research Center for Biorenewable Chemicals (CBiRC). Several examples of bioprivileged molecules being developed by CBiRC will be discussed.

1:25 PM 4-2: Production of renewable chemical building blocks from lignin by recombinantÂ *Pseudomonas putida*Â KT2440 and further upgrading to polymers

D. Salvachúa, C. Johnson, B.A. Black, N. Rorrer, K.J. Ramirez, W. Michener, T. Vander Wall, D.J. Peterson, H. Smith, D.R. Vardon and G.T. Beckham, National Renewable Energy Laboratory, Golden, CO, USA*

Lignin is currently underutilized in biochemical conversion processes to produce renewable fuels and chemicals from lignocellulose. However, the aromatic molecules found in this complex polymer are an excellent carbon source for some organisms, which are able to metabolize heterogeneous slates of aromatic molecules to a few central intermediates and subsequently direct them into central metabolism. In the current work, *Pseudomonas putida* KT2440 has been engineered to produce sixteen different metabolic intermediates from aromatic compounds (i.e. β-ketoadipic acid, PDC, muconic acid, etc). These intermediates - which are both native or heterologous to *P. putida* KT2440 – were produced in 10 L bioreactors utilizing a DO (oxygen saturation)-stat feeding strategy to avoid toxicity effects by the aromatic compounds and decrease catabolic repression by other carbon sources present in the cultivation media. Seven molecules were produced successfully, ranging in titer from 1 to 50 g/L. As a proof of concept, we have also demonstrated the production of muconic acid from real lignin streams. Furthermore, new analytical methods were developed to detect and quantify molecules for which methods have not been

previously described. Lastly, downstream separations, purification, and polymerization of the molecules resulting from these cultivations were also developed, which in some cases demonstrated performance advantaged properties over their petroleum-derived counterparts, suggesting a viable path forward for this integrated technology to upgrade lignin to value-added compounds.

1:50 PM 4-3: Improved Enzymatic Co-Production of Nanocellulose and Biofuel Precursors

P. Ciesielski[*]*, NREL, Golden, CO, USA; J.M. Yarbrough and T.A. Vander Wall, National Renewable Energy Laboratory, Biosciences Center, Golden, CO, USA; A. Mittal, Y. Bomble, S.R. Decker and M.E. Himmel, National Renewable Energy Laboratory, Golden, CO, USA*

The development ecofriendly methods for the production of fuels, chemicals, and materials from renewable resources will advance societal progress towards a sustainable bioeconomy. Carbohydrate-active enzymes have long been used to deconstruct cellulosic feedstocks to soluble sugars as precursors to biofuels and chemicals. However, the recent surge in interest in cellulose nanomaterials has led to investigations of these enzymes as alternatives to the strong mineral acids typically used in nanocellulose production processes. A major advantage of using enzymes as opposed to acid in such processes is that the hydrolysate produced as cellulose nanoparticles are liberated is largely compatible with current downstream conversion technologies employed in second generation biorefineries. In this presentation, I will compare the capacity for coproduction of nanocellulose and soluble sugars using two cellulase systems: the conventional “free enzyme” system of fungus *Trichoderma reesei* (*T. reesei*) and the multifunctional enzymes produced by the thermophilic bacterium, *Caldicellulosiruptor bescii* (*C. bescii*). Our findings indicate that that the *C. bescii* system outperforms the fungal enzyme system in terms of total cellulose conversion, sugar production, and nanocellulose production. Furthermore we show by several characterization methods that the nanocellulose produced by the *C. bescii* system is highly uniform compared to that produced by the *T. reesei* system. We attribute these differences in the yields and characteristics of the nanocellulose produced by the two enzyme systems to disparities of the binding and deconstruction modalities of the dominant enzymes in each system.

2:15 PM Break

2:45 PM 4-4: Multi-stream Integrated BioRefinery (MIBR) for Sustainable and Cost-effective Biofuels and Bioproducts

Q. Li, Texas A&M University, College Station, TX, USA; S. Xie and J.S. Yuan[*]*, Synthetic and Systems Biology Innovation Hub, Texas A&M university, College Station, TX, USA; Z. Liu, Texas A&M University, College station, TX, USA; Y. Pu, The University of Tennessee, Knoxville, Knoxville, TN, USA; B. Yang, Washington State University, Richland, WA, USA; A. Ragauskas, Oak Ridge National Laboratory, Oak Ridge, TN, USA*

The success of a modern biorefinery heavily depends on the availability of diverse product streams. The utilization of the lignin-containing biorefinery waste as feedstock for renewable products offers a unique opportunity to achieve a multi-stream integrated biorefinery (MIBR), where the lignin-containing biorefinery waste will be utilized for value-added biproducts to maximize economic return and sustainability. Specifically, we have advanced the fractionation, conversion, and processing technologies to enable different bioproduct streams. Several pretreatment and fractionation technologies were developed to fractionate lignin-containing biorefinery waste into low- and high- molecular weight fractions with more homogenous chemical characteristics. We have demonstrated that these lignin fractions are more amenable to different applications in bioconversion, asphalt binder modifier, and carbon fiber. The low molecular weight fraction is more amenable to bioconversion into PHA for bioplastics and lipid for biodiesel. Systems biology-guided microbial engineering has significantly improved lignin depolymerization, aromatic compound conversion, and bioproduct synthesis, which led to the record yields of lipid and PHA from biorefinery waste. In addition, the low molecular weight lignin can be used as unique asphalt binder modifiers to improve both high and low temperature performance of road pavement. The high molecular weight fraction can be used to fabricate carbon fiber with significantly improved mechanical performance and conductivity. Together, MIBR will reduce ethanol production cost through the creation of high value bioproducts, produce asphalt binder modifier with unique features, develop an innovative, new pathway to quality carbon fiber; and create a means to utilize all carbon in the feedstocks.

3:10 PM 4-5: Chemical Synthons and Recyclable Materials from Lignin

M. Abu-Omar[*]*, University of California Santa Barbara, Santa Barbara, CA, USA*

Transition metal catalysts have been an integral part of the success story of the petrochemical industry in the past century. For this century and the future, we must advance developments in renewable energy and the utilization of sustainable resources to make chemicals and materials. Approximately 1.4 billion tons of lignocellulosic biomass is an annually renewable source of energy and chemicals in the U.S. alone. The major components of biomass are cellulose, xylan, and lignin- all polymeric and contain high percentage of oxygen. Current biomass processing underutilizes lignin. We have developed selective reaction chemistries that convert lignin selectively into phenolic molecules/synthons. We have coined this process chemistry CDL for Catalytic Depolymerization of Lignin. Spectroscopic data coupled with mechanistic investigations revealed the roles of solvent and catalyst in this unique extractive-reaction, which provides high value from lignin. Renewable triphenol motifs (TPs) have been synthesized and converted to pre-polymer precursors that can be cured into thermosets with advanced thermo-mechanical properties that rival those from petroleum. A fully biobased epoxy thermoset has been prepared by esterification of lignin-derived

TP with vegetable oil to yield materials with tunable mechanical properties and glass transition temperature. The implication and use of lignin synthons to make renewable and recyclable thermoset polymers will be discussed.

3:35 PM 4-6: Engineering metabolic pathways and enzymes for the synthesis of biorenewable chemical precursors in yeast

N. Da Silva, University of California, Irvine, Irvine, CA, USA*
Polyketides and fatty acids are important biorenewable chemical precursors. Both are synthesized via complex polyketide or fatty acid synthases, with many using acetyl-CoA and malonyl-CoA as starter and extender units. We have combined engineering of the pathway and synthase enzymes, metabolic pathway engineering, and improved cultivation strategies to substantially increase titers and yields in the yeast *Saccharomyces cerevisiae*. Two important products have been the polyketide triacetic acid lactone (TAL) and free fatty acids (FAs) of varying lengths; these compounds can be converted via chemical catalysis to a wide range of products (from commodity to high value). For high-level TAL production, our work has focused on overexpression of native and variant *Gerbera hybrida* 2-pyrone synthases, extensive engineering of the yeast metabolic pathways for increased cofactor and precursor pools, and implementation of fed-batch cultivation strategies. These interventions increased TAL titer from 0.07 g/L to 10.5 g/L and yield from <1% to 44% of theoretical. To specify the chain length of free fatty acids, heterologous fatty acid synthases and thioesterases were introduced. Synthesis, degradation, and activation pathways were engineered to increase free fatty acid titers. Recent work has included engineering of native regulatory systems to increase synthesis of both polyketides and fatty acids, implementation of CRISPR-based combinatorial methods, and enhancing yeast resistance to medium-chain fatty acid toxicity. In the presentation, we will highlight the critical pathways engineered, and examine the synergy between successful strategies for the various fatty acid and polyketide products.

4:00 PM 4-7: Choosing the Right Lignin for Phenolic Adhesive Formulation

M. Nejad, Michigan State University, East Lansing, MI, USA and S. Kalami, Mississippi State University, Starkville, MS, USA*
Choosing the Right Lignin for Phenolic Adhesive Formulation

Lignin is a natural polyphenolic compound with great potential as phenol replacement in phenolic adhesive formulations. However, depending on the source (hardwood, softwood or annual crops) or extraction process (kraft, organosolv, sulfite, soda and enzymatic hydrolysis) there are significant variations in lignin structure and properties. In this study, more than 10 different lignin samples were completely characterized and used to formulate lignin-based phenolic adhesives by replacing 100% of phenol with lignin. Among tested lignins, a corn stover lignin isolated through enzymatic hydrolysis proved to be the most suitable lignin for replacing the entire portion of phenol in phenolic adhesive formulation. The formulated lignin-based adhesive had similar wet and dry strength similar to a benchmark phenol-resorcinol formaldehyde adhesive. Based on our characterization results, lignins which have high hydroxy-phenyl content or very high guaiacyl content which have two ortho positions (H-lignin) and one ortho position (G-Lignin) available for reaction with formaldehyde are good candidates for replacing phenol. Additionally on weight basis, replacing 100% of phenol with lignin resulted in reducing the formaldehyde consumption by 75%.

5:30 PM - 7:30 PM Exhibits Open

Grand Ballroom Foyer, Lobby Level

6:00 PM - 8:00 PM Session: PS2: Poster Session 2/Reception

Grand Ballroom A-E Lobby Level

M1 Ethanol production by *Clostridium autoethanogenum*: process and the parameters

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Clostridium autoethanogenum is able to metabolize syngas/CO and synthesize ethanol, some factors affecting its cell growth or product formation were investigated in this study. Although xylose is an easily-used carbon source for *C. autoethanogenum*, the primary product in xylose metabolism was acetate, and nitrogen source didn't have much influence in ethanol production. Two-step fermentation, i.e., growing on xylose and then fermenting with CO, was performed in a 3 L bioreactor to study the batch fermentation of *C. autoethanogenum*. Results indicated that cell growth and acetate production occurred in the first stage, whereas ethanol was primarily produced in the second stage when xylose was exhausted and CO became the sole carbon source. pH value and oxidation-reduction potential (ORP) dropped with cell growth and acetate production, while ethanol production was accompanied by the decrease of acetate concentration and the rise of pH and ORP. But due to the limitations of bioreactor and operating conditions, only 1.71 g/L of ethanol was obtained. To eliminate the limitations, the bioreactor was modified and equipped with a specific device which could keep a constant pressure in the headspace. One-step fermentation was carried out in the modified bioreactor using CO as the sole carbon and energy source. In spite of reduced growth rate and

cell density, *C. autoethanogenum* produced more ethanol than it did in two-step fermentation. The maximum ethanol concentration achieved was 7.36 g/L, and during the whole fermentation process acetate concentration remained lower than 1.1 g/L.

M2 A statistical approach for the identification of cellulolytic enzyme inhibitors using switchgrass dilute acid prehydrolyzates as a model system

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The identification of biomass pretreatment-generated compounds that impede cellulose hydrolysis is critical for improving the overall biomass saccharification process. The aim of this study was to correlate the identification and concentrations of switchgrass dilute acid pretreatment-generated compounds to cellulolytic enzyme inhibition and to tie this back to processing parameters. Twenty-four dilute acid prehydrolyzates were prepared with switchgrass at temperatures from 140°C to 180°C, processing times from 10 to 40 min, and sulfuric acid concentrations of 0.5% or 1% (v/v). Results showed that all the switchgrass prehydrolyzates significantly reduced cellulolytic enzyme activities when assayed against model substrates. Exoglucanase was the most sensitive with its activity reduction ranging from 58% to 88%; the inhibitory effect on β-glucosidase and the cellulase cocktail ranged from 32% to 63% and 16% to 41%, respectively. Polyphenolic compounds were the most detrimental pretreatment-generated products to the cellulolytic enzymes, especially to exoglucanase. Limited enzyme inhibition, with acceptable biomass digestibility were observed with pretreatment conditions corresponding to 160°C. The statistical based approach used in this study proved to be a valid method to assess the effect of pretreatment-generated compounds on cellulolytic enzyme activities, linking their generation to pretreatment processing parameters.

M3 Biocompatible depolymerization of lignin to enable microbial conversion

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Lignocellulosic biomass constitutes the largest renewable carbon source on earth and there is a growing interest in developing processes for conversion of its components into value-added products. Among these processes, lignin valorization is essential for biorefinery economics but remains a major challenge due to its heterogeneity and recalcitrance to chemical or enzymatic depolymerization. The use of microorganisms with the natural ability to assimilate aromatic compounds has been proposed as a strategy for lignin valorization; however, microorganisms cannot efficiently consume lignin in solid state or containing high molecular weight components. It is thus necessary to develop depolymerization approaches that can be compatible with assimilation and conversion by microbial systems. In this work, we developed a base-catalyzed depolymerization (BCD) strategy for a process-relevant lignin-rich stream obtained from corn stover via deacetylation, mechanical refining, and enzymatic hydrolysis approaches. A range of mild temperatures at two base concentrations were examined to identify conditions that could generate a biocompatible depolymerized lignin stream. After determining the BCD conditions that release high concentrations of monomeric aromatic compounds, a screen was conducted to identify aromatic-catabolizing microorganisms that can tolerate the resulting lignin liquors. Microbes that grew well in this stream were subjected to a deeper metabolic characterization to assess the extent of lignin conversion and whether they could elicit changes in the lignin molecular weight distribution. Our results indicate that mild BCD is a potential method to solubilize solid lignin and generate a biocompatible substrate that can be upgraded into value-added bioproducts.

M4 Rapid room temperature solubilization and depolymerization of polymeric lignin at high loadings

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Lignin valorization is of great importance to improve overall biorefinery economics but very challenging due to the relatively poor solubility of lignin in most solvents. In this work, we demonstrated, for the first time, that the rapid room temperature solubilization of lignin at high solid loadings (>30 wt%) can be easily achieved in a single step using ethylene glycol (EG). In addition, the solubilized lignin can be rapidly and quantitatively recovered with the addition of ethanol. Detail computational and nuclear magnetic resonance (NMR) spectroscopic studies confirm that strong hydrogen bond interactions between EG and the free hydroxyl groups present in lignin contribute to the high lignin dissolution. As a potential application of this room temperature solubilization property of lignin in EG, hydrogen peroxide mediated depolymerization of the dissolved lignin at a low temperature (80 °C) was tested and the effect of EG molecules on depolymerization of lignin was also studied theoretically. The findings of this work provide mechanistic insights of hydrogen bond interactions in high lignin solubilization and depolymerization.

M5 Effect of washing and pH on the enzymatic hydrolysis of liquid hot water pretreated switchgrass at high solids content

V. Larnaudie, M.D. Ferrari and C. Lareo, Universidad de la República, Montevideo, Uruguay*
The enzymatic hydrolysis of switchgrass after liquid hot water pretreatment (LHW) was studied at high solids content. High solids content in hydrolysis and its further conversion can lead to energy savings on product separation and purification. It also can have a significant effect on both, economic and environmental parameters of production processes with energy intensive separation stages. The effect of sequential washings (up to four washes) of pretreated solids (solid to water 15% (w/w, dry basis), 200°C, 5 min) with deionized water (10 g water per g dry solids) on enzymatic hydrolysis was studied. Enzymatic hydrolysis assays were performed with 17.5% solid content (w/w, dry basis), 25 FPU/g glucan of cellulase (Cellic CTec 2®), buffer citrate pH 4.8. The hydrolysis yields increased with the number of washings after 72 h, from 56% without washing up to 78% and 85% for one and two washes, respectively. Additional washes did not improve significantly the yield ($p < 0.05$). The effect of pH (4.0, 4.8, 5.0, 5.5, 6.0, 7.0 obtained by adding buffer citrate and sodium hydroxide) at different solids contents (5, 20, 25% (w/w, dry basis)) on enzymatic hydrolysis was also studied. Solids were washed two times with 10 g water per g dry solids. The highest hydrolysis yields obtained at 72 hours for each solid content depended on the pH used: 82% for 15% of solids and pH 4.8, 82% for 20% of solids and pH 6.0, and 56% for 25% of solids and pH 6.0, respectively.

M6 Biomimetic cleavage of lignin with small organic thiols

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Lignin is an energy- and carbon-rich resource that has the potential to replace petrochemicals and fuels. Deconstruction of lignin for conversion into valuable products has remained challenging due to expensive inputs and/or caustic depolymerization techniques. Lignin-degrading enzymes provide an excellent model for a simple and green depolymerization method. The β-O-4 linkage of the lignin polymer is the most abundant and easiest to cleave. In the β-aryl ether cleavage pathway of *Sphingobium sp* strain SYK-6, the tripeptide glutathione reductively cleaves β-O-4 bonds in a three-step process: 1) oxidation of the alpha-carbon hydroxyl group, 2) glutathione nucleophilic attack on the beta-carbon, displacing the phenoxide, and 3) reduction of the above-formed glutathione ether's S-C bond with a second glutathione, releasing the 2nd lignin fragment and the glutathione disulfide. This work focuses on replicating this enzymatic chemistry using small organic thiols. With 2-mercaptoethanol and related thiols, oxidized lignin dimer models undergo 100% conversion with yields of product fragments ranging from 30% to 100% depending on the specific reaction conditions. Applied to real lignin, the process achieves approximately 60% molecular weight reduction. This work exemplifies the first reductive biomimetic approach to lignin degradation by mimicking the nucleophilic thiol-mediated ether cleavage.

M7 The "substrate sensing" of *Trichoderma reesei* – the relationship between substrate architecture, fungal morphology, and enzyme production

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As previously observed, *Trichoderma reesei* seems to have the ability to adjust its morphology, and the profile of secreted enzymes, to reflect the characteristics of the lignocellulosic substrate it is cultivated on. This "substrate sensing" could have the potential of tailoring specialized enzyme cocktails, which can efficiently hydrolyze a broad range of industrially relevant biomass feedstocks. However, to achieve this goal, the underlying regulation mechanisms must be better understood.

We investigated the relationship between substrate architecture (*i.e.* 3D microstructure and composition of the surface accessible to fungal hyphae/enzymes), fungal micromorphology, and the enzyme production. Cultivations were performed on bleached softwood pulp and steam-pretreated wheat straw, using the "wildtype-like" *T. reesei* QM6a and a *cre1* knock-out mutant. Substrate architecture was visualized by a novel technique based on two fluorescence-tagged carbohydrate binding modules (CBMs) that specifically bind different carbohydrate structures, and confocal laser scanning microscopy (CLSM). CLSM was further used to visualize the fungal micromorphology, and computer-aided image analysis enabled semi-quantitative evaluation (*i.e.* degree of branching, single cell dimensions). Using the CLSM methods it was observed that i) substrate architecture as well as fungal micromorphology varied depending on the substrate used, ii) fungal hyphae growth was highly substrate associated, resulting in a 3D microscale network, and iii) the 3D hyphae-substrate-network changed over the cultivation time. When the influence of these variations on enzyme production (protein content, filter paper activity, measured in the cultivation supernatant) was investigated, a semi-quantitative correlation between fungal micromorphology and protein production was apparent.

M8 A Novel fungal GH7 Cellobiohydrolase: Towards Improvement of Cellulose Conversion

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Fungal cellobiohydrolases from glycoside hydrolase family 7 (GH7) are the primary enzymes found in the industrial enzyme formulations used to produce sugars destined for production of biofuels and products. In this work, a novel fungal GH7 cellobiohydrolase was selected from a large natural diversity collection, and produced in *Trichoderma reesei* under the control of

a constitutive eno promoter-driven expression system developed at NREL. The new enzyme was tested and compared to the GH7 cellobiohydrolase from *T. reesei* (TrCel7A) and several other enzymes from the same family. When tested with the synthetic substrate, p-nitrophenyl-β-D-lactopyranoside, the enzyme demonstrated higher specific activity, as well as a higher kcat than the reference enzymes. Moreover, pretreated corn stover and Avicel hydrolysis tests performed at 40, 50, and 60˚C, and in combination with different endoglucanases, demonstrated that the new enzyme has a higher extents of conversion of cellulose when compared to other enzymes, especially in the case of Avicel. Insights regarding mechanism of action were studied using a bioinformatics approach and single-crystal x-ray diffraction analysis underscored features of this new cellulase from a structure/function relationship perspective.

M9 Determinants and products in the cellulose oxidation by the lytic polysaccharide monoxygenase TrAA9A

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Lytic polysaccharide monooxygenases (LPMOs) are one of the rare enzyme types that can oxidize polysaccharides. The prerequisite of the catalysis presence of suitable electron donor and molecular oxygen or hydrogen peroxide. They are applied to total hydrolysis of lignocelluloses, where they remarkably enhance the hydrolysis. The products of the LPMOs are soluble and insoluble neutral and oxidized mono- oligo and polysaccharides, depending on the specificity of the enzyme and type of substrate.

TrAA9A is a LPMO enzyme produced by filamentous fungus *Trichoderma reesei*, which is the common industrial producer of lignocellulolytic enzymes. We have studied the properties of the TrAA9A and formation of soluble and insoluble oxidation products in different reaction conditions in presence of donors, oxygen and hydrogen peroxide. The results indicated that the performance of TrAA9A is dependent on various factors and the overall product formation depends on not only of the enzyme properties but also behaviour of the electron donor in given reaction conditions. Both soluble and insoluble reaction products were detected and dependent on the type of substrate. The impact of the observations on applications the TrAA9A are discussed.

M10 Investigation of Reaction Pathways for Lignin Hydrodeoxygenation during Simultaneous Biomass Pretreatment and Lignin Depolymerization

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Integration of pathways for the production of lignin co-products into cellulosic biofuels processes is one route to potentially improve the economic viability of integrated biorefineries. One of the primary challenges to utilizing lignin as a source for the production of phenolic monomers is the difficulty in achieving both high product yields and selectivities. This is due to lignin's heterogeneity and its substantial modification during pretreatment/extraction processes that can result in the formation of C–C bonds that frustrate efforts for lignin depolymerization. Recently, a related set of catalytic reduction approaches have been identified that can overcome this hurdle and achieve exceptionally high monomer yields (*i.e.*, approaching theoretical maximum from C–O cleavage) by utilizing high-quality lignin extracted directly from the cell wall without lignin pre-extraction under mild conditions and appropriate catalysts. The central features of this approach include (1) "high-quality" lignin from delignification in organic solvents; (2) hydrogenolysis and hydrodeoxygenation of the solubilized lignin over a metal catalyst; (3) potentially generation of surface hydrogen created in situ through reforming of the alcohol solvent on the catalyst surface. In this work, experimental results using a range of catalysts (Ni, Co, Ru, Pd on inert supports), feedstocks (whole biomass, model lignin dimers), and reaction conditions (solvent, addition of H_2) are integrated with density functional theory (DFT) simulations to yield insight into the reaction pathways for lignin hydrogenolysis and hydrodeoxygenation at differing hydrogen surface coverage, which we hypothesize determines reaction pathways for some metals based on preliminary data.

M11 Glycosylation is vital to the unique biochemical properties of *Caldicellulosiruptor bescii* CelA

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Caldicellulosiruptor bescii CelA is the most active thermophilic cellulase yet discovered. It is the most abundant extracellular enzyme in the exoproteome of this hyperthermophilic cellulolytic bacterium and contains bifunctional activity with processive endoglucanase (GH9-CBM3) and exoglucanase (GH48) domains. The relatively long, low complexity linker regions of CelA are extensively *O*-glycosylated, predominantly with galactose moieties. Biochemical and biophysical characterization has demonstrated that glycosylation is essential to the overall physicochemical properties of this hyperthermophilic cellulase including thermal stability, resistance to proteolytic cleavage, and hydrolytic activity. Analysis of intra- and intermolecular endo-exo cellulase synergism of this multi-domain cellulase, employing intact as well as truncated variants revealed the presence of an intramolecular synergism on the hydrolysis of some but not all biomass substrates.

M12 Impacts of Corn Stover Variability on Operational Reliability of a 0.5 MT/d Continuous Pretreatment Reactor

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The recent experiences of the DOE-supported Integrated Biorefineries (IBRs) suggest the ability of the facilities to run continuously without significant unplanned downtime remains a critical problem. The variability in feedstock physical and chemical attributes is largely responsible for the problematic operation of these IBRs, with many of the critical problems observed during biomass handling and feeding into deconstruction reactors. This variability in feedstock properties has multiple sources, including intrinsic genetic and environmental variability in feedstocks, and the effects of harvesting, transportation, and storage. The Feedstock-Conversion Interface Consortium (FCIC) has been developed to to address the technical risks in developing and scaling up biomass harvest, storage, preprocessing, and conversion technologies with the goal of improving the overall operational reliability of integrated pioneer biorefineries. One of the tasks in the FCIC is establish operational reliability and conversion performance baselines for low-temperature primary deconstruction (through enzymatic hydrolysis) of variable corn stover feedstocks. Progress of the baseline experiments performed in a 0.5 MT/d continuous, horizontal pretreatment reactor including operational and sugar yield data will be presented.

M13 Cellobiohydrolase Cel7A mutations and their impact on substrate conversion

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Breakdown of crystalline cellulose is the crucial step in the hydrolysis of lignocellulosic biomass, which is catalyzed by cellobiohydrolases (CBHs) along with endo-β-1,4-endoglucanases and β -1,4-glucosidases. Among the CBHs, glycosyl hydrolase family 7 is the most well studied and have dominated industrial applications of cellulases. These enzymes are known to have slow rate of catalysis, which greatly reduces the efficiency of the cellulose breakdown process. This can be partially attributed to high binding affinity to substrate or decreased product generation due to catalysis per se. Using a random evolution approach, we have generated 9 sets of mutations that have differential activity in response to two different types of substrates. For instance, a mutant 1-1d, which has high specific activity (~200%) and higher k_{cat} (~250%) on *p*-nitrophenyl-β-(1-4)-D-lactopyranoside (*p*NP-L), shows decreased activity (~10%) on cellulosic substrates (Avicel and pretreated corn stover), when compared to wild-type *Trichoderma reesei* Cel7A. On the other hand, another mutant 1-11, has 30% lower specific activity and 50% lower k_{cat} on *p*NP-L than the wild type on *p*NP-L, but higher (~ 7%) cellulosic substrate conversion rates. Structural analyses of these mutant enzymes reveal that position of mutations play a crucial role in their activity. Whereas 1-1d mutation is adjacent to the active site inside the protein, 1-11 mutation resides within the junction of the two catalytic arms of the protein. Analysis of these and other mutants will be presented.

M14 Melanin degeneration through bleaching activity of lignolytic enzyme complex for skin whitening

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Melanin has biological functions, which has protecting tissues from harmful ultraviolet, thermoregulation, cation chelators and antibiotics. Melanin which was decolorized by many lignolytic enzyme such as laccase and peroxidases researched by other researchers. This decolorization reaction showed by lignolytic enzyme complex which consist of laccase and dye-decolorizing peroxidase is not enough for melanin deradation. Laccase-peroxidase enzyme mixture has synergic effect came from hydroxyl group in melanin through autoxidation. Minicellulosome, which was amplified from *Clostridium cellulovorans*, is one of greater potential through structural benefits that enables enzyme complementary effect. Laccase (CueO) from *Escherichia coli* and dye-decolorizing peroxidase from *Bacillus subtilis* are merged with dockerin domain from *C. cellulovorans* endoglucanase B by overlap PCR. And CBM were replaced to 4B4 melanin binding peptide (MBP) for more melanin binding to efficient melanin degradation. MBP was 35 % more bound to melanin than CBM3 in scaffoldin. Dockerin attached cCueO, cDyP, ordinary scaffoldin and MBP attached scaffoldin was purified His-tag purification method and confirmed by SDS-PAGE. To confirm that an enzyme complex was formed, Native-PAGE was used. This final assembled complex caused a significant increase in the level of melanin degradation with 18.1 mg/mL melanin decrement and this result is approximately 2.1-fold higher than single laccase at optimal enzyme complex composition. Developed enzyme complex system may exhibits much greater degradative potential in skin whitening area. Based on this result, this recombinant enzyme complex is suitable for next whitening agent in skin cosmetics industry.

M15 Discovery of novel laccases combining *in silico* screening of metagenomics libraries and their application in lignin degradation

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Laccases have a range of applications in green chemistry such as laccase-mediated systems for improved process economics for biofuel and bio-plastic production. Laccases can be used as an effective pretreatment method for lignin degradation required

for efficient utilization of lignocellulosic biomass to produce cellulose-based chemicals, but also for obtaining molecules with potential as new natural mediators or as scaffolds for organic synthesis processes.

In this study, a total of 20 metagenomic libraries, isolated from a range of environments, were screened for novel laccases. Using a combination of sequence-, domain-, and signature-based searches, a set of the most promising sequences was selected for functional characterization. Five laccases were selected and their ability to deconstruct Kraft lignin isolated from *Eucalyptus* was determined. All five laccases degraded the polymer in the presence of redox mediators. Different parameters such as pH, temperature or reaction time of the process were assessed and reaction conditions optimized. Data will be presented on the characterization of these enzymes, their activity on lignin substrate and the products that were produced.

M16 Effect of urea pretreatment on conversion of wheat straw for methane recovery

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Urea was used to pretreat wheat straw with the advantages of structure deconstruction, its nitrogen source, and prevention of pH drop in subsequent anaerobic digestion (AD). Scanning electron microscopy, X-ray diffraction analysis and Fourier transform infrared spectroscopy measurements indicated that urea pretreatment is able to degrade the lignocellulosic structure, which was beneficial for the improvement of methane production. Urea pretreatment led to the satisfactory performance of AD with wheat straw as substrate. The maximum methane production of 305.5 L/kg volatile solids (VS) was obtained using 1% (w/w) urea loading, which was 45.2% higher than the untreated. Time used for achieving stable status (≥50%) was shortened by urea pretreatment. Higher levels of urea pretreatment (3% and 5%) were less efficient and resulted in the formation of pseudo-lignin according to FTIR. These results indicate that urea pretreatment of lignocellulosic materials is a feasible technology for the purpose of methane production.

M17 Characterization of a novel β-glucosidase from a Mexico native *Clavispora lusitaniae* strain for bioethanol production

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Cellobiose is one of the most abundant sugars resulting from the degradation of cellulose, which is an important step in the bioethanol production process. For this reason, the search of novel microorganisms capable of producing ethanol from cellobiose is necessary; a pathway in which β-glucosidase is a key enzyme.

In this work, we have characterized a β-glucosidase from a *Clavispora lusitaniae* strain, which was isolated from mezcal musts in Oaxaca, Mex. *C. lusitaniae* produced an intracellular β-glucosidase when grew in a minimum media containing 10 g/L of cellobiose, while the synthesis of this enzyme was repressed by glucose. This β-glucosidase presents optimal activity at pH 5.5 and 45 °C; after 90 min under these conditions, the enzyme maintains over 50 % its activity. In anaerobic conditions, *C. lusitaniae* produced 3.4 g/L of ethanol and the β-glucosidase presented the same activity levels as those observed in aerobic conditions (~15 U/mL). Results of this work confirm the presence of a β-glucosidase in a Mexico native *Clavispora lusitaniae* strain, and put forward this yeast as a potential candidate for lower-cost cellulosic ethanol production.

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M18 Copper-catalyzed alkaline oxidative pretreatment efficiently delignifies zip-lignin poplar and produces a lignin stream suitable for reductive depolymerization using biomimetic catalysts

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Our two-stage copper-catalyzed alkaline hydrogen peroxide (Cu-AHP) pretreatment process effectively delignifies hybrid poplar and results in high glucose and xylose yields following enzymatic hydrolysis. Two key strategies have been employed to improve the economic sustainability of this process. In the first approach, we tested hybrid poplar engineered to contain readily cleavable ester bonds in the backbone of lignin (zip-lignin). Employing zip-lignin poplar resulted in increased delignification, improved glucose accessibility, and higher glucose yields relative to the wild-type line. Analysis of the biomass by glycome profiling and microscopy both support our conclusion that the presence of the ester linkages increases delignification and glucose accessibility during pretreatment, highlighting the potential for zip-lignins to reduce processing costs. In the second approach, we are developing biomimetic mild organic catalysts for the reductive depolymerization of lignin into smaller fragments suitable for subsequent valorization. Utilizing small organic thiols to mimic enzymatic etherase chemistry, we demonstrated 100% conversion and up to 95% yield of lignin model compounds. In addition, using this approach we also observed a considerable decrease in molecular weight of authentic poplar lignin, demonstrating the promise of mimicking small molecule-mediated enzymatic ether cleavage to reductively depolymerize lignin.

M19 Exploring abundant marine ascidian bioresources for second generation bioethanol and food-grade prebiotic oligosaccharide production

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The scandinavian native tunicate species *Ciona intestinalis* is a very common and fast growing marine invertebrate animal with cellulose content of up to 60% in the ash-free outer part – the tunic. The tunic is an industrial waste stream that remains after the inner part is used for feed applications and can be utilized for ethanol production, but also constitutes a promising source for oligosaccharides with prebiotic potential. In collaboration with Marin Biogas AB that has already demonstrated a harvesting and dewatering system for large-scale cultivation of *Ciona*, we developed a process that integrates thermal pretreatment, enzymatic decomposition and fermentation for efficient ethanol production. The pretreatment enabled the removal of non-carbohydrate components of the tunic and provided a relatively clean cellulose-rich solid fraction. Consequently, it favored the enzymatic hydrolysis and the subsequent ethanol yield in combination with developing an industrial concept for high gravity hydrolysis. Moreover, the pretreated tunic fractions were used for the production of cello-oligosaccharides (COS) by designing tailor- made efficient enzyme cocktails that boost the product formation. COS belong to non-digestible oligosaccharides with high prebiotic potential and an established beneficial effect in modulating the gut microbiome of both humans and animals. Our results demonstrate that a combination of processive EGs with CBHI in appropriate proportions can enhance the production of COS from pretreated tunicate biomass, showing that the fractionation efficiency of thermal pretreatment combined with a fine-tuned substrate degradation can be used for obtaining cost-competitive food-grade prebiotic COS from non-edible, high abundant novel sources.

M20 Biochemical characterization of a novel GH7 endoglucanase (Af-EGL7)Â from *Aspergillus fumigatus*

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Sugarcane bagasse (SEB) has been considered as the lignocellulosic residue for the Ethanol 2G, produced by breaking down biomass into fermentable sugars. We observed that, *A. fumigatus* secrete a set of glycoside hydrolases to degrade SEB. In this study, a novel gene encoding an endo-1,4-β-glucanase (Afu6g01800) from *A. fumigatus* was cloned into the pET-28a(+) vector and expressed in *E. coli* Rosetta^{TM} (DE3) pLysS strain. Sequence analysis indicated that Af-EGL7 enzyme belonged to GH7 family. *Af-egl7* gene encodes a 460 amino-acid protein with a CBM1 domain at residues 424-460. After protein purification, Af-EGL7 molecular mass was estimated as 51.95 kDa by SDS-PAGE. Enzyme was optimally active at pH and temperature ranging between 4.5–5.5 and 40–60 °C, respectively. The addition of Mn^{2+} significantly enhanced cellulase activity in 233 % and addition of SDS caused a fully inhibition. Substrate specificity analysis revealed a higher activity on β-glucan than on Xyloglucan and CMC, suggesting that Af-EGL7 could be classified as a β-1,3-1,4-glucanase. Time course expression profile was established in different culture media by qRT-PCR. *Af-egl7* gene was induced by different polysaccharides (SEB, CMC, β-glucan, Xyloglucan and Avicel) in a time-dependent manner. In SEB conditions, the *Af-egl7* was highly induced (2500x), suggesting a great potential to hydrolyze complex biomass. The characterization of a novel endoglucanase from the thermophilic fungus *A. fumigatus is* an important tool to optimize bioprocessing applications, as sugarcane bagasse breakdown.

M21 Deletion of a Single Glycosyltransferase in *Caldicellulosiruptor bescii* Eliminates Protein Glycosylation and Growth on Crystalline Cellulose, Implications for Cellulase Activity and Thermostability

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Protein glycosylation pathways have been identified in a variety of bacteria and are best understood in pathogens and commensals in which the glycosylation targets are cell surface proteins, such as S-layers, pili, and flagella, of pathogens. Very little is known about enzyme glycosylation in bacteria. CelA is one of a number of unique multifunctional enzymes produced by *Caldicellulosiruptor bescii,* and is largely responsible for its ability to grow on lignocellulosic biomass without conventional pretreatment. We recently discovered that extracellular CelA is heavily glycosylated. Using bioinformatics we identified a gene

that had not previously been identified as a glycosyltransferase and whose function was unknown. Deletion of this gene in the *C. bescii* chromosome eliminated protein glycosylation including glycosylation of CelA and resulted in failure to grow on crystalline cellulose. Elimination of glycosylation of CelA had a dramatic effect on protein stability. With the genetic tools available in *C. bescii*, this system represents a unique opportunity to study bacterial enzyme glycosylation in a thermophile and on an enzyme of industrial interest.

M22 Photobiocatalysis of cellulose by LPMOs – characterization, product formation and secondary reactions

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Enzymatic deconstruction of biomass holds great promise as a technology to sustainably produce fuels and chemicals. Key players in nature's toolbox for biological conversion of lignocellulose are the lytic polysaccharide monooxygenase (LPMOs), just as they are used in synergy with cellulases to boost the hydrolysis of cellulose. Their catalytic activities differ considerably, dependent on the provided electron donation system and, combining LPMOs with pigments and applying light can greatly enhance their oxidative performance.

For a light-driven LPMO system we present how the amount and profile of products released from cellulosic substrates is affected by several parameters, i.e. the influx of electromagnetic energy, constant or sequential light exposure as well as the type of pigment used and the dry matter content of the substrate. Furthermore, we will discuss the occurrence of secondary reactions derived from reactive oxygen species that can contribute to the product formation but may not be directly related to the LPMO catalysis.

Lastly, we will provide insights into strategies to increase the cellulose conversion efficiency of commercially available and industrially relevant enzyme cocktails when supplemented with pigments and driven by light.

M23 Understanding Polyhydroxyalkanoates (PHAs) Biosynthesis from Biomass-derived Compounds in *Pseudomonas putida* KT2440 via Dynamic Flux Balance Analysis

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Various bacteria have the potential to bio-transform cellulosic biomass to Polyhydroxyalkanoates (PHAs) as carbon and energy storage material under unbalanced growth conditions. In this study, under limited or sufficient nitrogen conditions, *Pseudomonas putida* KT2440 exhibited different PHAs accumulation patterns using biomass model compounds benzoate and glucose with glycerol as the carbon source. Composition and concentration of PHAs with different chain length revealed following an advanced gas chromatography-mass spectrometry (GC-MS) analytical method. Dynamic flux balance analysis further interpreted the PHAs biosynthesis processes in various carbon sources, paving the way in future systems biology design for biomass conversion to bioplastics.

M24 Release of low molecular weight products from Kraft lignin using a laccase-mediator system

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Lignin represents the most abundant source of aromatic substance in biomass. A major source of lignin is from the pulp and paper industry, where it is extracted along with hemicellulose by the Kraft process as a black liquor. This is generally considered a low value or waste product which can be combusted to generate energy for the paper mill's energy requirements. Valorisation of this lignin to a higher value product would improve overall economics for this industry. One route is to deconstruct lignin into monomeric components which can be polymerised to produce a range of industrial materials. This can be achieved via chemical or using oxidative enzymes such as laccases. Lignin molecules are generally relatively inaccessible to the catalytic site preventing direct interaction with the enzyme. Laccase activity can be greatly increased by including a mediator which is oxidised to a stable intermediate with high redox potential and directly reacts with lignin. Here, we screened a range of potential laccase mediators using the reactive black assy. The most active compounds were further screened for deconstruction of lignin using a higher molecular weight fraction (≥ DP3) which was separated from total kraft lignin derived from Eucalyptus. Results will be presented on the release of monomeric phenolic compounds and the formation of insoluble condensation product, following incubation with a *Trametes versicolor* and other laccases. Data will show that the product profile is dependent on pH, temperature and length of incubation.

M25 Insight into the high-pressure CO_2 pre-treatment of sugarcane bagasse for a delivery of upgradable sugars

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A comprehensive and integrated valorisation of lignocellulosic materials using environmentally friendly conversion technologies is the main challenge for the development of sustainable biorefineries. This work aims to contribute to this objective and provides an insight into sugarcane bagasse pre-treatment carried out with greener and more sustainable CO_2/H_2O system. Temperatures and residence times at a fixed initial CO_2 pressure were studied to verify their effects on pre-treatment efficiency with regard to the chemical composition of both water-soluble and pre-treated solid fractions as well as to the susceptibility of the latter to enzymatic hydrolysis using Cellic CTec3 and HTec3 (Novozymes) at high total solids. Also, trends in enzymatic hydrolysis were also analysed in function of biomass crystallinity. This work provides an integrated approach in analysis of upgradable sugars released in inseparables steps of biomass processing, *i.e.* pre-treatment and subsequent enzymatic saccharification. At optimal pre-treatment conditions (190 °C, 0 min), 17.2 $g \times L^{-1}$ sugars were released in the water-soluble fraction mainly as pentoses in monomeric and oligomeric forms. The enzymatic hydrolysis of solids produced at these pre-treatment conditions gave 76.8 $g \times L^{-1}$ glucose in the substrate hydrolysate. The overall sugar yield released in both pre-treatment and enzymatic hydrolysis was 73.9 mol%. These results were compared to the chemical effect of hydrothermal and physical effect of N_2-aided hydrothermal processes and showed that the greener processing of biomass pre-treatment with CO_2 is advantageous for the integrated valorisation of industrial residues and delivery of upgradable sugars within the biorefinery concept.

M26 Engineering of yeast strains for cost-efficient lignocellulosic ethanol production

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For cost-efficient lignocellulosic ethanol production, Toyota developed yeast strains improved D-xylose, L-arabinose and acetic acid utilization and reduced glycerol production with metabolic engineering. Furthermore, we adapted genetically modified yeast strains to improve the rate of D-xylose utilization and enhance tolerance for lignocellulosic hydrolysate inhibitors (e.g. acetic acid, furfural and 5-hydroxymethyl furfural) with evolutionary engineering.

Recent advances in our study are improvement tolerance of yeast strains to high-temperature and inhibitors with evolutionary engineering. The evolved strain can utilize acetic acid with high concentration of inhibitors in lignocellulosic hydrolysate. Consequently, the evolved strain can achieve higher ethanol yield and rapid fermentation with various kinds of lignocellulosic hydrolysate.

For commercial production, we confirmed all inserted genes were stably integrated in the genome of yeast strains. Moreover we confirmed the robustness of fermentation ability of the evolved strain at 46kL tank scale. Our research continues and we are continuously improving our strains to adapt the industrial needs of commercial production.

M27 Reprogrammed pathways of genetically engineered industrial yeast for xylose utilization

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Conventional industrial yeast *Saccharomyces cerevisiae* is superb in glucose consumption but limited in uptake and utilization of xylose. Introduction of heterologous genes is commonly used to enable xylose metabolism. In this study, we report reprogrammed pathways for a genetically engineered industrial yeast derivative stain NRRL Y-50463 that enabled its utilization of xylose using pathway-based qRT-PCR array analyses. Strain Y-50463 has a genetic background with a synthesized yeast xylose isomerase gene *YXI* in its chromosome XV and a set of plasmid-carried heterologous genes from *Scheffersomyces stipitis*, including xylitol dehydrogenase, xylulokinase, and two xylose transporter genes *XUT4* and *XUT6.* The extremely high levels of constitutive expression of *YXI* served as a necessary initiating step for xylose metabolism facilitated by the heterologous xylose transporter and utilization genes. The highly activated transketolase and transaldolase genes *TKL1*, *TKL2, TAL1* and *NQM1* as well as their complex interactions in the non-oxidative pentose phosphate pathway were critically important for the serial steps of sugar transformation to drive the metabolic flow of sugar into glycolysis. The introduced *YXI*-led heterologous gene set changed gene expression profiles of the yeast. Consequently, the altered gene interactions favored the non-oxidative pentose phosphate pathway in Y-50463, which enabled xylose to be transformed into glycolysis for increased ethanol production. Our results also suggest the industrial yeast, with many desirable characteristics, can be further improved to serve as a better delivery vehicle for new strain development in efficient lignocellulose-to-advanced biofuels production.

M28 Endophytic fungi ofÂ *Paullinia cupana*Â andÂ *Rhizophora mangle* and their potential for (hemi)cellulolytic enzymes production using pretreated sugarcane bagasse as substrate

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Depending on the types of lignocellulosic biomass and pretreatment methods, the compositions and their physical properties vary significantly. Therefore, custom enzymatic mixtures should be employed for the efficient hydrolysis of the biomass. Endophytic fungi offer excellent prospects for finding new strains producing enzymes. Through millions of years of coevolution

with plants, these fungi evolved a catalytic inventory to support their role as primary degraders of plant polysaccharides. *P. cupana* is an Amazon native plant and its fruits are used by the industry of soft and energy drinks. *R. mangle* is an estuarine species that can tolerate saltwater and extended flooding. Their endophytes has not yet been fully explored for production of lignocellulosic biomass-degrading enzymes. The objectives of this work were to evaluate a collection of endophytic fungi on the ability to produce lignocellulolytic enzymes in solid agar, and evaluate (hemi)cellulolytic activities in submerged fermentation using sugarcane bagasse pre-treated by steam explosion as carbon source. 266 strains from *P. cupana* and 38 from *R. mangle* were analyzed in solid media containing carboxymethylcellulose, Avicel, starch, xylan, or pectin, and were identified by sequence analysis of the ITS rDNA region. Eighteen strains were selected for cultivation on submerged fermentation conditions using sugarcane-pretreated bagasse, and FPase, β-glucosidase, and xylanase activities were determined. Interestingly, the best-selected fungi in comparison with *Trichoderma reesei* Rut-C30 belong to taxonomic groups that have not been exploited for industrial enzymes production (*Bionectria*, *Diaporthe*, and *Fusarium)*, indicating that endophytic fungi are a prolific source of new strains for the saccharification process

M29 Pathway discovery and strain engineering in *Pseudomonas putida* KT2440 for the valorization of S-lignin-derived aromatics

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Lignin is an abundant heterogeneous polymer found in plant tissue and formed through the polymerization of *p*-coumaryl, coniferyl and sinapyl alcohols compounds (H, G and S-lignin types, respectively) by combinatorial oxidative radical coupling. *Pseudomonas putida* KT2440, a robust soil bacterium, has the natural ability to utilize aromatics from lignin biomass as carbon and energy sources. The biological funneling of the H and G-lignin-derived aromatics into the central intermediates protocatechuate or catechol is well studied in this microorganism. Additionally, metabolic engineering has been extensively applied in order to valorize such biomass into added-value fuels and chemicals.

We recently discovered that *P. putida* KT2440 is also able to metabolize S-lignin derived aromatics. This offers great opportunity for the efficient conversion of S-lignin derived aromatics into compounds of industrial interest. We have performed pathway engineering and enzymatic characterization to elucidate S-lignin degradation pathways in *P. putida* KT2440. Going forward, metabolic engineering and enzyme evolution approaches will be implemented to develop strains for the bioproduction of polymer precursors from S-lignin derived aromatics.

M30 Down Regulating the Ethanol Pathway in *Zymomonas*

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Zymomonas mobilis has been engineered for efficient conversion of lignocellulosic biomass to ethanol because of its natural tolerance to ethanol and high sugar concentrations, fast sugar utilization rate, low biomass yield, high product yield, and ability to grow and ferment under anaerobic conditions. By applying classical recombinant DNA technologies and advanced genetic tools, we have not only augmented *Z. mobilis* for efficient ethanol production from cellulosic biomass hydrolysates, but also further introduced and expressed heterologous genes into this potential commercial strain for the production of other biofuel molecules or precursors (e.g., hydrocarbons). *Z. mobilis* is known to be an outstanding producer of a key intermediate in the Entner–Doudoroff pathway, pyruvate. However, despite our efforts to overexpress pathway enzymes for the production of new molecules, ethanol remains the dominant product from fermentation, confirming the strong activity of pyruvate decarboxylase (PDC) in *Zymomonas*. PDC shunts pyruvate to ethanol, leaving little substrate for other pathways. This enzyme has been shown to be highly expressed in *Zymomonas*. Here, we present a strategy for targeting the PDC gene for down regulation with the intention to increase the availability of pyruvate as the substrate for desirable (non-ethanol) products. We demonstrate the effect of PDC down regulation on the improved yield for the production of several biofuel related molecules.

M31 Engineering *Pseudomonas putida* KT2440 for catabolism of of alkylated-phenolics

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Lignin is an abundant but underutilized source of renewable carbon. For the production of next-generation bio-based fuels and chemicals, pyrolysis of ligno-cellulosic material is a promising technology, however processing can often yield large amounts of undesirable by-products including acids, aldehydes, and phenols. Phenol or its alkylated derivatives are not only toxic but incapable of being metabolized by most organisms. Here we utilized *P. putida* KT2440, a soil bacterium that can metabolize a broad slate of molecules derived from biomass, including lignin, and whose robustness in the presence of toxic chemicals is well documented. In this work, we integrated non-native metabolic pathways for the complete catabolism of alkylated phenols into value-added products.

M32 Discovery of novel biomass-degrading enzymes from anaerobic rumen fungi via metatranscriptomics

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The microbial community that inhabits the cow's rumen, an anaerobic region of the cow's stomach where feed is efficiently fermented, is composed of a complex mixture of microorganisms. Whereas a significant number of biomass-degrading rumen bacteria have been isolated, only a few anaerobic rumen fungi have been isolated and cultured under laboratory conditions. This explains why very little is known about this group of microorganisms. With recent advances in isolation and cultivation techniques and the development of cultivation-independent omics techniques, it has become clear that anaerobic fungi possess a large repertoire of biomass-degrading enzymes and are among the most prolific degraders of recalcitrant plant material.

In an ongoing study we are employing metatranscriptomics to enhance our understanding of anaerobic fungi and their molecular machinery. Analysis of ~4.5 Gbp of assembled fungal metatranscriptome data, generated from rumen-incubated biomass, revealed a total of ~79,500 transcripts containing at least one of 73 conserved domains that are specific for genes with biomass degrading activity. Notably, we also identified transcripts with multi-domain structures specific for components of cellulosomes, multiprotein complexes that have been primarily studied in anaerobic bacteria.
We are currently in the process of selecting the most promising candidates of the identified biomass-degrading genes for synthesis and subsequent physicochemical characterization of the corresponding recombinant proteins. Results from the functional characterization will provide us with a framework to obtain a better understanding of the physiological role of the tested proteins and the function of anaerobic fungi during biomass degradation in the rumen ecosystem.

M33 Efficient bioconversion of enzymatic hydrolysate to aldonic acids by *Gluconobacter oxydans*

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We will discuss the method of producing gluconic acid and xylonic acid using the bacterial strain *Gluconobacter oxydans* NL71 while carrying out microbial fermentation. One of the by-product 2-ketogluconic acid formation was prevented by addition of 10 g/L $ZnCl_2$. When using synthetic media containing 75.3 g/L glucose and 26.7 g/L xylose, about 93.9% of gluconic acid and 93.4% of xylonic acid conversion were obtained in 36h with the supplementation of 10 g/L $ZnCl_2$. When using acid pretreated corn stover hydrolysate containing 73.2 g/L glucose, 30.8 g/L xylose as carbon source, 63.0 g/L (93.9 %) of gluconic acid and 33.8 g/L (93.4 %) of xylonic acid utilizing almost all the sugars in the hydrolysate. The results show the production of high value products like gluconic acid (used as food additive, cleaning mineral deposits and acidity regulator) and xylonic acid (used in bakery products, an acidulant in meat products, and cement formulation) using lignocellulosic materials. In another study, we tried to improve the yield of 2-ketogluconic acid using *Gluconobacter oxydans* NL71 fermentation, by supplementing H_2O_2 to the synthetic medium and enzymatic hydrolysate. We found that H_2O_2 helped to produce 2-ketogluconic acid when using synthetic medium, but did not work well when using dilute acid pretreated corn stover hydrolysate as carbon source. Details about different strategies that has been explored to improve the production of 2-ketogluconic acid using dilute acid and ammonia pretreated corn stover hydrolysate as carbon source and the role of $ZnCl_2$, H_2O_2 on *Gluconobacter oxydans* performance will be discussed.

M34 Comparison of Chimeric CBH I in Yeasts and the Effects of CBH I Fusion with Endoglucanase

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We have characterized the expression differences, biochemical properties and catalytic proficiency of *T. reesei*/*T. emersonii* chimeric CBH I (*TrTe*CBH I) in yeasts and built a multi domain enzyme that links the *T. reesei* endoglucanase 2 (*Tr*EG2) to the *TrTe*CBH I. Characterization of chimeric *TrTe*CBH I in three industrially used yeasts, *Y. lipolytica, L. starkeyi and S. cerevisiae* shows interesting differences in catalytic activity and glycosylation properties. The fusion protein experiments show that *Tr*EG2-*TrTe*CBH I fusion protein can significantly improve the yields of active glycoside hydrolase in *L. starkeyi*. Together these results help in industrial yeast selection for biomass utilization and shed light on how linking proteins could improve yields. Overall, our results are a step forward in engineering an efficient cellulolytic yeast and better understanding the interplay between glycosylation, expression level, stability and biomass deconstruction proficiency.

M35 Engineering *Clostridium saccharoperbutylacetonicum* for enhanced isopropanol-butanol-ethanol (IBE) production from lignocellulosic biomass through acetic acid pretreatment

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The demand for renewable energy has evoked the interests for biofuel production from lignocellulosic biomass. During biomass pretreatment, chemical reagents are generally used to overcome biomass recalcitrance barrier. These reagents, even at low concentration, are usually severe inhibitors for following fermentation. On the other hand, the acetone-butanol-ethanol(ABE) production through clostridial-fermentation has attracted lots of attention because the produced biobutanol is an outstanding fuel source (with various advantages over ethanol) and also valuable chemical feedstock. However, acetone within the ABE mixture cannot be used as biofuel due to its corrosive effects on engine parts. Thus, it is considered as undesirable. In this study, we developed an innovative acetic-acid based pretreatment method. Comparing to other commonly used reagents, acetic-acid provides mild condition and thus generates less inhibitors from degradation; meanwhile, the residual acetic-acid could serve as

carbon source for enhanced production. Further, we metabolically engineered *Clostridium saccharoperbutylacetonicum* N1-4 (a well-known hyper-ABE-producing strain) to produce isopropanol-butanol-ethanol(IBE, which can be used directly as fuel source) using the cutting-edge CRISPR-Cas9 technology. After optimizing pretreatment and fermentation, we were able to produce IBE efficiently using our engineered strain from switchgrass hydrolysates generated through acetic-acid pretreament. Overall, a total of 9.4g/L IBE(based on 20g/L biomass) and 0.47g/g solvent yield obtained in batch fermentation. To the best of our knowledge, our results represents the highest IBE yield in batch culture with biomass as subtract that reported. This research can lead to significant economic benefits for lignocellulosic biofuel production, and potentially result in an enabling process for bio-economy industry.

M36 The production of lipids from the growth of oleaginous yeasts on the hydrolyzates of steam pretreated biomass substrates

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Rhodotorula graminis proved to be a promising strain for the production of lipids, after the microorganism was grown on the water soluble fraction of the SO_2 catalyzed steam pretreated biomass (using corn steep liquor (CSL) for nitrogen source). *R. graminis* produced higher amounts of lipids compared to nine other oleaginous yeasts, despite the presence of furfural and 5-hydroxymetyl furfural (5-HMF), which are known to inhibit the growth of microorganisms. Although increasing of CSL content from 8 g/L to 160 g/L increased the growth rate (OD_{600}) of *R. graminis* about three times, the lipid content of *R. graminis* decreased from 32.5% to 12.8%. This suggested that the amount of nitrogen in the media had an inverse relationship with *R. graminis* lipid accumulation. This phenomenon was also observed when *Yarrowia lipolytica* and *Lipomyces starkeyi* were used. The poster will describe how the nitrogen content of the media influences lipid production when *R. graminis* is grown on the water soluble fraction of the SO_2 catalyzed steam pretreated biomass.

M37 Discovering Pathways for Biological Conversion of Poplar Wood to Lipids by Co-Fermentation of *Rhodococci* Strains

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Biological routes for utilizing both carbohydrates and lignin are important to reach the ultimate goal of bioconversion of full carbon in biomass to biofuels and biochemicals. In this study, a series of natural and engineered *Rhodococcus* strains (e.g. *R. opacus* PD630*, R. jostii* RHA1*,* and *R. jostii* RHA1 VanV$^-$) with lignin degradation and/or lipid biosynthesis capacities were selected to establish a co-fermentation module that enabled a platform for fundamental understanding of bioconversion pathways of glucose and lignin to lipids. Profiles of metabolites produced by *Rhodococcus* strains following growth on different carbon sources (e.g. alkali lignin, flowthrough pretreated poplar slurry) revealed several unexpected fermentation products, suggesting novel metabolic capacities and unexplored metabolic pathways in these organisms. Although *Rhodococci* showed preference to glucose over lignin, nearly half of the lignin was quickly depolymerized to monomers by these strains for cell growth and lipid accumulation after glucose was nearly exhausted. Proteomic profiles showed that lignin depolymerization by *Rhodococci* involved multiple peroxidases with accessory oxidases. Besides the β-ketoadipate pathway, the phenylacetic acid (PAA) pathway played a predominant role in the in vivo ring cleavage activity. Deficiency of reducing power and cellular oxidative stress led to lower lipid production while using lignin as the sole carbon source compared with that of using glucose. Results in this study suggest that synthetic reconstruction and balanced modification of key regulators and enzymes in lignin depolymerization, aromatic compound metabolism, lipid biosynthesis, and other relevant processes will enable efficient conversion of both lignin and carbohydrates to lipids by *Rhodococci*.

M38 Membrane engineering: a key technology for the development of robust *Saccharomyces cerevisiae* strains

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Acetic acid released from biomass during pre-treatment and hydrolysis results in lignocellulose-based fermentation media that typically contains 5-10 g/l acetic acid. Weak organic acids such as acetic acid enter the cell predominantly by passive diffusion through the cell membrane and can heavily affect cell physiology. Furthermore, products of the cell factory could be toxic to the cell, or act synergistically with acetic acid or other inhibitors and make the growth conditions even harsher for the microorganism.

Acetic acid released from the biomass reduces the efficiency of fermentation by *Saccharomyces cerevisiae*. Increased tolerance to weak organic acids can generally be achieved in three ways: decrease intake, increase efflux or metabolize the weak organic acids.

Our prior research has shown that membrane composition is a crucial factor for acetic acid prermeability. Furthermore,

membrane composition and premeability are imperative determinants of yeast tolerance to this compound. In the present study, we are investigating metabolic engineering strategies to modulate the composition of yeast membrane towards desired physico-chemical properties. In particular, the work presented aims to reduce acetic acid permeability by increasing membrane thickness and rigidity. The strategy is guided by molecular dynamics simulations and it will be achieved by increasing the mean fatty acid length of membrane lipids by enhancing activity and substrate availability to metabolic reactions that preferentially use longer fatty acids in the production of glycerophospholipids. In our contribution we will present our latest membrane engineering results and discuss membrane engineering as a vital strategy to obtain key properties in industrial microorganisms.

M39 An Experimental and Computational Study of the Intracellular Pathways in *Methylomicrobium buryatense*, a Promising Methanotroph

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Over the past 30 years methanotrophs have moved from a “black box” organism to being on the cusp of becoming the next biocatalyst in a promising biotechnical world. Of the many different methanotrophs, *Methylomicrobium buryatense* 5GB1 is a promising strain because of its relatively fast growth rate in medium that is resistant to contaminants and due to its potential to generate organic acids, ectoine, and desirable lipids for biodiesel.

In this work we provide a systematic approach in analyzing the carbon use and growth patterns of 5GB1 with an in house gas mixing system that safely creates a variety of headspace conditions with methane, oxygen, and nitrogen. Of particular interest are the yields of methane to biomass, to carbon dioxide, and to organic liquid products in batch and continuous cultures. The approaches used overcome challenges with headspace analysis and increased CO_2 solubility. In doing so, an overall balance of 95%-105% was consistently achieved.

With an accurate carbon balance, the specific gas pickup and metabolite production rates were utilized to examine possible electron transfer routes to oxidize methane in a genome scale metabolic model. Additionally, metabolic modeling provides initial evidence on intracellular carbon distribution through the central core carbon network and ATP requirements. Through this analysis, both experimental and computational data provide insights on the phenotype of 5GB1 under different experimental conditions, adding to the knowledge base required to design processes for improved methane bioconversion.

M40 Lipid production by oleaginous yeast from lignocellulose and crude glycerol for sustainable biodiesel and fish-feed

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First generation biodiesel provides a low per-hectare energy yield, is competing with food production and can promote rain-forest cutting. Aquaculture is utilising large resources of either wild caught fish or food-grade plants to cover the demands for oil and protein in the feed. Oleaginous yeasts can accumulate lipids to more than 50% of their biomass, utilising waste raw materials such as crude glycerol from biodiesel production or lignocellulose (including hemicellulose); these lipids can be used both for second generation biodiesel production and as ingredient in fish feed. We have tested a variety of ascomycetous and basidiomycetous oleaginous yeast strains for growth and lipid production on hemicellulose hydrolysate and crude glycerol. We tested novel methods for *in situ* determination of lipid content and composition. Basidiomyceteous yeasts such as e.g. *Rhodotorula* and *Rhodosporidium* spec., usually produced lipids more rapidly and to higher concentrations from lignocellulose and crude glycerol than ascomycetes such as e.g. *Lipomyces* spec. Fermentation techniques were introduced to test lipid production under reproducible conditions.[1] In an analysis of a biorefinery approach, including also biogas and electricity production from fermentation residues, the energy output from biolipid production from lignocellulose had an energy balance similar to ethanol production (41% of the total energy in the biomass)[2]. We have also tested lignocellulose-based yeast oil as ingredient in fish feed, and did not find any negative impact on the cultivated fish.

References:

1) Brandenburg et al. 2016. Yeast 33, 451-462

2) Karlsson et al. 2016. Biotechnol Biofuels 9, 229

M41 Engineering oleaginous yeast strains in the *Yarrowia* clade for enhanced growth and lipid production from lignocellulosic biomass

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Oleaginous yeasts have attracted increasing attention for their ability to accumulate intracellular lipids from abundant lignocellulosic biomass feedstocks. However, fermentation inhibitors— generated during conventional biomass pretreatment processes—have been observed to inhibit yeast growth. For example, the industrial yeast *Yarrowia lipolytica*, which is a model species for lipid production, is inhibited by very low concentrations (<0.5 g/L) of the xylose degradation product furfural. Chemical methods to condition the hydrolysate are expensive. Engineered strains may lower processing costs, but gene targets have not been previously identified to increase inhibitor tolerance in oleaginous yeasts. In this study, an oleaginous strain from

the *Yarrowia* clade—producing up to 3-fold higher lipid titers than control strain *Y. lipolytica* W29 in a diluted biomass hydrolysate—was engineered for *in situ* detoxification of inhibitory furan compounds. To this end, several heterologous gene targets were independently expressed under control of the strong *Y. lipolytica* TEF promoter, and transformants were screened for growth performance in liquid culture with or without a furfural challenge (10 mM). The best recombinant strains showed a significant reduction in lag time and increase in biomass productivity and growth rate ($P < 0.01$) as compared to the control strain in the presence of furfural. The robust, engineered *Yarrowia* yeast was further characterized to evaluate other relevant traits, including *in vitro* enzyme activity and lipid production kinetics in dilute acid-pretreated biomass hydrolysate. In summary, this work describes engineering and characterization of robust oleaginous strains in the *Yarrowia* clade for lipid and biofuel production from renewable biomass.

M42 Develop CRISPR-Cas Genome Engineering Tools and Engineer Solventogenic Clostridia for Enhanced Biofuel and Biochemical Production

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Clostridium is a genus of Gram-positive, rod-shaped, anaerobic bacteria. Many of the species are excellent workhorses with great potentials for industrial biofuel and biochemical production. The growing interests in biofuel and biochemical research in recent years stimulated the development of the genetic engineering tools that can be employed to understand clostridial physiology and strain improvement. However, this is generally difficult due to the lack of efficient transformation protocols and amenable genetic engineering systems. Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated (Cas) system is an immune system in bacteria and archaea that can efficiently cleave foreign DNA entering the cells. Recently, CRISPR-Cas has been extensively explored as a revolutionary genome engineering tool for both eukaryotic and prokaryotic organisms. In our lab, we focus on the development of efficient genome engineering tools for solventogenic clostridia based on the CRISPR-Cas system, and employed the developed toolkits to enhance the desirable phenotype of the target strains related to biofuel and biochemical production. This includes the production of butanol and derivatives, long-chain alcohols, esters, etc. For example, in one of the engineered strains, the n-butanol production in a regular batch fermentation reached 26.2 g/L, which is, to our best knowledge, the historically record highest among all the reported from the literature. The detailed results from several relevant projects will be presented in this talk. Our developed protocol has a broader applicability to other microorganisms with underdeveloped genetic engineering tools. Our work can provide essential references for the researchers in the relevant research communities.

M43 Photosynthetic C5 Redirection Enhances Terpene Productivity

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Photosynthetic terpene production represents one of the most energy and carbon efficient route for converting CO2 to hydrocarbons. We hereby use squalene as a model compound to demonstrate the concept of C5 redirection, where photosynthetic carbon will be redirected to terpene biosynthesis through the conversion of xylulose-5-phosphate into DXP. Squalene biosynthesis was chosen as the ideal carbon sink for the proof-of-the-concept due to the broad commercialization of the compounds and the rapid turnover of the enzyme for enhancing downstream productivity. The farnesyl pyrophosphate synthase(FPS) and Squalene synthase(SQS) were over expressed to catalyze the last two reactions of isoprenoid metabolic pathway with and without 3,4-dihydroxy-2-butanone 4-phosphate synthase(RibB) mutant enzyme to catalyze the conversion of xylulose 5-phosphate(X5P) or ribulose 5-phosphate(R5P) to DXP. The design overcomes the inefficiency of the first step of MEP pathway, improved carbon conversion efficiency of the pathway by 1/6, and enhance the carbon flux into terpene biosynthesis. The results showed that RibB over-expressed lines have 66% increase in the squalene yield with 9.9% decrease of photosynthesis rate. Metabolomics analysis confirmed the decrease of ribulose/xylulose and ribose. In addition, the increase of pyruvate and 3-hydroxy-3-methyl glutarate(HMG), suggesting that an effective C5 redirection to MEP allowed the accumulation of upstream compounds in competing pathways. Overall, the study opened new avenues to redirect photosynthesis carbon into alternative carbon sinks for high-value products. Further optimization and design of the pathway to increase photosynthesis carbon fixation and C5 sugar recycling could help to mitigate the reduced photosynthesis and to increase terpene yield.

M44 Discovery of a natural allele in yeast that improves biofuel production and tolerance to ionic liquids

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Imidazolium ionic liquids (ILs) are effective pretreatment solvents used to extract cellulose from plant biomass for saccharification and subsequent microbial fermentation. However, ILs such as 1-ethyl-3-methyl imidazolium chloride ($[C_2C_1im]Cl$) are toxic to biofuel producing bacteria and yeast, causing impaired cell growth and production efficiency. We and others have shown that natural strains of *Saccharomyces cerevisiae* isolated from a wide variety of ecological niches have extensive genomic sequence differences that can project into a range of industrially beneficial phenotypes, including tolerance to inhibitory chemicals present in lignocellulosic hydrolysates. We phenotyped a panel of 136 sequenced *S. cerevisiae* isolates and identified strain 378604X, which tolerates high concentrations of $[C_2C_1im]Cl$. A library of 378604X genomic DNA fragments

were transformed into the [C_2C_1im]Cl-sensitive *S. cerevisiae* strain, BY4741, and screened for enhanced IIL tolerance. From this screen, two genes, *SGE1* encoding a multidrug efflux pump and *"ILT1"* encoding a predicted membrane protein of unknown function, were found to increase IIL tolerance when overexpressed and increase sensitivity when deleted. By comparing the genotypes and phenotypes of our *S. cerevisiae* strain panel, we identified two linked SNPs within *SGE1* that correlate with IIL-tolerance. Introducing the tolerant *SGE1* SNPs into the sensitive BY4741 strain by CRISPR/Cas9 significantly improves IIL-tolerance and ethanol production. Biochemical studies suggest that these SNPs stabilize the Sge1 protein, potentially allowing greater efflux of toxic IILs out of the cytoplasm. Our results make way for more efficient microbial biofuel production, and highlight the potential of untapped natural sequence variation for useful biotechnological functions.

M45 Ranking microbial organisms for optimal production of target compounds with RetSynth

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The biological production of compounds used in fuels are essential in decreasing the United States dependence on foreign oil. Using microbial organisms to synthesize these compounds is an attractive method in achieving energy independence that is cost and time efficient. Metabolic activity has been characterized or inferred for thousands of potentially industrially viable microbial organisms. Determining which organism will facilitate the highest yield of a target compound is difficult. There are a number of factors to consider, including the organisms' growth times and metabolic structure that may result in one organism producing higher yields. RetSynth is a tool that identifies enzymes/genes that, if added to a specified organism, result in production of a target compound. It also predicts the yield of a compound in an organism with the added genes using flux balance analysis (FBA). Using this tool, we are able to identify from set of potential host microbial organisms the ones that will give the highest rates of production of a given target compound. We have previously used related machine learning techniques to determine a set of compounds that were predicted to be good fuel blending agents. Here we use RetSynth to determine how each could be produced biologically. We identified genes that need to be added to a number of organisms, including *E. coli* DH1, *Pseudomonas putida* KT2440, and *Streptomyces venezuelae* ATCC 10712, to achieve production of target fuel blending agents and then simulate their metabolism to identify which organism will generate the highest theoretical compound yields.

M46 Characterizing Metabolic Pathways of *Rhodococcus jostii* RHA1 Converting Biorefinery Wastes to Lipids

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Biorefinery waste valorization is an attractive but challenging topic for economic biorefinery design which improves the carbon efficiency of the entire process. *R. jostii RHA1* is well known for its robust growth, adaptions to various environmental conditions, and remarkably versatile ability in catabolizing a wide range of carbon sources. In this study, growth of *R. jostii* RHA1 and its variant Δ*atf8* mutant on mixed carbon sources which mimicked the biorefinery wastes, including lignin-derived aromatics (vanillin, vanillic acid), furans (furfural, 5-HMF), and weak acid (acetic acid), as well as ethanol refinery solid residues from dilute-acid pretreated corn stover were investigated. The accumulation of lipids in these two strains influenced by different compounds was compared, and the complex metabolism network in *Rhodococcus* was discussed. In addition, the catabolic pathways of furfural and 5-HMF in *Rhodococci* were addressed.

M47 Deletion analysis of the itaconic acid production gene cluster components in *Aspergillus pseudoterreus*

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The filamentous fungus *Aspergillus pseudoterreus* has been successfully used for industrial production of itaconic acid for many years. The itaconic acid gene cluster has been identified by a transcriptomics approach through examining the time course of itaconic acid production. The cluster consists of four genes, including genes for cad (*cis*-aconitic acid decarboxylase), a predicted transcription factor (tf), mitochondrial organic acid transporter (mtt), and MFS (Major Facilitator Superfamily) type transporter (mfs). We investigated those genes in detail for their role in itaconic acid biosynthesis by making deletions in *A. pseudoterreus*. Gene deletion analysis demonstrated that *cad*, *tf* and *mtt* genes in the cluster are essential for itaconic acid production; deletion of any of them will either completely abolish the itaconic acid production or dramatically decrease itaconic acid levels to trace amounts. When whole cell extracts were incubated with *cis*-aconitic acid, those extracts from Δmtt and Δmfs will produce itaconic acid, but not the Δcad and Δtf mutant strain cell extracts. Next, overexpression of cytosolic citrate synthase and aconitase in the both Δcad and Δmtt will be done to further clarify the function of each component in this cluster.

M48 Systems and synthetic biology advancements to improve *Synechocystis* sp. PCC 6803 strain engineering in the industrially-relevant condition of diurnal light-dark cycles

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Cyanobacteria are an interesting chassis for industrial chemical production due to their ability to utilize sunlight and carbon

dioxide as substrates. However, much of the strain engineering has been done under low- and continuous- light laboratory conditions as opposed to the realistic day/night cycle of outdoor sunlight availability. Our lab previously demonstrated that engineered free fatty acid production is decreased in daily light-dark cycles as opposed to continuous light relative to wild-type. This observation motivated system and synthetic biology developments to improve strain engineering efforts specifically in realistic day/night cycles. Toward this goal, we have improved systems biology understanding and developed synthetic biology tools for use in day/night cycles. Specifically, we discovered and characterized four native *Synechocystis* sp. PCC 6803 promoters which enable light-activated gene expression in daily light-dark cycles. We engineered a photobioreactor system which enables diurnal sinusoidal light cycles with peak-light intensities reaching over 1,500 µmol photons m^{-2} s^{-1} and report interesting *Synechocystis* sp. PCC 6803 growth in these conditions. Lastly, we developed and implemented a multi-platform 'omics study investigating the dynamic behavior of *Synechocystis* sp. PCC 6803 in sinusoidal day/night cycles. Together, these advances contribute to the advancement of *Synechocystis* sp. PCC 6803 as an industrially-relevant chassis for chemical production.

M49 Developing cellulolytic *Yarrowia lipolytica* as a platform for consolidated bioprocessing of cellulose to valuable products

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The bioprocessing of cellulosic biomass is still insufficiently mature to allow its widespread industrial implementation. With the aim to develop a consolidated bioprocess for cellulose bioconversion, we first conferred cellulolytic activity to the most widely studied "nonconventional" oleaginous yeast *Yarrowia lipolytica*, and further exemplified how this cellulolytic *Y. lipolytica* strain can be used as a CBP platform for the production of target products. Our results reveal that overexpression of SCD1 and DGA1, two enzymes involved in lipid biogenesis, confers the obese phenotype to the cellulolytic *Y. lipolytica*. The resulting strain consumed 12 g/L cellulose and accumulated 14% (dry cell weight) lipids in batch conditions. The introduction of an extra copy of the endogenous *YlLIP2* gene into cellulolytic *Y. lipolytica* led to the production of a strain capable of producing lipase 2 on cellulose. When grown in batch conditions, the engineered strain consumed 16 g/L cellulose and produced 9.0 U/mL lipase over a 96-h period, a production yield representing 60% of that obtained on glucose. Finally, expression of the hydroxylase from *Claviceps purpurea* (CpFAH12) in cellulolytic *Y. lipolytica* procured a strain that can produce ricinoleic acid (RA). Using this strain in batch cultures revealed that the consumption of 11 g/L cellulose sustained the production of 2.2 g/L RA in the decane phase, 69% of what was obtained on glucose. Overall the engineered cellulolytic *Y. lipolytica* strains presented herein can be described as a promising prototype for the development of CBP aimed at converting cellulose into a wide variety of commercially-relevant products.

M50 Direct kinetic comparison of the two cellobiohydrolases Cel6A and Cel7A from *Hypocrea jecorina*

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Cellulose degrading fungi such as *Hypocrea jecorina* secrete several cellulases including the two cellobiohydrolases (CBHs) Cel6A and Cel7A. The two CBHs differ in catalytic mechanism, attack different ends, belong to different families, but are both processive multi-domain enzymes that are essential in the hydrolysis of cellulose. Here we present a direct kinetic comparison of these two enzymes acting on insoluble cellulose. We used both continuous- and end-point assays under either enzyme- or substrate excess, and found distinct kinetic differences between the two CBHs. Cel6A was catalytically superior with a maximal rate over four times higher than Cel7A. Conversely, the ability of Cel6A to attack diverse structures on the cellulose surface was inferior to Cel7A. This latter difference was pronounced as the density of attack sites for Cel7A was almost an order of magnitude higher compared to Cel6A. We conclude that Cel6A is a fast but selective enzyme and that Cel7A is slower, but promiscuous. One consequence of this is that Cel6A is more effective when substrate is plentiful, while Cel7A excels when substrate is limiting. These diverse kinetic properties of Cel6A and Cel7A might elucidate why both cellobiohydrolases are prominent in cellulolytic degrading fungi

M51 Rate-limiting step and substrate accessibility of cellobiohydrolase Cel6A from *Trichoderma reesei*

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The fungal Cellobiohydrolases (CBHs) are processive enzymes that catalyze the hydrolysis of cellulose. The two types of CBHs are denoted Cel6 and Cel7, respectively, which are the most abundantly produced and secreted cellulases in the fungus *Trichoderma reesei.* The overall hydrolysis process of CBHs can be simplified into three reaction steps: adsorption, the inner processive cycle, and dissociation, governed by three rate constants. In the present study, we applied a recently developed quenched-flow technique to investigate kinetics of Cel6A from *T. reesei* working on cellooligosaccharides and two different cellulosic substrates, the micro-crystalline cellulose Avicel and regenerated amorphous cellulose (RAC). We analyzed the burst kinetics of the enzymes on the cellulosic substrates, and investigated the rate limiting steps in the enzymatic hydrolysis process. In addition, the substrate accessibility on the two different substrates was examined by the kinetic analysis.

M52 Targeting the hemicellulosic fraction of biomass: economic assessment of hydrogenation and ethanolysis of furfural for production of ethyl levulinate

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Hydrolysis of hemicelluloses leads to a plethora of products, many of them undesirable in fermentation processes. Moreover, the main sugar commonly obtained from this process, xylose, is proven to have a low fermentation performance when compared to glucose. Xylose and other pentoses easily dehydrate to furfural, a furan which has demonstrated potential to be produced at low cost. This low-cost possibility opens opportunities to other uses, such as applications in the fuels market. Furfural itself cannot be used as fuel since it is very unstable, and therefore it requires a chemical upgrade. This work presents the technoeconomic assessment of the chemical upgrade of furfural to ethyl levulinate. In this process, furfural is hydrogenated to furfuryl alcohol, which is then mixed with ethanol in a reaction to open the furan ring to yield ethyl levulinate. Hydrogenation is a crucial, costly step of this process, and two options of hydrogen source were considered: steam reforming of natural gas, representing a benchmark, and electrolysis of water using electricity produced from biomass as a renewable counterpart. Economic and sensitivity analysis determined a maximum buying price range for furfural to make the chemical upgrade economically feasible, aiming at an internal rate of return of 10% and considering the selling price of ethyl levulinate to be the same of diesel on an energy basis. These results demonstrated the potential of the hemicellulosic fraction of biomass in the production of a fuel additive of renewable origin.

M53 A feasibility and life cycle assessment of poplar-based production in Lewis County, WA

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Modeling results derived under the Advanced Hardwood Biofuels Northwest consortium revealed that Lewis County, WA is an ideal candidate for construction of a poplar-based biorefinery. Lewis County has favorable climatic conditions, including a relatively high average rainfall, eliminating the need for crop irrigation and ample acreage for growing poplar. Poplar is a promising feedstock selection due to its ability to thrive under minimal conditions and its short rotation coppicing. The case for building a biorefinery in Lewis County is especially compelling if the biorefinery can be co-located with a natural gas power plant, where inexpensive excess medium pressure steam generated from the power plant can be utilized by the biorefinery, eliminating the need for a boiler. Environmental and economic impacts of producing ethyl lactate from purpose-grown poplar farms will be investigated. The feedstock assessment will determine the quantity of poplar that could be grown, where, and at what costs. It will also determine the quantity, price, and availability of nearby residues including agricultural and hardwood residues from harvest, nearby sawmills, and wood product manufacturers. Poplar tree farms providing ecosystem services such as flood mitigation or treatment of wastewater effluent from nearby facilities will also be investigated. The life cycle assessment will determine life cycle carbon emissions, water consumption, and fossil fuel consumption. Results will be compared to N-Methyl-2-pyrrolidone, corn-derived ethyl lactate, and corn-stover derived ethyl lactate. Lastly, IMPLAN software will be used to determine the direct, indirect, and induced effects associated with the new biorefinery.

M54 Technoeconomic analysis method to supply a biorefinery with sufficient high-quality biomass at the lowest possible cost.

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Selection of biorefinery location is dependent upon the availability of sufficient quantities of biomass that meet quality specifications at a reasonable cost. This is evidenced by the siting of pioneer biorefineries in the Midwest corn belt where abundant corn stover is available. This study used a technoeconomic analysis (TEA) driven approach to determine the best combination of feedstocks and preprocessing operations to supply a biorefinery located in South Carolina with 800,000 tons of high quality biomass per year at the lowest possible cost. Potential biomass available in South Carolina included corn stover, switchgrass, logging residues, pine, and construction and demolition (C&D) waste. It was assumed that combinations of these feedstocks would feed a biorefinery producing bio-oil using fast pyrolysis. The Biomass Logistics Model developed at the Idaho National Laboratory (INL) was utilized to determine the costs to harvest, transport, preprocess, and deliver each feedstock to the biorefinery gate. To ensure appropriate quality, it was assumed that the biomass would be treated to remove ash and other inhibitors using methods previously developed at INL including air classification and acid leaching. TEA models for these processing methods were used to estimate the costs to upgrade biomass quality. The model favored the use of C&D and logging residues despite their low quality since their lower overall cost made it more cost effective to upgrade their quality rather than use more expensive pristine biomass. It was also determined to be more cost effective to use a biomass blend rather than any one single feedstock.

M55 Scotland – a land of opportunity for Biorefining

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The Scottish National Plan for Industrial Biotechnology is on a mission to grow the industrial biotechnology industry to 900 million pounds by 2025. This will be achieved by developing new biotechnology solutions as well as adopting existing biotechnology solutions in current supply chains. A substantial part of the industry growth could come from resource recovery

and biorefinery activities. In order to guide and stimulate the development of these opportunities in Scotland a unique feedstock mapping model has been developed with the aim of identifying key data sources on arisings (waste or co-products) of materials streams with potential value, assess the quality of these data sources and review composition, map quantified material arisings regionally and also use known fate to generate figures of available bio-resources. This model in combination with a strong capabilities in chemical manufacturing, synthetic biology and marine science puts Scotland in a strong position to develop its bioeconomy. In addition, an overview of activities aimed at establishing pilot and demonstration scale facilities will be presented

M56 Economic opportunities for a sweet sorghum-based biorefinery in Florida

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Sweet sorghum is a grass with tall (3-5 m) stems that accumulate soluble sugars. These sugars can be easily extracted and fermented directly to ethanol or other renewable chemicals. The bagasse that remains can be used as a lignocellulosic feedstock. Sweet sorghum can be grown as a complement to sugarcane, or as a dedicated bioenergy crop, especially in areas that are too cold for sugarcane. Improved sweet sorghum cultivars developed at the University of Florida (UF) can generate over 1,000 gallons of ethanol per acre (10,000 L/ha) from juice and bagasse combined.

In order to determine sweet sorghum's economic potential, we have performed a techno-economic analysis of producing ethanol from sweet sorghum bagasse based on data obtained at the UF Stan Mayfield pilot biorefinery. The process combines phosphoric acid pretreatment followed by liquefaction plus simultaneous saccharification and co-fermentation by a recombinant *E. coli* strain. Several scenarios were modeled in SuperPro Designer and the most optimistic scenario resulted in a minimum ethanol selling price that was close to the price of gasoline.

An economic analysis conducted by ABF Economics indicated that the economic activity associated with a commercial biorefinery that produces 20 million gallons of ethanol per year from sweet sorghum bagasse could support >700 jobs and generate $6.3M in tax revenues. A life cycle analysis by Life Cycle Associates was performed to quantify the reduction in greenhouse gas emissions relative to the use of gasoline. The combined data underscore the economic potential of sweet sorghum. Supported by USDA-BRDI project 2011-10006-30358.

M57 Genome-wide association study of biorefinery-relevant biomass traits in *Salix viminalis*

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Salix species (willows), primarily cultivated for bioenergy purposes (i.e. burning), also represent promising feedstocks for biorefineries, due in part to their high rates of biomass production and short harvest cycles. To study the genetic background with regard to breeding for improvement of traits relevant to biorefinery usage, a genome-wide association study (GWAS) was performed. 323 accessions of *Salix viminalis* collected in Europe and Russia were clonally propagated and planted in a field experiment in Uppsala, Sweden (59°48' N, 17°39' E) using a randomized complete block design. Two-year-old plant material from 4 blocks (n=1120) was evaluated for sugar release using NREL's high-throughput pretreatment and hydrolysis platform (180°C, 17.5 min, log R_0=3.6), lignin syringyl:guaiacyl ratio using py-MBMS, compositional analysis using FT-NIR, as well as for other phenotypic measurements such as density. 19592 of the SNPs generated in a genotyping-by-sequencing effort were used in the association analysis together with phenotypic data, taking population structure into account. Significant SNP–phenotype associations were identified, and genes found in proximity to the SNPs include enzymes involved in energy and secondary metabolism as well as several transcription factors. *P. trichocarpa* transcription factor orthologs were shown to be expressed in wood forming tissues, where expression data was available. Broad-sense heritability (H^2) estimates ranged from 0.34 for xylose release to 0.69 for wood density. These results are promising for use in marker-assisted selection to accellerated breeding but will also give important information for generating hypotheses on the genetic mechanisms underlying these traits in *Salix*.

M58 Modeling and analysis of operating conditions effects of sugarcane bagasse gasification in a circulating fluidized bed using Aspen Plus^{TM}

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Gasification is a thermochemical process that converts a wide range of carbonaceous resources mainly into syngas, a gaseous mixture that contains H_2, CO, CO_2, CH_4 and light hydrocarbons, and can be applied in the generation of heat, power, and fine chemicals. Such versatility of the gasification technology, both in terms of feeding and applications, makes it an interesting thermoconversion option for underused feedstocks like sugarcane bagasse. Sugarcane bagasse, an abundant agricultural residue derived from the sugar-alcohol industry, is usually burned in boilers for power generation, a practice that results in low yields and ash production. Despite some experimental initiatives on sugarcane bagasse gasification, none of them focuses on obtaining high-quality syngas for higher added-value applications. Due to this necessity, this work aims at studying via

simulation the operating conditions that lead to a high calorific power syngas in a sugarcane bagasse gasification process that takes place in a circulating fluidized bed. To achieve this goal, a simulation model based on Gibbs free energy minimization was proposed in the Aspen Plus^{TM} software, and the effects of gasification temperature, pressure, steam-to-biomass ratio, and biomass moisture content on H_2/CO ratio, syngas lower heating value and energy demands were assessed in a 2^4 factorial design. The results have shown that sugarcane bagasse gasification is a potential upstream process for chemicals production, contributing as an alternative not only for the usual bagasse processing methods but also for the development of current bagasse gasification activities.

M59 Improving Sugarcane as a Feedstock

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Sugarcane is a tropical and subtropical crop described as one of the most efficient and sustainable biomass feedstocks for the producion of a broad range of high value biochemicals and bioproducts. With a complicated breeding history, commercial sugarcane cultivars are allopolyploid hybrids with a complex polyploid and an aneuploid genome of 10 Gbp with 100-130 chromosomes. The economic and technical challenge, for more widespread production of all sort of chemicals and polymers is to improve the deconstruction of the recalcitrant lignocellulosic matrix composed of cellulose, hemicelluloses, and lignin. Genetic approaches to improving the potential for disruption of sugarcane biomass rely on modifications to reduce lignin polymerization and enhance saccharification. To achieve this goal this study focuses on transcriptome analysis with total RNA sequencing (RNA-Seq) to study the differential expression of transcripts in different genotypes. Alternative splicing events detection, functional annotation, metabolic pathways analysis, and differentially expressed transcript isoforms are being characterized. A detailed analysis of the isoforms of the enzymes involved in key biosynthesis pathways will be presented. The identification of candidate genes for selection of modification is the key strategy of this study with the objective to improve cost and quality of sugarcane bioconversion.

M60 The IBSS Partnership: Creating the Foundation for the Southeast's Bioeconomy

T. Rials and J. McCord, The University of Tennessee, Knoxville, TN, USA*

A key barrier to expanding the South's bioeconomy is the availability of affordable, sustainable, high-performance feedstock. The IBSS Partnership, sponsored by the U.S. Department of Agriculture (USDA NIFA), has addressed this challenge through an expansive, integrated program that takes into consideration the unique aspects of biomass production in the region. To meet the demand for a new industrial sector producing fuels and chemicals, new biomass sources are needed that provide higher yields and offer improved performance for diverse technology platforms. Short-rotation woody crops like hybrid poplar and eucalyptus hold considerable promise for the region, as do herbaceous crops (switchgrass, sorghum, etc). Progress has been made toward identifying management practices that maximize growth and yield, improve harvest and logistics, and define quality metrics of performance. This work has effectively advanced the technology readiness of these innovative energy crops, and introduced landowners to the benefits afforded by alternative crops. Additionally, research has provided new insight into biomass characteristics that impact conversion efficiency, providing guidance for continued process improvements. This poster presentation will highlight key developments associated with biomass production and quality characteristics, as well as efforts directed at education and workforce development.

M61 The effect of cultivar and nitrogen on biorefining potential of wheat and triticale straw

H. Jørgensen, J. Van Hecke, L. Baldwin and J.K. Schjoerring, University of Copenhagen, Frederiksberg C, Denmark*

To meet the future demand for biomass for cellulosic biorefineries it is relevant to select for cereal cultivars with maximum output of grain and straw. In this respect nitrogen supply is an important factor affecting yields and quality.

The study comprises field trials with 14 cultivars of commercialized winter wheat and one triticale at three levels of N fertilizer conducted during three growth seasons. The triticale Trilobit had the highest average grain yield (10.7 t/ha, 6% above average), but more profoundly the straw yield was on average 60% higher (12.0 t/ha) compared to all wheat cultivars. All wheat cultivars had rather similar straw yields. Trilobit therefore has great potential with respect to overall biomass productivity (22.7 t/ha), but the wheat cultivar Creator also showed high yield (18.5 t/ha). Increasing the N application from 100 kg/ha to 160 kg/ha resulted in higher grain and straw yield, but beyond that limited effect on yield was observed.

The content of both cellulose and lignin was rather conserved among the cultivars and did not respond to N-application. The enzymatic saccharification efficiency of the straw revealed no effect of cultivar nor N application. Therefore, the straw yield is the most dominating factor determining the biorefining potential.

A high content of Si can be problematic for the valorization of the lignin residue. The study revealed that Si concentration decreased with increasing N-application. Clear cultivar differences were observed. Again Trilobit was among those with the lowest Si content and overall has very favorable traits for biorefining purposes.

M62 Characterizing Cellulose Surface Changes by Nonlinear Vibrational Spectroscopy

L. Zhang, Z. Xu and B. Yang, Washington State University, Richland, WA, USA; Z. Wang, Pacific Northwest National Laboratory, Richland, WA, USA; H. Wang, Fudan University, Shanghai, China*

In this study, Total Internal Reflection Sum Frequency Generation Vibrational Spectroscopy (TIR-SFG-VS) combined with non-TIR-SFG-VS enabled detection of the molecular structures of surface layers and the crystalline core of cellulose, which correlates with the biomass recalcitrance. From the SFG spectra in the C-H and O-H regions, it was found for the first time that the surface layers of Iβ cellulose differed in structural and spectroscopic signatures with its crystalline core. This work demonstrates the capacity of TIR and Non-TIR SFG-VS in selectively studying the structures and polymorphs of cellulose. This discovery is significant because it not only challenges the traditional understandings of cellulose materials that neglects the differences between its surface and core region as well as their effects on the cellulose deconstruction reactivity, but also provides a novel spectroscopic tool to measure the spectral signature of the bulk crystalline core and the surface layer of cellulose. In addition, dynamic detection of the structural changes of cellulose surface layers and crystalline core induced by thermal treatments was conducted. Results lead to transformative advances in understanding of biomass recalcitrance on the molecular level in terms of cellulose basic structures and conformations (i.e. surface and crystalline core assembling), including changes in biomass structure resulting from reduced recalcitrance phenotypes as well as hydrothermal pretreatment.

M63 Label-free characterization of cellulose as constituent biopolymer of lignocellulosic biomass using multiphoton Raman bioimaging

S. Vilms Pedersen, H. Karring, M.A.B. Hedegaard and E.C. Arnspang, University of Southern Denmark, Odense M, Denmark; S.D. Hafner, Aarhus University, Aarhus, Denmark; T. Jeoh, University of California - Davis, Davis, CA, USA*

Accurate biomass characterization, in terms of surface topography and chemical composition of constituent biopolymers, is of significant importance, both in industry and in research. Characterization at micron-scale is frequently done using different microscopy techniques, including atomic force microscopy (AFM) and different fluorescence techniques such as Stimulated Emission Depletion, Spinning Disk Microscopy or Total Internal Reflection.

A common drawback of the canonical fluorescence microscopy techniques is that successful imaging and visual identification of constituent biopolymers in complex substrates, is highly dependent on auto fluorescing fluorophores or staining- specificity and efficiency of selected dyes. Furthermore, both fluorescent probes and auto fluorescing fluorophores undergo photobleaching over time, which may complicate quantitative imaging. Coherent Anti-Stokes Raman Scattering (CARS) is a third order optical process, which can be used to probe molecular vibrations inherent to the constituent biopolymers of the substrate, enabling chemically specific imaging. CARS is a multiphoton technique generating signals that are stronger than typical spontaneous Raman scattering, allowing video-rate data acquisition and optical sectioning, irrespective of staining efficiency and specificity. The purpose of this work was to conduct non-contact based label-free imaging of cellulose, a constituent biopolymer of lignocellulosic biomass, using CARS. CARS-acquired images providing structural information are stacked into a hyperspectral array for multi-image analysis, providing chemically specific information about the image. Subsequently AFM microscopy can be applied to obtain detailed information about the surface of the substrate. Future work includes applying the developed CARS method to more complicated lignocellulosic matrices, containing several other biopolymers.

M64 Influence of nitrogen supply and harvesting time on the potential for biorefining of triticale to protein and sugar

H. Jørgensen, S.T. Thomsen and J.K. Schjoerring, University of Copenhagen, Frederiksberg C, Denmark*

This work focus on investigating the biorefinery advances of early whole crop harvest of cereal biomass in comparison to traditional harvest. The study shows that early harvest enables fractionation of the green biomass into a protein and sugar rich juice and a press pulp that is less recalcitrant towards carbohydrate conversion compared to mature straw. Combined with the possibility of harvesting a second cover crop in late autumn this increase overall biomass productivity and improve the biorefining of lignocellulosic biomass into protein for food/feed and sugars for production of fuels or chemicals as demanded in a bioeconomy.

The triticale cultivar Trilobit (hybrid of wheat and rye) was selected due to high biomass productivity, average 24.9 t DM/ha over a three-year period. Three levels of nitrogen application were tested in the field study. Samples were collected one and two months prior to final harvest and then at final harvest in August. The maximum dry matter biomass output was from the harvest one month prior to harvest of the mature crop (24% higher). Green biomass from the early harvest was fractioned into a sugar and protein rich juice and a press cake. Protein content in the juice was significantly influenced by the level of N-application and harvest time. Press cake and straw from the final harvest were subjected to hydrothermal pretreatment followed by enzymatic saccharification. Conversion efficiencies of the carbohydrates were higher in the green biomass compared to mature straw, thereby demonstrating green biomass to be less recalcitrant compared to mature straw.

M65 Next-generation bioenergy sorghums for successful cultivation in the southeastern United States

W. Vermerris, T. Silva, L. Stutts, A. Abril and M. Riley II, University of Florida, Gainesville, FL, USA; H. Cuevas, USDA-ARS, Mayaguez, Puerto Rico*

Sorghum is an attractive feedstock for the production of renewable fuels and chemicals due to its low input requirements, resilience to adverse environmental conditions, and great yield potential. The southeastern United States is well suited for bioenergy production because of its climate and abundance of land. Sorghum cultivation in this region requires adaptation to low-fertility soils, high disease pressure, and strong winds. We are using multiple approaches to accomplish this. 1) The genome-editing tool CRISPR/Cas9 is promising for creating mutations in genes of interest in many species. However, transgenic sorghum has been met with concern over transgene transmission to wild, weedy relatives. Hence, the generation of transgene-free plants through genome editing has potential to improve sorghum in a targeted, biosafe way. We are targeting *Cinnamate 4-hydroxylase* to reduce lignin concentration and/or alter lignin subunit composition to enhance biomass conversion. 2) Our breeding approaches have resulted in high-yielding sweet sorghum cultivars that are able to withstand hurricane winds and that are resistant to anthracnose, a fungal disease that can cause severe yield losses and that is omnipresent in the southeastern U.S. We have identified several novel resistance loci. 3) Sweet and biomass sorghums need to be tall to maximize the yield of fermentable sugars, but this prevents combine harvesting of the grain needed for the next generation. We have generated short-statured, combine-compatible inbred lines that can be crossed with each other to generate tall progenies for the production of renewable fuels and chemicals. Results from these different approaches will be highlighted.

Tuesday, May 1

7:00 AM - 8:00 AM Speaker Breakfast

Starfish, Lobby Level

7:00 AM - 5:00 PM Registration

Grand Ballroom Foyer, Lobby Level

8:00 AM - 11:25 AM Session: 5: Engineering Operations for Biomass Processing II

Conveners: Maria Cuellar Soares, TU Delft, The Netherlands;**Charlie Wyman**, UC-Riverside, USA and **Jack Saddler**, UBC, Canada

8:00 AM 5-1: Engineering a diverse chemical product platform capable of utilizing multiple carbon feedstocks via fermentation

S. Frykman[*]*, Renewable Energy Group, Life Sciences Division, South San Francisco, CA, USA*
REG is the leading North American producer of biomass-based diesel and is committed to offering cleaner, low carbon products. REG Life Sciences, its South San Francisco based subsidiary, is developing a pipeline of high-value and commodity renewable chemical products that complements the REG fuels business and leverages REG's existing expertise in manufacturing and commercialization.

REG Life Sciences has demonstrated the versatility of its microbial fatty acid platform for the production of both drop-in and higher value fatty acid derived molecules. However, some of these secreted compounds can be detrimental to the producing organism. Approaches to biological product detoxification, as well as the utilization of lower cost alternative feedstocks (such as crude glycerol and cellulosic sugars) will be discussed.

8:25 AM 5-2: A new hybrid lignocellulosic biorefinery design for the production of high-cetane diesel blendstocks via integration of bioconversion and hydrothermal liquefaction operations

J.R. Collett[*]*, J. Billing, A. Meyer and S. Jones, Pacific Northwest National Laboratory, Richland, WA, USA*
We report the production of a high-cetane, candidate diesel blendstock via continuous hydrothermal liquefaction (HTL) of lipid-rich microbial cell mass mixed with corn stover lignin, followed by hydrotreating to a finished fuel product. Our experimental data and recent precedents in the literature support the concept of a new, hybrid lignocellulosic biorefinery design in which lignin is separated from pretreated corn stover hydrolysate using a vacuum filter press, followed by bioconversion of filtered hydrolysate sugars to hydrocarbons via bioreactor cultivation of oleaginous yeast (which may accumulate up to 70% of their dry cell mass as triglycerides). The resulting lipid-rich bioreactor broth is then directly mixed with the corn stover lignin to create a smooth-flowing, pumpable intermediate that may be converted at high loadings to biocrude within a plug-flow HTL reactor. The biocrude is then upgraded to fuel via hydrotreating, while the HTL aqueous phase product is routed to an anaerobic digester for biogas production. This new, bio-thermal hybrid biorefinery design suggests an immediately feasible approach for maximizing the integration of feedstock lignin and residual cell mass into the final fuel, instead of burning them for plant steam as is commonly called for in

current lignocellulosic biorefinery designs for biochemical conversion. Preliminary techno-economic analysis of a hypothetical biorefinery of our design that would process 2,000 mt of dry biomass per day suggests a reasonable R&D path toward a hydrocarbon biofuel with an MFSP of $5/gge, with additional options for attaining $3/gge via the production of a coproduct such as a feed supplement.

8:50 AM 5-3: Intensifying bio-reactor concept with in-situ product recovery using phase separation

K. Steinbusch, A. Oudshoorn, F. Feskens-Snoeck and M. Perdigão Elisiário, Delft Advanced Biorenewables, Delft, Netherlands; R. Verlinden and P. van der Meer, Bioprocess Pilot Facility, Delft, Netherlands; M.C. Cuellar, Delft University of Technology, Delft, Netherlands*

A successful transition of the traditional chemical industry into a bio-based industry is mainly cost driven and will benefit from novel more intensive or integrated processes. One way to intensify processes is the use of in-situ product recovery (ISPR). By continuously removing product during fermentation, the volumetric productivity increases especially in case of product inhibition, inhibition of cell growth or limited product formation.

DAB and Delft University of Technology have developed an in-situ product removal (ISPR) reactor where product is removed via phase separation. The bioreactor has an internal configuration such that it creates its own hydrodynamic flow behaviour both provides internal broth recycle and creates a laminar flow regime enabling phase separation in the reactor.
Criteria for the reactor design were to develop a scalable bioreactor with mild recovery technology that facilitates cell reuse and can be integrated in (existing) fermenters. During the presentation, these design points will be evaluated. A 100L prototype was tested using sesquiterpene *E. coli* fermentation. While running the fermentation, the ISPR reactor was able to recover 90% of the auxiliary phase. The recovered 2nd phase was present as (coarse) cream with recovery rates per cross sectional recovery area of 54 L.m^{-2}.h^{-1}. With this, the pilot run demonstrated the technical feasibility as well as the potential of the ISPR reactor concept.

9:15 AM Break

9:45 AM 5-4: Pilot-Scale Process Integration for Enhanced Process Intensification and Operational Reliability for Biochemical Conversion of Lignocellulosic Biomass to Fuels and Chemicals

R. Elander, National Renewable Energy Laboratory, Golden, CO, USA*

Pilot-scale equipment and systems for the conversion of lignocellulosic biomass to sugars and subsequent biological and/or catalytic upgrading to fuels and chemical are often utilized for process integration development and scale-up of critical unit operations. In a biochemical conversion process, key unit operations often include biomass pre-processing, pretreatment, enzymatic hydrolysis, biological conversion, and upgrading of intermediates to final products. Additionally, process separations and product recovery steps are of increasing importance to produce a wider range of hydrocarbon fuel intermediates and value-added co products.

For lignocellulosic biomass conversion processes, the need to manage the continuous flow of solid feedstocks and process-intermediate slurries into and through scalable pilot plant equipment can be particularly challenging. Conversion performance data from an integrated pilot plant operated at process relevant conditions can provide insights into eventual commercial-scale challenges, particularly when operating at high solids loadings. The use of continuous, pilot-scale equipment also can provide insights into the effects of varying feedstock properties (composition, moisture, particle size/shape, ash content, etc.) on conversion performance as well as operational reliability throughout the conversion train. An understanding of these effects can be used to identify feedstock pre-processing approaches, changes in conversion process conditions, equipment modifications, and control strategies to improve long-term operational reliability while maintaining or even improving conversion performance. Process intensification and simplification strategies with potential to reduce CAPEX and/or OPEX, improve process performance, and increase the overall operational reliability of biochemical conversion processes will be presented.

10:10 AM 5-5: Reducing the fermentation costs for lignocellulosic ethanol production by closing the gap between fed-batch and continuous fermentation

J.D. Knudsen, O. Sibbesen, T. Hvid Andersen and B. Rønnow, Terranol A/S, Copenhagen, Denmark*

Terranol's commercial yeast strains for cellulosic ethanol production have repeatedly shown complete conversion of xylose and glucose in a short period of time and on various lignocellulosic substrates. Fed-batch fermentations have shown ethanol yields above 90% of theoretical in laboratory scale, as well as in pilot and demo scale. The fermentation results achieved in laboratory scale have proven scalable, with decidedly comparable performance obtained when running fermentations side by side in 2 l laboratory scale and 230 m^3 demo scale.

The highest ethanol productivity, during the batch process, is obtained in the co-fermentation phase, and the prolonging of this phase, in a controlled fed-batch process, was the first step to increase the yield and rate and reduce the yeast pitch compared to a batch process. The phase of highest productivity may be prolonged further, and theoretically maintained to infinity, in a

continuous process. Thus, there is potential for further increase in productivity and yield and reduction in yeast pitch, but a continuous process implies great challenges on process design and process control, and it may prove difficult to surmount these in a commercial scale. Various intermediate strategies in the space between fed-batch and continuous processes, with more relaxed requirements, could be more realistic with respect to feasibility and cost control in commercial scale.
By combining high-performing strains with well-designed fermentations, Terranol is pushing further towards a more efficient C5/C6 fermentation of lignocellulosic materials. The latest results will be presented.

10:35 AM 5-6: Uncertainty analysis as a tool to consistently evaluate lignocellulosic bioethanol processes at different system scales

D.B. Nickel, M. Janssen and C.J. Franzén, Chalmers University of Technology, Gothenburg, Sweden; R. Fornell, RISE Research Institutes of Sweden, Gothenburg, Sweden*
Lignocellulosic processes are highly prone to batch-to batch variability, e.g. of raw materials and enzyme activities. This variability can be propagated throughout system scales during process development and optimization, influencing the outputs of bioreaction models, techno-economic analyses and life cycle assessments. As these outputs are the main decision variables for designing and developing lignocellulose-based processes, tools are required to evaluate the influences of process variation at different system scales.

Uncertainty analysis quantifies the effects of model input variations on model outputs. It is an effective tool to consistently propagate process variation throughout scales and analyse its influence on model outputs. As an example, we use a model describing multi-feed simultaneous saccharification and co-fermentation (SSCF) of wheat straw. During the process enzymes hydrolyse the lignocellulosic material to release glucose which can be converted by microorganisms into ethanol. To investigate the impact of batch-to-batch variability in enzyme cocktails, we collected literature data on the enzymatic activity of Cellic CTec2. Retrieved data were propagated in models at bioreactor, techno-economic analysis and life cycle assessment scale. We show how uncertainty analysis can be used to guide process development by comparing different modes of operation. The method can identify economically feasible process ranges with low environmental impact while increasing the robustness of bioprocesses with high variation in raw material inputs. Furthermore, uncertainty analysis could help to identify relevant parameters to choose as response variables in experimental designs.

11:00 AM 5-7: High solid loading bioprocesses for cellulosic ethanol production at a high titer

M. Jin, S. Chen, X. Li, X. Chen, Y. Yuan, R. Zhai, Z. Xu and Z. Wen, Nanjing University of Science and Technology, Nanjing, China; L.D.C. Sousa and B. Dale, Michigan State University, Lansing, MI, USA; V. Balan, University of Houston, Houston, TX, USA*
Conversion of cellulosic biomass to biofuels, such as ethanol, has attracted much attention. The ability to operate at high solid loading is crucially important for cellulosic ethanol production, as it gives high ethanol titer, high volumetric productivity, less waste water to treat and potentially lower costs. However, sugar conversion and ethanol yield linearly decreases with increasing solid loading during cellulosic ethanol production, rendering high solid loading costly.

This talk presents our research efforts in examining the causes of decreased conversion associated with increased solid loading. Degradation products generated by lignocellulosic biomass pretreatment and ethanol produced from fermentation were the main causes of decreased sugar conversion during simultaneous saccharification and co-fermentation (SSCF). Removal of both degradation products and ethanol enabled high sugar conversion at high solid loading, and the estimated ethanol production cost continued to decrease with increased solid loading within parameters tested. Potential integration of the lignocellulosic ethanol process with the corn ethanol process was also studied to alleviate problems with the lignocellulosic ethanol process. Sugar streams from the corn ethanol process were used to dilute the inhibitor-containing lignocellulosic sugar stream and to increase the glucose concentration. The ratio of corn sugars to lignocellulosic sugars were optimized and different lignocellulosic biomass pretreatment methods were tested to evaluate the effects of their specific inhibitor profile. Blending of corn-derived sugars with lignocellulosic sugars increased overall fermentation rates, ethanol titers, and ethanol productivity. Co-production of corn ethanol and cellulosic ethanol could be a transition strategy for large-scale cellulosic ethanol production.

8:00 AM - 11:25 AM Session: 6: Bioproducts from unconventional and waste feedstocks

Conveners: Gregg Beckham, NREL, USA;**Jay Fitzgerald**, DOE-BETO, USA and **Dr. Ken Tokuyasu**, Nat Ag Food Res, Japan

8:00 AM 6-1: Metabolic engineering of *Pseudomonas putida* to convert aromatic compounds into fuels and chemicals

A.M. Guss, Oak Ridge National Laboratory, Oak Ridge, TN, USA*

Pseudomonas putida KT2440 is a highly robust bacterium capable of efficiently utilizing a variety of carbon sources, including aromatic compounds derived from lignin. Recently, *P. putida* has been engineered to valorize lignin streams from lignocellulosic biomass pretreatment processes, and further metabolic engineering holds promise for diversifying the portfolio of products that can be made from lignin. Medium chain length polyhydroxyalkanoates (mcl-PHAs) are natural carbon storage compounds produced by *P. putida* that can also be used as bio-plastics. Through a combination of gene deletions and heterologous expression, we have engineered *P. putida* to increase the yield of mcl-PHAs from both model aromatic compounds and from depolymerized lignin. This work is being leveraged to also produce medium chain length alcohols form aromatic compounds, since both biosynthetic pathways are offshoots of fatty acid biosynthesis. To further increase the compounds able to be made from lignin, we are also exploring production of molecules derived from the TCA cycle, and current progress will be discussed. To enable more rapid progress in *P. putida* metabolic engineering for the conversion of lignin into fuels and chemicals, we are also developing new genetic tools, including constitutive promoters, regulated promoters, and high efficiency genome integration tools for rapid evaluation of heterologous pathways. Together, this work is a critical step forward in demonstrating microbial approaches for lignin valorization to enable sustainable biorefineries.

8:25 AM 6-2: Production of naturally occurring carboxylic acids from anaerobic digestion of organic materials

C. Granda[*]*, Earth Energy Renewables, Bryan, TX, USA*

Anaerobic digestion (AD) is the most robust and versatile fermentation process for conversion of organic wastes and other biodegradable feedstocks. AD uses natural consortia of microorganisms that adapt very efficiently to any organic feedstock and operating conditions. Unlike other fermentation processes, AD requires no sterility, no genetically engineered organisms, and no extraneous enzymes. As a result, AD is also the most inexpensive bio-conversion process in the market. Typically, AD generates relatively low-value methane as its main product. Methane is the final product in the AD conversion stages, with acidogenic bacteria converting the organic feedstock into intermediate organic acids, and these acids, in turn, being converted to methane by methanogenic bacteria. The intermediate carboxylic acids, which are short- and medium-chain fatty acids (e.g., propionic, butyric, valeric, caproic, octanoic acids), are produced in higher yields (>2.5X) than methane and also, once recovered, they demand considerably higher prices (>15X) than methane, thus drastically improving AD value proposition. Earth Energy Renewables (EER) is commercializing its patented technology for efficiently producing and recovering these valuable carboxylic acids from AD. EER has successfully demonstrated that is able to readily produce these acids at high yields and with remarkable high purity without the need for expensive purification techniques. The acids are of excellent quality for the chemical market or to be used as feedstocks for the production of other valuable chemicals such as esters, alcohols, ketones, polyols and olefins and drop-in biofuels such as gasoline, diesel and jet fuel.

8:50 AM 6-3: Evaluating the potential of Glycogen Accumulating Organisms to Recycle Waste Carbon

R. Red Corn[*] *and A. Engelberth, Purdue University, West Lafayette, IN, USA*

Glucose is an important feedstock in the production of biological fuels and chemicals. However, demand for glucose is largely filled through harvesting starch and cellulose based crops which must be stored to match seasonal harvest with continuous demand. In the present work, we evaluate phenotypic glycogen accumulating organisms (GAOs) for their potential to convert the carbon in municipal waste to glycogen, an analogue of starch. It is envisioned that municipal organic waste could be anaerobically degraded to produce mixed volatile fatty acids (VFAs), which are taken up anaerobically by GAOs and then converted to glycogen aerobically. A GAO culture was selected for in a trickling filter reactor through continuous six hour cycles which consisted of feeding a model acid phase digestate, followed by two hours of anaerobic conditions and four hours of aerobic conditions. Then, glycogen accumulation was optimized in the GAO culture at varied pH and temperature conditions. The optimum pH and temperature were used to evaluate the yield of glycogen per unit carbon fed. The work indicates GAOs have the potential to convert biopolymers, food waste, sewage, paper products, and other waste carbon into a stable year round supply of glucose for biological fuel and chemical production.

9:15 AM Break

9:45 AM 6-4: Methane to Value Chemicals: Ectoine and Lipase Production by Methanoptrophic Bacterium *Methylomicrobium alcaliphilum* 20Z^{R}

M. Kalyuzhnaya[*] *and O. Demidenko, San Diego State University, San Diego, CA, USA*

Biological strategies for methane mitigation or its conversion into valuable compounds offer promising new technologies for global warming stabilization and possibly reduction. *Methylomicrobium alcaliphilum* 20Z^{R} is a rifamycin-resistant derivative of a gram-negative, gammaproteobacteria, which is now gaining momentum as a promising industrial catalyst, i.e., it is being developed as the microbial platform of choice for production of fuels and chemicals from methane in both industry and academia.

The major objective of the present study was to establish a catalytic platform for efficient utilization of coal-mine methane and simultaneous production of ectoine (a chemical chaperone for industrial enzymes or pharmaceuticals, a cryoprotectant, a hydrator in skin-care products, and a crop-protecting agent) and industrial enzymes (i.e., Lip L1 lipase) using *M.alcaliphilum*

$20Z^R$. Rational modifications of the methanotroph were carried out, including the rewiring the central metabolic pathways toward C1-to-C4 conversions, deleting ectoine degradation pathways, and enhancing ectoine biosynthesis. Simultaneous production of both LipL1 (0.02 g g^{-1} CDW h^{-1}) and ectoine (0.01 g g^{-1} CDW h^{-1}) upon continuous fermentation in methane-fed bioreactor was demonstrated. Finally, the growth parameters of the wild type and new traits were then tested with coal-mine gas. A 150% enhancement of the targeted product production was observed, most likely due to the higher presence of ethane in the gas mixture.
In summary, a reliable biological process for reduction of the methane content in the coal-mine environment was developed. This technology could provide both environmental (reduction of the global warming impact) and economical (production of value-added compounds) benefits.

10:10 AM 6-5: Carbon-efficient Methane Biocatalysis to Fuel and Chemical Intermediates

M.T. Guarnieri, National Renewable Energy Laboratory, Golden, CO, USA*
Nearly 120 million tons of oil-associated methane (CH_4) and 40 million tons of biogas generated from anaerobic digestion of waste streams are flared annually. In turn, this leads to underutilization of these abundant, high energy gas sources, and results in substantial greenhouse gas (GHG) emissions. Valorization of these gas streams thus represents substantial economic and energetic potential, while concurrently offering a means to reduce GHG emissions. However, the gaseous state of CH_4 makes for a lack of compatibility with current transportation and industrial manufacturing infrastructure, limiting its utilization as a transportation fuel and intermediate in biochemical processes. Microbial conversion of CH_4 to fuel- and chemical intermediates using methanotrophic bacteria offers a modular, down-scalable, and highly selective approach to valorize these gas streams, while concurrently reducing GHG emissions. To this end, we will present a series of metabolic, protein, and fermentation engineering approaches to target carbon- and energy-efficient conversion of methane and anaerobic digestion-derived biogas to fuel precursors and platform chemicals. Additionally, techno-economic considerations and potential opportunities for deployment as both a bolt-on and standalone technology will be presented.

10:35 AM 6-6: P4SB – From Plastic waste to Plastic value using *Pseudomonas putida* Synthetic Biology

N. Wierckx, On behalf of the P4SB consortium | RWTH Aachen University, Aachen, Germany*
275 million tons of plastic waste were produced in 2010 worldwide, with Europe accounting for about 55 million tons/year. The environmental impact of these primarily fossil-based plastics has been broadly discussed. While the vast majority of these polymers are not biodegradable, their strength and light weight provide comparative advantages. Poly(ethylene terephthalate) (PET), for instance, has contributed significantly to reducing energy expenditure during transport. Due to its thermoplastic nature PET is also easy to recycle. However, recycled PET is of lower quality and current recycled PET products struggle to compete with virgin PET on price and quality, leading to an overall European recycling rate of less than 30%. Polyurethanes (PU), are used extensively in a wide range of applications including construction, transportation, furniture, and medicine. Since many PU types have a thermoset nature with covalent bonds, one of the main concerns for this product is the notable lack of end-of-life recycling (<5%). Hence, new ideas that give room for new incentives are required for the recycling of plastics.

We propose to establish plastic waste as a novel bulk carbon source for industrial biotechnology. This strategy allows upcycling of plastic waste, by feeding degraded plastic to microbial bioplastic producers. In detail, the enzymatic degradation of PET and PU, and the possibilities to produce PHA from the resulting molecules will be presented. In addition, synthetic biology possibilities to improve the bioplastic producing microbe, *Pseudomonas putida*, will be shown. Finally, the potential contributions to a more sustainable plastic industry will be discussed.

11:00 AM 6-7: Enhancing carboxylic acid yields from duckweed by acidogenic digestion

O. Calicioglu, M.J. Shreve, T.L. Richard and R.A. Brennan, The Pennsylvania State University, University Park, PA, USA
Duckweeds are efficient aquatic plants for wastewater treatment due to their high nutrient uptake capabilities, growth rates, and resilience to severe environmental conditions. Their high starch, high cellulose, and low lignin content makes them an attractive alternative to agricultural residue and energy crops for conversion into bioethanol, as they do not require intensive pretreatment prior to saccharification. In this study, we investigate an alternative strategy, acidogenic digestion with undefined microbial cultures, to convert duckweed into carboxylates (i.e., volatile fatty acids, VFAs), precursors of higher-value chemicals and biofuels. Previous literature suggests that higher VFA concentrations can be achieved under alkaline conditions of pH 9 to 10, which should simultaneously suppress methanogenic activity to avoid VFA loss. However, the behavior of acidogenic microbial consortia at high pH is not well understood.

This study aims to evaluate the effects of operating conditions such as temperature (35°C or 55°C) and pH (5.3 or 9.2) on the acidogenic digestion of duckweed, to quantify conversion rates, and to identify shifts in dominant microbial taxa.
The highest duckweed-to-carboxylic acid conversion of 388 mg acetic acid/g total solids was observed under basic mesophilic conditions with 25 g/L solid loading, resulting in an average production rate of 0.59 g acetic acid equivalent/L·d. These values are comparable to those reported for other highly digestible organics such as food waste. The superior performance under these conditions was attributed to both chemical treatment and microbial bioconversion. Biochemical mechanisms behind these

observations will be explored through microbial community analysis (in progress).

9:00 AM - 3:00 PM Exhibits Open

Grand Ballroom Foyer, Lobby Level

1:00 PM - 4:25 PM Session: 7: Engineering Operations for Biomass Processing III

Conveners: Jack Saddler, UBC, Canada;**Charlie Wyman**, UC-Riverside, USA and **Maria Cuellar Soares**, TU Delft, The Netherlands

1:00 PM 7-1: Pretreatment Scale-up: An Integrated 1st & 2nd Generation Biofuel Project Case Study

D. Monceaux[*] and R. Agar, AdvanceBio, LLC, Milford, OH, USA

Technology and project development in the field of lignocellulosic-based fuels and chemicals continues, albeit at a slower pace due to the downturn in global petroleum prices which have served to undermine project economics. New lignocellulosic feedstocks made more readily available through the co-location of 2nd Generation projects at 1st Generation biofuels plants offer new project development opportunities. Integration of the biomass process technologies into the 1st Generation biofuel plant offers reduced capital expenditure and improved production economics. Pilot plant operations have proven to be essential in defining key physical and chemical properties of a new class of lignocellulosic feedstocks. In depth knowledge of the new feedstock's chemical as well as physical properties, which often introduce unforeseen challenges during the technology development, piloting and scale-up phases of the project, is critical to the selection and design of commercial scale systems. A scale-down pilot plant approach was employed in the development of data required to mitigate risk associated with implementing the commercial-scale horizontal screw pretreatment reactor process technology and ancillary material handling and process equipment.

1:30 PM 7-2: Feedstock-Conversion Interface Consortium: Providing Innovative Solutions to Address Operational Challenges Faced by Biorefineries

M. Resch[*], National Renewable Energy Laboratory, Golden, CO, USA and C. Li, Idaho National Laboratory, Idaho Falls, ID, USA

Many of the process bottlenecks experienced in nascent biorefineries are the result of feedstock handling failures due to the inherent chemical and physical variabilities of biomass energy crops such as corn stover (low temperature conversion) or pine residues (high temperature conversion). The interface between unit operations from the field to the conversion reactor throat is a hotspot for problematic operations of Integrated Biorefineries (IBRs).

Related research and development (R&D) efforts at 8 DOE national labs (ANL, INL, LANL, LBNL, NREL, ORNL, PNNL and SNL) have been coordinated into a Feedstock-Conversion Interface Consortium (FCIC). The FCIC goals are to identify and address the impacts that feedstock chemical, mechanical and physical variability have on supply logistics, storage handling, preprocessing and conversion equipment operation and process integration, so as to develop and validate improved integrated feedstock/conversion processes that increase the operational reliability. The DOE National Laboratories possess unique capabilities, which will be leveraged to establish results that surpasses what an individual lab or company can deliver.
This talk will give an overview of the R&D in five areas: 1) Feedstock Variability and Specification Development, 2) Feedstock Physical Performance Modeling, 3) Process Integration, 4) System-wide Throughput Analysis, and 5) Process Control and Optimization, which all aim to coordinate with industry advisors and stakeholders to deliver solutions with the intention of near term industry adoption. These advances will lead to a robust U.S. Bioeconomy enabling agricultural development, domestic job creation, energy security and the reduction of greenhouse gas emissions.

2:00 PM 7-3: Key factors for achieving high operating reliability and conversion yields in scaling up pretreatment processes

Q. Nguyen[*], M. Anderson, P. Bonebright, W.A. Smith, R. Kinoshita and N.A. Yancey, Idaho National Laboratory, Idaho Falls, ID, USA; R. Elander, E.M. Kuhn, D.A. Sievers, X. Chen, N. Nagle and M.P. Tucker, National Renewable Energy Laboratory, Golden, CO, USA

Pioneer biorefineries have encountered many challenges in quickly achieving plant design throughput and biofuel conversion yields. The difficulties are caused by many factors: variable physical and mechanical properties of biomass feedstock, lack of data on the impact of biomass properties on equipment and process performance, matching capacity and capability in equipment integration, sub-optimal equipment design and operation, and less-than-optimal integrated equipment and process control.

This presentation will discuss key impactful factors on major unit operations such as feedstock storage, conveying, size reduction, pretreatment reactor feeding and discharge, and will provide recommendations for R&D activities, improvements in equipment design and in-line instrument and sensors to provide data necessary for successful scaling up bioconversion technologies to commercial operation. Various pretreatment process modifications to potentially mitigate operational challenges that are commonly encountered will also be discussed.

2:30 PM 7-4: Pretreatment of lignocellulosic biomass at pilot scale: design considerations vs operational challenges.

R. Verlinden[*] and A. Happel, Bioprocess Pilot Facility, Delft, Netherlands

There is a large interest in processes using second generation renewable biomass feedstocks to release sugars. To address the scale up challenges of this process, BPF has a state-of-the-art pilot scale system and is involved in many projects which design demo-scale or large-scale facilities.

The pretreatment pilot at the BPF is based upon a two-stage technology with high temperature and mild chemical treatment (acid/base). The pilot unit is a scaled-down model based upon pulp digesters of which hundreds of systems have been commissioned on industrial scale. The pretreatment is in this case typically followed by enzymatic conversion to sugars using enzyme cocktails which are being improved continuously.
Examples of design considerations for this type of pretreatment technology are:

- Type of feedstock and supply chain.
- The chosen severity conditions.
- The use of co-solvents such as alcohols or gases such as SO_2.
- The setup of the pretreatment process;

The above choices can give rise to several operational challenges, for example:

- Feeding a wet stream of biomass into a high pressure reactor, while maintaining the high pressure steam conditions in the reactor.
- The release of biomass from high pressure.
- The presence of residual dirt in the biomass.
- Solid liquid separations, pretreatment conditions and size reduction are relevant.

Piloting is essential to recognize and mitigate operational challenges before confrontation with expensive issues at large scales. The relation between design considerations and our practical experience on operational issues will be discussed in detail during the presentation.

3:00 PM 7-5: Assessing alternative biomasses for wheat straw based bioethanol refinery

H. Zhang, C. Felby and S.T. Thomsen[*], University of Copenhagen, Frederiksberg, Denmark; A. Lunde, Maabjerg BioEnergy, Holstebro, Denmark; C. Holland, University of Copenhagen, Frederiksberg C, Denmark; P. Cabaneros, Technical University of Denmark, Lyngby, Denmark; M. Ambye-Jensen, Aarhus University, Aarhus, Denmark

Feedstock flexibility can lower operational cost for biorefineries, as it secures feedstock sourcing and increases competition on the supplier side. In Denmark, a full scale 2G bioethanol plant is expected to run primarily on wheat straw (WS). In this study, we investigate the compatibility of 16 alternative biomasses with a wheat straw based platform (different types of straw, sawdust, grass fiber, chaff, deep litter, and industrial wastes). All biomasses have been pretreated under conditions optimized for WS, *i.e.* hydrothermal treatment at 15% DM, 190°C, for 10 min, to mimic their addition to a WS based production line.

Untreated and pretreated biomasses are analyzed via strong acid carbohydrate analysis, comprehensive microarray polymer profiling, elemental analysis, water retention value, as well as enzymatic hydrolysis. The liquid fractions from the pretreatment are analyzed for monomeric and oligomeric sugar content, inhibitor level and fermentability.
The alternative biomasses vary in their carbohydrate content and accessibility, which reflect their intrinsic recalcitrant traits. These traits and relations to the conversion efficiency are discussed. Furthermore, ethanol potential is estimated and included to a number of techno-economic parameters. Based on the results, some biomasses are found compatible in a wheat straw biorefinery, while others must be disregarded or processed differently. For example, barley straw performs better, oat straw equal and rye straw is poorer, compared to WS, while these straw types have similar price and logistic systems. Contrary to this, biogas fibers are economically beneficial, but the carbohydrates are highly inaccessible even after hydrothermal pretreatment.

3:30 PM 7-6: Comprehensive analysis of sugarcane bagasse pretreatment by auto-hydrolysis and acid-catalyzed steam explosion at equivalent combined severity factors

L. Ramos[*] and D. Fokinck, Federal University of Paraná, Curitiba, Brazil

Steam explosion has been examined by many for the optimal fractionation of sugarcane bagasse within the biorefinery concept. Exogenous acid catalysts are usually applied to improve pretreatment efficiency but the benefits of using such catalytic systems are not always clear compared to auto-hydrolysis. In this study, experiments performed at the same combined severity factor (CSF) of 0.76 ± 0.02 were compared: three auto-hydrolyses (SEB), three catalyzed by dilute sulfuric acid (SEB/SA) and one catalyzed by dilute phosphoric acid (SEB/PA) that was used as reference. Steam explosion at the same CSF produced substrates with similar chemical composition, crystallinity index, rheological behavior and glucose yields by enzymatic hydrolysis at 15% total solids (TS) using Cellic CTec3, except for SEB/SA experiments that were carried out at lower temperatures and shorter times. Hence, CSF values were very useful to adjust the strength of auto-hydrolysis and acid-catalysis steam explosion, however, this tool was not valid for all range of temperatures, residence times, acid catalysts and acid concentrations employed in this study. In principle, better results were obtained by auto-hydrolysis but pretreatment had to be performed at higher temperatures and residence times. The composition of pretreatment water-solubles varied among different pretreatment conditions with regard to their contents in carbohydrates, aliphatic acids, furans and aromatic compounds. Finally, hydrolysis of SEB at 15% TS in a lab-scale bioreactor required the lowest power consumption compared to both SEB/SA and SEB/PA because these latter substrates were more heterogeneous and contained more fiber aggregates.

4:00 PM 7-7: Strategies for Bridging the Bio-processing "Valley of Death"

J. Spooner, ICM inc., St Joseph, MO, USA*

The synergy between large commodity based infrastructure and smaller niche production can be leveraged to the advantage of both, as evidenced in petroleum and wet corn mill refining. The current dry-grind ethanol industry is a large volume low margin business with only a limited number of products available. The operators of these facilities continue to increase efficiency and yields, using both conventional and cellulosic conversion technologies. This is prone to resulting in back end bottlenecks, allowing front end excess capacity. Through process intensification this over capacity of feedstock handling can be leveraged to begin producing small volume, high margin specialty products. Purity of media to perform bio-conversions can also be tailored depending on process requirements, resulting in similar opportunity costs regardless of purity. The combination of process intensification and co-location can have a significant impact on overall economic viability, allowing new processes to reach commercial production faster, cheaper.

1:00 PM - 4:25 PM Session: 8: Separations and catalysis of bio-derived intermediates

Conveners: Gregg Beckham, NREL, USA;**Jay Fitzgerald**, DOE-BETO, USA and **Dr. Ken Tokuyasu**, Nat Ag Food Res, Japan

1:00 PM 8-1: Renewable Bubbles, Bottles and Rubber Bands from Biomass

P. Dauenhauer and D. Vlachos, University of Minnesota, Minneapolis, MN, USA*

Conversion of lignocellulosic biomass utilizes hybrid biological & thermochemical catalysts to transform sugars to the common chemicals comprising everyday products. In this work, sugars are catalytically transformed to many of the common chemicals and materials used in everyday products including PET plastics, surfactants and synthetic rubber. Novel solid acid catalysts and supported metals are utilized to promote catalytic dehydration and hydrogenation to selectively produce targeted compounds. In particular, Diels-Alder cycloaddition of furan dienes in tandem with sequential dehydration yields para-xylene[1,2], the key monomer in polyester polymers. Acid-catalyzed acylation of furans with fatty acids (obtained from renewable oils) produces alkylfurans as precursors to novel surfactants[3]; these unique surfactant structures exhibit high surfactancy and stability not present in conventional petroleum-derived ionic surfactants. And selective hydrogenation and dehydration produce useful olefin precursors to synthetic rubbers[4,5] including butadiene and isoprene for renewable automobile tires. The presentation combines experiment and computation to identify the mechanisms of formation of various products by combining a variety of skills from the Catalysis Center for energy Innovation (www.efrc.udel.edu).

References

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2. J. Cho, et al.. *ChemCatChem* DOI: 10.1002/cctc.201601294 (2016).
3. S. Park, et al. *ACS Central Science* DOI: 10.1021/acscentsci.6b00208 (2016).
4. Abdelrahman, et al. *ACS Sustainable Chemistry & Engineering* DOI: 10.1021/acssuschemeng.7b00745
5. O. Abdelrahman, et al. *ACS Catalysis* DOI: 10.1021/acscatal.6b03335

1:30 PM 8-2: Acrylate production from lignocellulosic feedstocks

V. Sànchez i Nogué, T.R. Eaton, N.A. Rorrer, C. del Cerro, E.M. Karp and G.T. Beckham, National Renewable Energy Laboratory, Golden, CO, USA*

Lactic acid is an important commodity chemical, currently used in a wide range of applications in the food, cosmetic, agricultural, and pharmaceutical industries. But this carboxylic acid holds a greater market potential as starting chemical for the production of acrylates. Moreover, microbial production of lactic acid represents an emerging route for the production of bio-based polymers, and selection of highly efficient microbial strains becomes essential, especially when lignocellulosic feedstocks are being used. To that end, we present a hybrid process for the production of three bio-based acrylates commonly used in different polymer applications. In the bioconversion step, a final titer of 99.4 g L^{-1} lactate was obtained from a batch fermentation of corn stover hydrolysate to lactate by a thermophilic *Bacillus coagulans* strain, representing an overall productivity of 3.1 g L^{-1} h^{-1}, and a metabolic yield of 0.91 g g^{-1} total sugars. Recovered lactic acid was subjected to three upgrading steps into methyl acrylate at > 90% yields, and further processed to acrylonitrile through a nitrilation step with a yield of 94%. Transesterification of methyl acrylate into acrylic acid was also investigated.

2:00 PM 8-3: Delivering our second commercial bioprocess even faster: key learnings from Genomatica's butylene glycol program

R. Pacheco, Genomatica, Inc, San Diego, CA, USA*

In mid-2017 Genomatica announced successful scale-up of its new GENO BG™ bioprocess to make 1,3-butylene glycol (BG) directly from renewable feedstocks, producing multiple tons for market sampling. This new process, not previously disclosed, was quickly recognized with the ICIS Innovation Award for its technology, scale-up, and potential commercial impact in cosmetics and personal care.

GENO BG's success built on Genomatica's previous experience delivering a commercial process for 1,4-butanediol (BDO). That process is used in Novamont's 30,000 ton per year plant, which had a notably smooth and rapid startup in late 2016. New learnings and techniques enabled GENO BG to be commercialized even faster than GENO BDO.

Both BDO and BG are demanding targets. They are high and medium volume markets with well-established fossil-based production processes and challenging cost targets to be competitive.

This talk will share approaches on how to deliver bioprocesses that meet these especially challenging commercial requirements. Key to Genomatica's successes has been an integrated whole-process approach that intimately intertwines and co-optimizes technoeconomic analysis, modeling and simulation, optimal pathway selection and strain design, downstream process design and new approaches to scale-down techniques to master scale-up. The central lesson is that an integrated approach is key; it's not just about the bug. Genomatica is using these same approaches to address additional chemical targets, including polyamide intermediates.

2:30 PM 8-4: Fractionating, Purifying, and Solvating Lignins via the ALPHA Process: Significant Improvements in the Properties of Lignin-Based Carbon Fibers

M.C. Thies, J. Ding, J. Jin and A.A. Ogale, Clemson University, Clemson, SC, USA*

Among renewable feedstocks, lignin can play an important role as a precursor for carbon fibers, owing to its significant carbon content. Unfortunately, the commercial-grade lignins available today (primarily Kraft lignins) are polydisperse and have high metals content, rendering them unsuitable for high-value applications like carbon fibers.

Thies and co-workers (2016) have developed the Aqueous Lignin Purification with Hot Acids (ALPHA) process for both fractionating and cleaning lignin recovered from pulp-and-paper mills and lignocellulosic biorefineries. ALPHA uses hot acetic acid–water mixtures of tunable concentration to form two liquid phases that split the lignin into two fractions: a solvent phase containing a lower molecular weight (MW) portion and a polymer phase containing a higher-MW portion of the lignin. The solvated, higher-MW polymer phase is readily processable into fibers via dry spinning, using a modification of the procedure developed by Ogale and co-workers (2014) for dry-spinning acetylated Kraft lignin. Furthermore, metals impurities are preferentially extracted into the solvent phase, so that <20 ppm Na in the spun polymer phase can be obtained via ALPHA.

The tensile strength and modulus of carbon fibers spun from "ALPHA" lignin were found to consistently increase with lignin molecular weight, with the highest-MW cuts producing carbon fibers almost 50% stronger than any reported to date from lignin. The effects of phenolic content and molecular linearity on final carbon-fiber properties were also measured. Further improvements in properties are expected from ongoing spinning experiments using ultrapure (i.e., <20 ppm Na), high-MW ALPHA lignin to make carbon fibers.

3:00 PM 8-5: Recovery & Purification of Fermentation-Derived Food Ingredients

B. Bhattacharjee, Amyris Inc., Emeryville, CA, USA*

Amyris is leveraging its strain engineering technology to deliver a number of sustainably sourced, commercially-relevant food ingredients via fermentation. The recovery, separation and purification of these molecules present a set of technical and cost challenges that are often distinct from those of high-volume commodity molecules: early fermentation strains typically show low product titers and variable impurity profiles that complicate recovery. The short time to market necessitated by the rapidly evolving food and beverage market requires parallel execution of strain, fermentation and DSP process development and makes extensive optimization on first-generation products challenging. The constraints of food-grade manufacturing also limit the unit

operations that can be used in downstream processing, and those are often further constrained by the range of available unit operations at contract manufacturers, for early multi-ton campaigns.

At Amyris, product recovery and purification of specialty ingredients is typically scaled from liter to multi-ton scale, over the course of a 12-24 month cycle. This paper examines some of the technical challenges typically encountered during DSP development and scale-up, the working solutions proposed at each scale to enable rapid but robust market entry, and some of the long-term strategies for driving down the cost of bringing fermentation-derived food ingredients to market.

3:30 PM 8-6: Characterization and Separation of Lignin after Alkali and Ionic Liquid Pretreatment

J. Yan, L. Liang, T.R. Pray and N. Sun, Lawrence Berkeley National Laboratory, Berkeley, CA, USA; E.M. Karp and G.T. Beckham, National Renewable Energy Laboratory, Golden, CO, USA; J.E. Coons, Los Alamos National Laboratory, Los Alamos, NM, USA*

Lignin is Nature's most abundant aromatic polymer, and replacement of petroleum-based chemicals with those derived from lignin represents a virtuous challenge for chemists. After biomass pretreatment under basic condition, most or significant amount of the lignin will be stripped from the feedstock and retained in the aqueous stream. In this work, lignin-rich stream was obtained from alkali and ionic liquid (IL) pretreatment process on corn stover. After pretreatment, the slurry was cooled down and solids removed. Three different technologies are being investigated for solids removal: ultrasonic separation (US), tangential flow filtration (TFF) and centrifugation. Ultrasonic separation is being used to remove fines (i.e., particles < 100 microns) for improved TFF membrane permeability or increase flow rates for centrifugation. Ultrasonic separation of fines can be achieved with low energy due to the high acoustic contrast between the lignocellulosic solids and water medium. TFF was also employed for the fractionation of lignin molecules. Samples were taken from processing streams and analyzed using different techniques. In general, weight-average and polydispersity decreased with the decrease of membrane pore size. Lignin properties from the two different pretreatment processes were compared to understand the pretreatment mechanisms with distinct catalysts.

4:00 PM 8-7: Recovery of bio-based intermediates from microbial cultivations

A.J.J. Straathof, Delft University of Technology, Delft, Netherlands*

Many chemicals can potentially be produced from biomass using carbohydrate fermentation. Comparison to petrochemical production and to ethanol fermentation shows stringent constraints not only on the minimum fermentation yield but also the maximum allowable downstream costs. Product recovery needs to achieve a high yield at low investment, low waste production and low energy costs, even though fermentation may lead to dilute aqueous solutions due to limited microbial tolerance to product. Different situations exist for different product groups; and a further distinction can be made on basis of feasible recovery techniques. Choosing for a specific situation the best recovery technique remains challenging; recognizing the specific situation and the analogy to solved separation problems is helpful.

In addition, in case of low achievable concentrations, *in-situ* product removal ("extractive reaction") can be tried to lengthen fermentation time. Many proof-of-concepts have been described in literature, and compared to base case processes. A generic approach will be present to assess early on in which cases *in-situ* product recovery will be favorable.
Examples that will be given of fermentation products for which new specific recovery processes are being developed are itaconic acid, 2-butanol and methyl propionate.

6:00 PM - 7:00 PM Banquet Reception

Grand Ballroom Foyer, Lobby Level

6:00 PM - 7:00 PM Exhibits Open

Grand Ballroom Foyer, Lobby Level

7:00 PM - 9:00 PM Annual Banquet and Award Presentations; Banquet Speaker Jim Lane, Biofuels Digest

Grand Ballroom, F-G Lobby Level

Wednesday, May 2

7:00 AM - 8:00 AM Speaker Breakfast

Starfish, Lobby Level

7:00 AM - 5:00 PM Registration

Grand Ballroom Foyer, Lobby Level

8:00 AM - 11:25 AM Session: 10: Synthetic and Systems Biology in Biomass Conversion I Sponsored by Cargill

Conveners: Prof. Lisbeth Olsson, Chalmers, Gothenburg, Sweden;**Pirkko Suominen**, Cargill, Plymouth, MN, USA and **Dr. Steven Brown**, LanzaTech, Skokie, IL, USA

8:00 AM 10-1: Membrane engineering as a tool to address cellular robustness

L. Olsson, J. Maertens, L. Lindahl and M. Bettiga, Chalmers University of Technology, Gothenburg, Sweden; S. Genheden and L.A. Eriksson, University of Gothenburg, Gothenburg, Sweden*

When moving towards a biobased economy, where fuels, chemicals and materials are produced from biomass, the substrate is more challenging for the microorganism than traditional raw materials and cells are exposed to inhibitory compounds in form of furans, weak acids and phenolics, which leads to decreased microbial performance. We attempt to modulate cellular robustness, which relates to the cell's ability to perform under challenging conditions through metabolic and evolutionary engineering and design of fermentation conditions.

This presentation will focus on one strategy to improve cellular robustness that include decreased uptake or increased efflux of inhibitory compounds, which is a strategy that has proven to be important for modulating weak acid tolerance. Acetic acid, a major inhibitor in lignocellulosic hydrolysates, enters the cell mainly by passive diffusion across the plasma membrane and inhibits yeast by mechanisms such as reduction of intracellular pH, accumulation of the acetate anion, and by signaling effects triggering cell death.

We have investigated the connection between membrane lipid composition, acetic acid permeation rate and acetic acid tolerance. We have explored nature to learn how to engineer the membrane lipid composition to reduce acetic acid permeation rate by learning from the acetic acid tolerant *Zygosaccharomyces bailii*. The proposed lipid composition has been evaluated using molecular dynamic simulations of the lipid bilayers and through metabolic engineering in *Saccharomyces cerevisiae*. We also suggest alternative strategies of membrane engineering leading to more stiff and thicker membranes.

8:30 AM 10-2: Identification of inhibitory mechanisms in the conversion of lignocellulosic hydrolysate to ethanol by *Zymomonas mobilis* using comparative multiomic fermentation with synthetic hydrolysates

Y. Zhang, J. Vera, J. Serate, E. Pohlmann, D. Xie, T.K. Sato and R. Landick, DOE Great Lakes Bioenergy Research Center, Madison, WI, USA*

A variety of biomass feedstocks can be used for producing bio-based chemicals and biofuels. Previously we compared microbial fermentation performance in hydrolysates produced from various feedstocks, including corn stover, switchgrass, miscanthus, sorghum, and mixed prairie. These feedstocks were harvested from the same year, as well as some feedstocks that were harvested form several different years. This previous work revealed differences in hydrolysate chemical composition, including lignocellulose-derived inhibitors (lignotoxins), that varied based on the feedstock as well as the year of harvest. Differing levels of lignotoxins in different feedstock hydrolysates greatly affected microbial fermentation performance, especially for xylose conversion. To further investigate the mechanism of lignotoxins affecting xylose utilization, we generated synthetic hydrolysates (SynH) which contains amino acids, acetate/acetamide, sugars, higher osmolarity, osmoprotectants, and a cocktail of lignotoxins at the concentrations detected in authentic AFEX-pretreated corn stover hydrolysate (ACSH). Comparative multiomic fermentations of *Zymomonas mobilis* using SynH with or without lignotoxins, were performed, and samples were collected for end product analysis, transcriptomic, metabolomic, and proteomic analyses. Fermentation results indicated that SynH with lignotoxins largely recapitulates the growth and physiology of *Z. mobilis* in ACSH, and showed poorer xylose utilization than SynH without lignotoxins. The more detailed analysis of multiomic data will be presented, which will help us to identify bottlenecks for how lignotoxins impact the conversion of biomass to biofuel.

9:00 AM 10-3: Strategies for assessing and improving microbial utilization of lignin-derived monomers

K. Davis, L.R. Jarboe, Y. Xue, X. Bai, M. Rover, R.C. Brown, R. Smith and Z. Wen, Iowa State University, Ames, IA, USA; A. Gerstein, University of Minnesota, Minneapolis, MN, USA*

Biomass contains lignin, a polymer of phenolic molecules, which provides stability and protection to the plant. Many economic models indicate that the addition of value to lignin is essential for the economic viability of the conversion of biomass to renewable fuels and chemicals. Some microorganisms, such as *Pseudomonas putida*, can convert or metabolize some aromatic and phenolic molecules. Previous reports have shown that *P. putida* can be engineered to funnel multiple phenolics

through its central metabolic pathways [1]. A funneling approach can be advantageous because thermally decomposed lignin can be composed of hundreds of different molecules many of which can be aromatic or phenolic in nature. Phenolic-rich fractions of pyrolyzed biomass have low solubility and therefore cannot be easily accessed by microorganisms in aqueous cultures. In addition, microorganisms can be negatively affected by inhibitors present in processed biomass streams. Here, we show strategies for both assessing and improving the microbial utilization of the phenolic-rich fractions. We have adapted a fast disk diffusion assay which simultaneously assess utilization and inhibition. The disk diffusion assay can be used to target favored molecules to produce by pyrolysis. The assay can also be used to learn more about the microorganism of interest because traditional culturing methods make it difficult to test molecules which can be both a carbon source and an inhibitor.
Refs: 1. Johnson, C. W.; Beckham, G. T. *Metabolic Engineering* **2015,** 28, 240-247.

9:30 AM 10-4: Engineering sucrose metabolism in yeast for enhanced ATP yields

A. van Maris, KTH Royal Institute of Technology, Stockholm, Sweden*
Anaerobic industrial fermentation processes do not require aeration and intensive mixing and the accompanying cost savings are beneficial for production of chemicals and fuels. However, the free-energy conservation of fermentative pathways is often insufficient for production and export of the desired compounds and/or for cellular growth and maintenance. Many relevant options to improve efficacy and kinetics of sucrose metabolism in *Saccharomyces cerevisiae* and, thereby, the economics of sucrose-based processes remain to be investigated. An essential first step is to identify all native sucrose-hydrolysing enzymes and sucrose transporters in this yeast, including those that can be activated by suppressor mutations in sucrose-negative strains. A sucrose-negative strain was developed as a platform to test metabolic engineering strategies and for fundamental studies into sucrose hydrolysis or transport. To increase free-energy conservation during fermentation of the industrially relevant disaccharide sucrose by *Saccharomyces cerevisiae*, we first replaced the native yeast α-glucosidases by an intracellular sucrose phosphorylase from *Leuconostoc mesenteroides* (LmSPase). Subsequently, we replaced the native proton-coupled sucrose uptake system by a putative sucrose facilitator from Phaseolus vulgaris (PvSUF1). Replacement of intracellular hydrolase with a phosphorylase increased the biomass yield on sucrose by 31%. Additional replacement of the native proton-coupled sucrose uptake system by PvSUF1 increased the anaerobic biomass yield by a further 8%, resulting in an overall increase of 41%. By experimentally demonstrating an energetic benefit of the combined engineering of disaccharide uptake and cleavage, this study represents a first step towards anaerobic production of compounds whose metabolic pathways currently do not conserve sufficient free-energy.

10:00 AM 10-5: Central metabolic shift in response to oxygen transfer rate switches the production of the triacylglycerols by *Corynebacterium glutamicum*

Y. Li, D. Zhang, J. Zhao, S. Gao and Y. Zhuang, East China University of Science and Technology, Shanghai, China; J. Plassmeier, Conagen Inc., Bedford, MA, USA; A. Sinskey, Massachusetts Institute of Technology, Cambridge, MA, USA*
We have previously engineered the non-native oleaginous microorganism *Corynebacterium glutamicum* ATCC 13032 (*C. glutamicum*) to accumulate triacylglycerols (TAGs) for the first time by completing and constraining a *de novo* TAGs biosynthesis pathway. The final engineered strain was shown to yield 3 g/L total fatty acids corresponding to an intracellular fatty acids content of 18% of the dry cell weight. However, TAGs production varied between the flask fermentation and the bioreactor fermentation. Here, we demonstrated the central metabolic shift in response to oxygen transfer rate in bioreactor culture, which switches the production of the TAGs by *C. glutamicum*. First, a high oxygen supply enhanced the TCA cycle and led to the maximum cell density plus trace intracellular TAGs. Second, the oxygen deprivation due to a low oxygen transfer rate rewired the carbon flux via malate, where additional NADPH have been generated through the malate enzyme. Two approaches based on these findings have been applied to improve the TAGs production. First, a two-stage oxygen supply strategy has been developed to enhance the TAGs accumulation, where the initial optimum level of oxygen supply ensured the proper redistribution of the carbon flux, and the latter high oxygen supply accelerated the re-utilization of the by-product organic acids. Second, the malate enzyme gene has been upregulated by the deletion of its transcriptional regulator malR, which boosted the supply of NADPH for the synthesis of fatty acids.

10:30 AM 10-6: Enhanced organic acid production via altered expression of *alg3* and *laeA* in industrial *Aspergillus* species

Z. Dai, S. Deng, K. Pomraning, J.K. Magnuson and S.E. Baker, Pacific Northwest National Laboratory, Richland, WA, USA*
Aspergillus species are well known to produce organic acids such as citric acid and itaconic acid in large quantity and have been explored as production hosts for a variety of industrial chemicals. Recent progress in genomics and proteomics of selected *Aspergillus* species has laid the foundation for further improvement of organic acid production. In this study, we evaluated the effects of the global regulator LaeA and asparagine-linked glycosylated protein 3 (Alg3) on production of citric acid in *Aspergillus niger* and itaconic acid in *Aspergillus terreus*. Deletion of *alg3* significantly increased the production of citric acid in *A. niger* and itaconic acid in *A. terreus*. In contrast, deletion of *laeA* dramatically reduced the production of citric acid in *A. niger* or itaconic acid in *A. terreus*. Furthermore, over-expression of *laeA* dramatically augmented the production of citric acid in *A. niger* or itaconic acid in *A. terreus*. Proteomic or transcriptomic analyses of either *alg3* gene deletion in *A. niger* or *laeA* over-expression in *A. terreus* was used to assess the effect on global gene expression. Finally, a combination of *alg3* deletion and *laeA* over-expression further improved production of citric acid in *A. niger* or itaconic acid in *A. terreus*. The results suggest that the

combination of *alg3* deletion and *laeA* over-expression may be applicable to improved production of other organic acids in *Aspergillus* species.

11:00 AM 10-7: Enhancing metabolic engineering in *Clostridium* using cell-free systems

A. Karim[*] and M. Jewett, Northwestern University, Evanston, IL, USA

The slow speed of design-build-test (DBT) cycles is a fundamental challenge facing metabolic engineering. This challenge is exacerbated in industrially-relevant, non-model organisms such as *Clostridium* which lack a robust toolset for manipulation. To address this, we report a new *in vitro* prototyping and rapid optimization of biosynthetic enzymes approach (termed iPROBE) to inform cellular metabolic engineering in *Clostridium*. In our approach, cell-free cocktails for synthesizing target small molecules are assembled in a mix-and-match fashion from crude cell lysates selectively enriched with pathway enzymes. The rapid ability to build pathways *in vitro* using iPROBE allows us to quickly generate data describing pathway operation under several operating conditions. However, to date no easy method of analysis provides informative bridging of cell-free data to cellular metabolic engineering. We address this limitation by developing a quantitative metric that combines titer at reaction completion, rate during the most productive phase of pathway operation, and enzyme expression as measured by protein solubility (TREE score). By reducing the complexity of available cell-free data to one value we can now quickly screen and rank pathways in the cell-free environment and provide useful information for cellular metabolic engineering. We demonstrate iPROBE and the use of the TREE score for the production of 3-hydroxybutyrate and *n*-butanol in *Clostridium*. We anticipate that iPROBE will facilitate efforts to define, manipulate, and understand metabolic pathways for accelerated DBT cycles in the cell-free environment before engineering organisms.

8:00 AM - 11:25 AM Session: 9: Deconstruction, Fixation, and Bioproduct Formation I

Conveners: Emile van Zyl, Stellenbosch, S. Africa;**Prof. Venkatesh Balan**, Michigan State University, USA and **Prof. Claus Felby**, U Copenhagen, Denmark

8:00 AM 9-1: Investigating the evolution of cellulose properties throughout enzymatic hydrolysis

J. Nill[*] and T. Jeoh, University of California, Davis, Davis, CA, USA; H.Y. Holman, Lawrence Berkeley National Lab, Berkeley, CA, USA

The mechanisms of cellulose hydrolysis by cellulases remain unsolved, in large part due to limited understanding of the substrate in the reaction. Exocellulases, such as *Trichoderma reesei* Cel7A, must adsorb to the surface of insoluble cellulose and complex with an accessible cellulose reducing end before hydrolysis can proceed. However, not all binding leads to hydrolysis and to differentiate, we define all sites at which a cellulase initiates hydrolysis as productive binding sites and those at which hydrolysis does not occur as non-productive binding sites. We have previously shown that the total number of productive binding sites varies depending on cellulose source and processing history. Here, we investigate the changes in the productive binding capacity of cellulose throughout hydrolysis. We further probe these changes by investigating the link between the productive binding of cellulases and cellulose surface chemistry and morphological features.

9:00 AM 9-3: Pretreatment severity and its effect on the enzymatic hydrolysis of spruce and wheat straw

K. Kruus[*], VTT Technical Research Centre of Finland Ltd., VTT, Finland; M. Kellock, K. Marjamaa and T. Tamminen, VTT Technical Research Centre of Finland Ltd, Espoo, Finland; H. Zhang and C. Felby, University of Copenhagen, Frederiksberg, Denmark

Lignocellulosic biomass requires pretreatment prior to enzymatic hydrolysis in order to open up the cell wall structure for enzymatic hydrolysis. Thermochemical pretreatment is commonly used due to its low cost and efficient degradation of hemicelluloses. In our previous work, we have seen that pretreatment of lignocellulose altered the lignin structure and enhanced non-productive binding of enzymes onto lignin [1]. Enzyme adsorption onto lignin is a major disadvantage in the enzymatic hydrolysis by preventing efficient hydrolysis of cellulose and recycling of enzymes. Enzymes are still a major cost in sugar production from lignocellulosic biomass. This could partially be overcome by minimizing non-productive binding and promoting the reuse of enzymes. We report here how the pretreatment severity affects on lignin structure and on non-productive enzyme adsorption onto lignin. Spruce and wheat straw biomass were hydrothermally pretreated in temperatures ranging from 180 to 220 °C in the presence or absence of a dilute acid catalyst.

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[1] Rahikainen, J. L., Martin-Sampedro, R., Heikkinen, H., Rovio, S., Marjamaa, K., Tamminen, T., Rojas, O. J. and Kruus, K. 2013. Inhibitory effect of lignin during cellulose bioconversion: The effect of lignin chemistry on non-productive enzyme adsorption. Biosource technology, 133, 270-8.

9:30 AM 9-4: Lytic polysaccharide monooxygenase involves in lignin oxidation for efficientlignocellulosic

biomass degradation by white-rot fungi

S. Xie, Texas A&M University, College Station, TX, USA; H. Yu and X. Zhang, Key Laboratory of Molecular Biophysics of MOE, School of Life Science and Technology, Huazhong University of Science and Technology, Wuhan, China; J.S. Yuan, Synthetic and Systems Biology Innovation Hub, Texas A&M university, College Station, TX, USA*

Lytic polysaccharide monooxygenases (LPMOs) are attracting widespread interest due to their oxidative activity on polysaccharides and their potential application in promoting biomass conversion. Current studies generally believe that LPMOs only oxidize the polysaccharides during biomass degradation, whilst no study investigates their role in lignin oxidation. This study revealed that LPMOs were co-regulated with lignin-degrading enzymes in white-rot fungi through transcriptomics analysis. We also showed that the LPMO gene numbers are well correlated with the numbers of gene encoding the lignin-depolymerizing major enzymes in biomass-degrading microbes. The in vitro experiments suggested that LPMOs could synergize with lignin-degrading enzymes for lignin oxidation, which was confirmed by the lignin functional group characterization with phosphorus-31 nuclear magnetic resonance. These findings further explored the roles of LPMO from polysaccharide to lignin oxidation, and made us rethink the position of LPMO in fungal extracellular redox environment for biomass degradation.

10:00 AM 9-5: Increased saccharification efficiency of cellulose by using hydrogen peroxide to activate LPMOs in a commercial cellulase cocktail

G. Müller, P. Chylenski, B. Bissaro, A. Várnai and V.G.H. Eijsink, Norwegian University of Life Sciences, Aas, Norway; L. Hansen and S.J. Horn, Norwegian University of Life Sciences, Ås, Norway*

The discovery of the oxidative enzymes named Lytic Polysaccharide Monooxygenases (LPMOs; 1) has improved the efficiency of current commercial cellulase cocktails for saccharification of lignocellulosic biomass. However, the notion that LPMOs use molecular oxygen as a co-substrate and require two externally supplied electrons per catalytic cycle is a challenge for the development of large-scale industrial processes. Building on the recent discovery that H2O2, rather than O2, is the preferred co-substrate of LPMOs (2), we show how cellulose degradation by the LPMO-containing commercial cellulase cocktail Cellic® CTec2 can be controlled and enhanced by adding H2O2 to the reaction. Optimal control of H2O2 supply leads to high LPMO rates in the absence of molecular oxygen with only sub-stoichiometric consumption of reductant, while oxidative self-inactivation of the LPMO is prevented. We report saccharification rates and yields for a model substrate (Avicel) and industrial lignocellulosic substrates that, at low H2O2 feed rates, are higher than those seen under standard aerobic conditions. In an industrial setting, controlled supply of H2O2 to a reactor is easier and cheaper than supplying molecular oxygen and reductants. Additionally, the use of H2O2 to activate LPMOs makes it possible to design novel combined saccharification and fermentation processes.

References:

1) Vaaje-Kolstad, G., et al. (2010). An oxidative enzyme boosting the enzymatic conversion of recalcitrant polysaccharides. Science, 330(6001), 219-222.
2) Bissaro, B., et al. (2017). Oxidative cleavage of polysaccharides by monocopper enzymes depends on H2O2. Nature chemical biology, 13, 1123.

10:30 AM 9-6: Exploring light-driven biomass degradation systems

D.A. Russo, K.B. Möllers, C. Felby and P.E. Jensen, University of Copenhagen, Frederiksberg, Denmark; R.K. Singh and M.J. Bjerrum, University of Copenhagen, Copenhagen, Denmark; B. van Oort and R. Croce, Vrije Universiteit Amsterdam, Amsterdam, Netherlands*

The development of sustainable biomass conversion technologies is crucial to decrease our dependency on petroleum-based chemicals and fuels. In this context, lytic polysaccharide monooxygenases (LPMOs) are powerful enzymes capable of degrading recalcitrant lignocellulose into monomers to produce over 200 added value chemicals and fuels. These monooxygenases are copper metalloproteins that reduce O_2 and insert one hydroxyl group into the polysaccharide chain to break the glycosidic bond. Additionally, for their catalytic cycle, electrons from external donors are required to reduce the copper-containing active site. Although traditionally LPMOs rely on enzymatic electron donors (e.g. cellobiose dehydrogenase), recently it has been shown that utilising photo-excited pigments can increase activity up to a hundred-fold. Here we report a series of studies dedicated to the development of sustainable light-driven biomass degradation. First, we will detail the redevelopment of traditional enzymatic assays to encompass the new paradigm of light-driven biomass degradation. Second, in an exploration of naturally occurring photosensitizers, we will show how chlorophyll precursors (different metallo-protoporhyrins) and chlorophyll-protein complexes, such as the abundant light-harvesting complex II, can drive the light-induced enzymatic activity. Finally, through biophysical methods, we will aim to provide insight into the interaction and kinetics of the pigment-LPMO coupling. Our findings will deliver novel solutions to establish light-driven biomass degradation as a source of sustainable chemicals and fuels.

11:00 AM 9-7: Real-Time Imaging Reveals Lytic Polysaccharide Monooxygenase Promotes Cellulase Activity by Increasing Cellulose Accessibility

B. Song, B. Li, X. Wang, W. Shen, S. Park, C. Collings, A. Feng, J.D. Walton and S.Y. Ding, Michigan State University, East Lansing, MI, USA; S. Smith, South Dakota School of Mines and Technology, Rapid City, MI, USA*

Supplementation of enzyme cocktails with lytic polysaccharide monooxygenase (LPMO) can increase the efficiency of cellulase mixtures for biomass conversion. Previous studies have revealed that LPMOs cleave polysaccharide chains by oxidization of the C1 and/or C4 carbon of the monomeric units. However, how LPMOs enhance enzymatic degradation of lignocellulose is still poorly understood. In this study, we combined enzymatic assays and real time imaging using atomic force microscopy (AFM) to study the molecular interactions of an LPMO (*Tr*AA9A from *Trichoderma reesei*) and a cellobiohydrolase I (*Tl*Cel7A from *T. longibrachiatum*) with bacterial microcrystalline cellulose (BMCC) as a substrate. Cellulose conversion by *Tl*Cel7A alone was enhanced from 46% to 54% by addition of *Tr*AA9A. Conversion by a mixture of *Tl*Cel7A, endoglucanase and β-glucosidase was increased from 79% to 87% by using *Tr*AA9A-pretreated BMCC for 72 h. AFM imaging demonstrated that individual *Tr*AA9A molecules exhibited intermittent random movement along, across, and penetrating into the ribbon-like microfibril structure of BMCC, which was concomitant with the release of a small amount of oxidized sugars and the splitting of large cellulose ribbons into fibrils with smaller diameters. The dividing effect of the cellulose microfibril occurred more rapidly when *Tr*AA9A and *Tl*Cel7A were added together compared to *Tr*AA9A alone; *Tl*Cel7A alone caused no separation. *Tr*AA9A increases the accessible surface area of BMCC by separating large cellulose ribbons, and thereby enhances cellulose hydrolysis yield.

9:00 AM - 10:00 AM Exhibits Open

Grand Ballroom Foyer, Lobby Level

10:00 AM - 12:00 PM Exhibit Dismantle

Grand Ballroom Foyer, Lobby Level

11:30 AM - 1:00 PM Planning Committee Meeting

1:00 PM - 4:25 PM Session: 11: Deconstruction, Fixation, and Bioproduct Formation II

Conveners: Prof. Claus Felby, U Copenhagen, Denmark;**Prof. Venkatesh Balan**, University of Houston, Houston, TX, USA and **Emile van Zyl**, Stellenbosch, S. Africa

1:00 PM 11-1: Tension wood structure and morphology conducive for better enzymatic digestion

H. O'Neill, S.V. Pingali, D. Sawada, U. Kalluri, U. Volker, P. Langan and B. Davison, Oak Ridge National Laboratory, Oak Ridge, TN, USA*

Tension wood is a type of reaction wood formed in response to bending or leaning of growing stems. It contains a higher glucan content and a lower amount of lignin, and is more easily broken down into fermentable sugars compared to native wood. Here, we have employed structural techniques; small-angle neutron scattering (SANS) and wide-angle X-ray diffraction (WAXD) to elucidate structural and morphological aspects of tension wood that result in higher sugar yields. SANS data exhibited a tri-modal size distribution of scattering features. The smallest size, 22 Å observed in all samples concurred with the WAXD results of the control and opposite side samples and was interpreted as the cellulose elementary microfibril diameter. The intermediate size feature of 45 Å, which is most pronounced in the tension side sample and consistent with WAXD results for tension side sample, indicates an association of neighboring elementary microfibrils to form larger crystallite bundles. The largest size, 61 Å, was not observed by WAXD and was interpreted as the presence of pores in the tension wood sample. In conclusion, clear differences were observed in the structure and morphology of tension wood compared to native wood. Cellulose crystallinity is increased, and there is evidence of pores that are not observed in native wood. It is likely that the presence of pores combined with lower lignin content in tension wood substantially improves enzyme accessibility leading to higher yields in enzymatic cellulose digestion.

1:30 PM 11-2: The Mechanism by which a novel GH51 arabinofuranosidase can cope with di-substitutions in arabinoxylans

C. Santos, M. Domingues, R. Pirolla, P. Giuseppe and M. Murakami, Brazilian Bioethanol Science and Technology Laboratory from the National Center for Research in Energy and Materials, Campinas, Brazil*

Arabinofuranosidases (EC 3.2.1.55) are exo-enzymes that remove non-reducing L-arabinofuranosyl (L-Araf) residues from arabinoxylans allowing the endo-enzymes to access the substrate backbone. However, very few Abfs are able to recognize and to cleave the L-Araf di-substitutions in arabinoxylans. In addition, the structural basis for this action mode yet remains obscure. In this work, we discovered a novel GH51 Abf that can cope with such di-substitutions and its crystal structure was solved unveiling the presence of a lateral pocket to accommodate the di-substituted xylose residue. Besides the ability to cleave di-

substitutions in arabinoxylans, this novel Abf can also efficiently break down mono-substituted residues, being an interesting enzyme for industrial applications involving plant cell wall depolymerization. Enzyme cocktail supplementation studies demonstrated its positive role in increasing reducing sugar release from sugarcane biomass treated with *Trichoderma reesei* cocktail supporting the importance of the recalcitrance imposed by di-substitutions in arabinoxylans during enzyme depolymerization.

2:00 PM 11-3: Enzyme diversity and industrial potential of bacterial glucuronoyl esterases

J. Arnling Bååth, S. Mazurkewich, L. Olsson and J. Larsbrink, Chalmers University of Technology, Gothenburg, Sweden*

The suggested biological role of glucuronoyl esterases (GEs) is to cleave the lignin-carbohydrate (LC) ester bonds between glucuronic acid residues in hemicelluloses and lignin. Breaking LC bonds is proposed to enhance efficient processing of plant biomass, and specific enzymatic cleavage of the LC ester bonds would therefore be of large industrial interest. GEs are classified in the carbohydrate esterase family 15 (CE15) in the Carbohydrate-Active Enzymes database (CAZy). CE15 members, and thereby putative GEs, are found in a range of fungi and bacteria. To date, a few fungal GEs have been characterized on model substrates and in a few cases, on native lignin-carbohydrate complexes (LCC) extracted from trees. However, studies on bacterial GEs are lacking, despite the much larger number of bacterial CE15 sequences in CAZy compared to fungal entries.

In this study, we focused on previously uncharacterised bacterial CE15 enzymes, which have as low as 19 % sequence identity. The targeted enzymes were kinetically characterized on a variety of synthetic substrates and their activities were also evaluated on lignocellulosic biomass. In addition, we investigated the biological roles of different GEs encoded by the same bacterial species through transcriptomic analyses of cells grown on multiple carbon sources. Combined with structural biology and substrate-binding studies, the results will provide novel insights into the enzyme diversity in CE15, as well as provide increased knowledge on the industrial relevance of GEs.

2:30 PM 11-4: The effect of adsorbed hemicellulose on the binding and activity of Cellobiohydrolase I, Cel7A, fromÂ *Trichoderma reesei*Â to cellulose

S. Malgas and B. Pletschke, Rhodes University, Grahamstown, South Africa; V. Kwanya Minghe, University of Lorraine, Nancy, France

Hydrothermal pre-treatments have been developed to decrease lignocellulosic biomass recalcitrance by solubilizing and disrupting the majority of the hemicellulose on the biomass thus increasing cellulase accessibility. However, a small quantity of the hemicellulose may still remain and become adsorbed to the cellulose, leading to cellulase inhibition. In this study, we produced hemicellulose bound cellulose, using glucuronoxylan and galactomannan, to simulate hydrothermally pre-treated hardwoods and softwoods, respectively, and evaluated how these may affect cellulose hydrolysis by *Trichoderma reesei* cellobiohydrolase I, Cel7A. Based on XRD, FTIR, SEM and Simon's staining, hemicellulose binding onto cellulose affected the physical properties of the biomass, and consequently its hydrolysis rate. As a result of hemicellulose binding onto cellulose, the adsorption of Cel7A was significantly affected (up to a 42 and 60% reduction for xylan and mannan, respectively), leading to lowered activities (~40% reduction), especially for xylan. The bound hemicellulose may be released from the cellulose during agitation and hydrolysis; we therefore evaluated the effect of free hemicellulose on Cel7A activity. Xylan was more inhibitory to Cel7A than mannan, demonstrating non-competitive inhibition, while mannan demonstrated uncompetitive inhibition. The recalcitrance effect of bound hemicellulose could thus be completely relieved by the addition of hemicellulolytic enzymes (i.e. a *Bacillus stearothermophilus* T6 xylanase, XT6, and an *Aspergillus niger* mannanase, Man26A) during hemicellulose bound cellulose hydrolysis. This study showed that hemicellulose remains a critical factor regarding biomass recalcitrance and that the addition of hemicellulolytic activities in commercial enzyme cocktails is required, especially the mannanolytic activities lacking from most enzyme cocktails.

3:00 PM 11-5: Strategies for developing thermophilic fungal strains as source of novel cellulolytic and auxiliary enzyme cocktails

B. Singh Chadha, Guru Nanak Dev University, Amritsar, India

This study reports thermophilic fungi as important source of lignocellulolytic enzymes. The secretome analysis using LC–MS/MS orbitrap showed that fungi produced a spectrum of glycosyl hydrolases (cellulase/hemicellulase), auxiliary activity (AA) polysaccharide lyases (PL) and carbohydrate esterases (CE) indicating the presence of diverse functional classical and oxidative cellulolytic mechanism. The secretome was fractionated by ion exchange chromatography and analyzed for ability to hydrolyze pretreated lignocellulosics to identify potentially important auxiliary proteins. Furthermore one of the thermophilic fungal strain was developed as benchmark source of cellulases. The cellulases produced were used for developing and evaluating cocktails with the mono-component AA9, xylanases, cellobiohydrolases, feruoyl esterase derived from different thermophilic fungi for hydrolysis of acid and alkali treated rice straw and bagasse. The released sugars in the hydrolysate were evaluated for enhanced ethanol production using conventional and BOLT-ON fermentation processes. The study demonstrated that developing catalytic efficient cocktails from thermophilic fungi has immense potential in reducing the cost of enzymes for developing economically viable 2G ethanol technology.

3:30 PM 11-6: Characterization and catalytic upgrading of deep eutectic solvent extracted sorghum lignin to phenolic compounds

L. Das, J. Stevens, W. Li and J. Shi, University of Kentucky, Lexington, KY, USA; M. Li, Y. Pu and A. Ragauskas, Oak Ridge National Laboratory, Oak Ridge, TN, USA*

Deep eutectic solvents (DES) is intrinsically cheaper than many ionic liquids (ILs) due to low precursor cost, simple synthesis and improved recyclability. Meanwhile DES can be as effective as IL towards dissolving lignin from plant materials. However, the lignin depolymerization mechanism in DES, the structural and chemical properties of DES-extracted lignin (DES-EL), and the possible valorization pathways of DES-EL towards value-added products were not well understood. This study aims to characterize the lignin streams from DES (1:2 ChCl:Lac) treated sorghum bagasse and further upgrade the extracted lignin to phenolic compounds. As revealed by HSQC, ^{13}C and ^{31}P NMR analysis, DES cleaved nearly all ether linkages in native lignin, resulting in significant size reduction; meanwhile the S and G units underwent structural changes and were likely transformed to condensed sub-units. To elucidate the mechanism, guaiacylglycerol-β-guaiacyl ether model compounds were incubated in DES at predetermined conditions to identify the reaction pathways and end products. We further evaluated catalytic conversion of DES-EL to phenolic compounds *via* catalytic transfer hydrogenolysis in presence of isopropyl alcohol. Among the three tested catalysts (Ru/C, Pd/C, and Pt/C), Ru/C proved most effective in deconstructing DES-EL, with oil, char, and gas yields of 36.28, 46.43, 17.29 wt%, respectively. Major lignin degradation products in the oil were phenol, 4-ethylphenol, 4-ethyl-2-methoxyphenol, 2-methoxy-4-propylphenol, and 4-hydroxy-benzenepropanoic acid, demonstrating cleavage of DES-EL to monomeric compounds. This study provides a mechanistic understanding of lignin depolymerization in DES and demonstrates catalytic upgrading of DES-EL to low molecular weight phenolic compounds.

4:00 PM 11-7: Newly developed Compacted Biomass with Recycled Ammonia (COBRA) pretreatment process

J. Zhang, East China University of Science and Technology, Shanghai, China, L.D.C. Sousa, Department of Chemical Engineering and Materials Science, Michigan State University, Lansing, MI, USA and V. Balan, University of Houston, Houston, TX, USA*

Pretreating lignocellulosic biomass helps to open up the cell wall and help enzymes to hydrolyze the substrate more efficiently. Since milled biomass occupies large volume in a reactor due to its low density and fibrous nature, the amount of biomass that could be pretreated in a given reactor volume is very limited. AFEX pretreated corn stover (typically using 1:1 ammonia to biomass loading) contains native cellulose I and when subjected to enzyme hydrolysis, about 72% glucan and 65% xylan is converted to monomeric sugars using Cte2 and Htec2 enzymes (15mg/g of glucan in 24 h). The same corn stover when subjected to Extractive ammonia (EA) pretreatment (using 6:1 ammonia to biomass loading) produce cellulose III and gave 85% glucan and 75% xylan conversion using the same enzyme loadings. Here, report a newly developed pretreatment process called COmpacted Biomass with Recycled Ammonia (COBRA) pretreatment that gave similar sugar conversion like EA pretreated corn stover. We used compacted corn stover pellets in that reactor that requires lesser ammonia to biomass ratio (1:1) when compared to EA process. The advantage of using the new COBRA process compared to EA include (1) less ammonia requirement for pretreatment (1:1 vs 3:1 to 6:1); (2) can operate at lower pressures (~400 psi vs 1000-1200 psi) and (3) requires lesser reactor volume per amount of pretreated biomass (about 5-15 times smaller reactor volume) due to using compacted biomass pellets. These advantages will help to reduce both operating and capital costs required for pretreating biomass.

1:00 PM - 4:25 PM Session: 12: Synthetic and Systems Biology in Biomass Conversion II

Conveners: Prof. Lisbeth Olsson, Chalmers, Gothenburg, Sweden;**Pirkko Suominen**, Cargill, Plymouth, MN, USA and **Dr. Steven Brown**, LanzaTech, Skokie, IL, USA

1:00 PM 12-1: Synthetic promoters and circuitries enabling controlled and tuneable expression in metabolic engineering and enzyme production in fungal hosts

M. Penttilä, A. Rantasalo, J. Kuivanen, C. Landowski, M. Valkonen, J. Jäntti and D. Mojzita, VTT Technical Research Centre of Finland Ltd, Espoo, Finland*

Sustainable production of chemicals, materials and industrial enzymes is increasingly performed by genetically engineered cell factories. In order to obtain highest possible overall efficiencies and controllable production, it is important to have tools that enable orthogonal expression of the target genes, i.e. expression that is not impaired by host gene regulation and physiology or external culture conditions. We have developed a synthetic expression system (SES) that consists of synthetic transcription factors and promoters, which enables tuneable expression at a range of expression levels. The system is also extended to include synthetic repressors and promoter elements for repression of target genes. A combination of the induction and repression elements to a bi-stable genetic circuit system was also constructed, which enables switching between expression and repression of alternative sets of pathway genes. Examples are given how the synthetic expression system can be used for pathway engineering in *S.cerevisiae*. Furthermore, the SES system works in a vast range of fungal hosts, from *Saccharomyces*

cerevisiae, *Pichia* to *Trichoderma reesei.* In *Trichoderma*, production of industrial enzymes is obtained on glucose at similar levels than is obtained from the strong cellobiohydrolase promoter *cbh1* in cellulase inducing conditions.

1:30 PM 12-2: Mechanism-Guided Design of Highly Efficient Protein Secretion for Biomanufacturing

S. Xie, Texas A&M University, College Station, TX, USA and J.S. Yuan, Synthetic and Systems Biology Innovation Hub, Texas A&M university, College Station, TX, USA*

Bacteria remains one of the most attractive hosts for commercial protein production, due to the rapid proliferation, economic media, and available genetic toolkit. Secretory protein production is particularly advantageous for biomanufacturing as it reduces the separation cost, avoids the endotoxin contamination, and enables the continuous production preferred by industry and regulatory agencies. Despite extensive research, secretory production of heterologous protein in either Gram-negative or Gram-positive bacteria remains highly challenging, where the highest yield is still very low. With Gram-positive bacteria Rhodococcus opacus PD630 as a model species, we demonstrated that systems biology-guided holistic design of transcription, translation, secretion and folding of ligninolytic laccase balanced the process, minimized the toxicity, and enabled efficient secretion with a record yield of bacterial secretory protein at 13.7 g/L. Proteomics and secretomics were used to identify and screen the efficient promoter/RBS and signal peptides for target protein, respectively. The Tat protein transporter machinery in the bacterial membrane was optimized to eliminate the overloading of transporter due to the high heterologous protein expression level. In order to mitigate the toxicity of laccase to the cell, we designed a two-step fermentation system in which laccase was produced as apoprotein in bacterial cytoplasm and to be fully functional after secretion. The fermentation conditions were further optimized to enable a 13.7 g/L total secreted protein yield. The protein yield leapfrogged the current technologies in secretory production of heterologous protein using bacteria system and could have broad applications in biomanufacturing of enzymes, protein therapeutics and various high value proteins

2:00 PM 12-3: Systems Biology Guided Engineering of CO2 to Hydrocarbon Conversion in Cyanobacteria

M. Li and X. WANG, Texas A&M University, College Station, TX, USA; J.S. Yuan, Synthetic and Systems Biology Innovation Hub, Texas A&M university, College Station, TX, USA

Atmospheric CO_2 has reached a historic high level due to fossil fuel burning and deforestation. Cyanobacteria is a photosynthetic bacterium that has been proven to have strong potential as cell factory to produce diverse bio products such as ethanol, butanol etc. Terpenes are large and class diverse class of organic compounds wildly used as in perfumery and medicine. In cyanobacteria, terpenes are produced through a secondary metabolic MEP pathways. To produce high titers of terpenes, high carbon flux from Calvin Benson cycle to MEP pathways is desired. In our study, rewiring the carbon partition from CB cycle to MEP further enhanced the limonene production. Metabolomics study of the previous limonene producing strain showed a huge accumulation of sugar such as sucrose after several days of culturing accompanied with a decrease of limonene productivity. Sucrose is a salt response molecule in cyanobacteria. By block the sucrose synthesis pathway and redirecting the carbon into the MEP pathway, we found there was no sucrose accumulated and a slightly slower growth rate comparing to the original strain, and reached a record yield of 1100(μg/L/day/OD730).It indicates rewiring the carbon from sugar storage to Calvin Benson cycle, and then to the MEP pathways increases the carbon flux for limonene production. Through various metabolic engineering strategies, we also established a heterotrophic system in cyanobacteria in which cyanobacteria are capable to consume the sucrose and xylose in medium and further enhance limonene production. Our systemic strategies of engineering CO2 to hydrocarbon conversion provides valuable hints for terpene productions.

2:30 PM 12-4: Metabolic engineering of farnesene manufacturing strains to enable commercial production on cellulosic feedstocks

Y. Xiong, D. Diola, B. Friedrikson, C. Garcia De Gonzalo, P. Kumagai, C.L. Liu, C.W. Lu, A. McGill, Q. Mitrovich, D. Pinel, W. Sun, A. Tsong, J. Walker and G. Wichmann, Amyris Inc., Emeryville, CA, USA; F. Moesler, Renmatix, King of Prussia, PA, USA; B. Perrotte and S. Rifflart, Total New Energies USA, Inc., Emeryville, CA, USA

The DOE-funded MegaBio collaboration among Amyris, Renmatix, and Total New Energies is focused on developing a manufacturing-ready process for production of farnesene from cellulosic biomass at a cost equivalent to Amyris's current cane syrup-based manufacturing process. Farnesene is a unique building block chemical with applications in hundreds of branded products including fuel, tires, cosmetics, and fragrances. Renmatix's unique Plantrose process hydrolyzes wood and produces cost-competitive lignin-free C6 and C5 sugar streams. Some organic acids and other molecules generated during cellulosic sugar processing are inconsumable and inhibitory to our yeast biocatalyst. To achieve a sustainable cost advantage, we are integrating metabolic pathways that allow our farnesene manufacturing strains to utilize xylose as well as organic acids and other inhibitors as additional carbon sources. By taking both rational-engineering and directed-evolution approaches, we have enabled performance improvements in our farnesene manufacturing strains on cellulosic feedstocks.

3:00 PM 12-5: Engineering *Pseudomonas putida* EM42 for co-utilization of lignocellulose-derived sugars

P. Dvořák and V. de Lorenzo, Spanish National Centre for Biotechnology (CNB-CSIC), Madrid, Spain

Sugars and aromatic compounds derived from lignocellulosic waste can serve as cheap substrates for biotechnological

production of value-added chemicals. However, well-defined microbial platforms that could efficiently perform such task are scarce. *Pseudomonas putida* KT2440 is a GRAS-certified, robust soil bacterium with versatile metabolism and high stress tolerance. This strain has been employed for production of fine chemicals from glucose and for processing lignin-derived aromatics. But its potential for valorization of other products of lignocellulose decomposition is limited due to the lack of adequate metabolic traits.

The aim of this study was to empower *P. putida* with novel biocatalytic functions for its application in biotechnological recycling of lignocellulosic waste. To meet this goal, we combined a synthetic biology-inspired metabolic engineering approach with an *in-house P. putida* KT2440-derived chassis *P. putida* EM42 - a strain with streamlined genome and superior physiological properties. Endogenous and heterologous catabolic pathways for D-xylose were tested in this platform strain. Major bottlenecks hindering efficient D-xylose utilization were removed and fast growth on the pentose was achieved. Functional screening of cellulases from distinct sources revealed b-glucosidase that enabled rapid growth of EM42 also on D-cellobiose and parallel accumulation of polyhydroxyalkanoates within the cell. A *P. putida* strain capable of co-utilization and valorization of major lignocellulose-derived sugars was obtained for the first time when such new parts were combined with interventions in carbohydrate transport systems. This study provides a showcase of how rational orchestration of *P. putida* metabolism expands the catalytic scope of this bacterium towards industrial applications.

3:30 PM 12-6: Engineering and evolving *Pseudomonas putida* KT2440 for efficient production of muconic acid

G. Bentley, C. Johnson, D. Salvachúa and G. Beckham, National Renewable Energy Laboratory, Golden, CO, USA; J.R. Elmore and A.M. Guss, Oak Ridge National Laboratory, Oak Ridge, TN, USA*

In addition to fuels, we rely on non-renewable resources for many chemicals and materials. Nylon-6,6 has become ubiquitous, and is polymerized from adipic acid and hexamethylenediamine. Unfortunately, beyond the non-renewable sources of these monomers, adipic acid production represents a major source of the potent greenhouse gas nitrous oxide. Biologically produced cis,cis-muconic acid (referred to as muconic acid) can be hydrogenated under mild conditions to adipic acid. We have engineered the Gram negative, environmentally resilient bacterium *Pseudomonas putida* KT2440 for the production of muconic acid from glucose. The Shikimate pathway can be harnessed for muconic acid production by dehydrating 3-dehydroshikimate to protocatechuate, which can then be decarboxylated to catechol. A 1,2-dioxygenase catalyzes the cleavage of catechol to muconic acid. Muconic acid was produced when this pathway was expressed in *P. putida* KT2440. However, yields, growth, and productivity remained low. A variant of the above engineered *P. putida* KT2440 was therefore subjected to adaptive laboratory evolution with growth-based selection. A growth lag observed in the parent strain was reduced more than 2-fold in the evolved lineages Whole genome sequencing revealed a deletion of over 153,000 base pairs. Surprisingly, muconic acid titers were significantly higher than the unevolved parent strain. Combining metabolic engineering approaches with adaptive laboratory evolution has produced a unique strain with a reduced genome that allows for enhanced growth and dramatically improved muconic acid production. Understanding the function of mutations observed in the evolved strain may illuminate additional mechanisms to enhance muconic acid production and growth of *P. putida* KT2440.

4:00 PM 12-7: Redirecting carbon metabolism in *Zymomonas mobilis*:A promising new pathway to fuels and chemicals from biomass

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Zymomonas mobilis is known for its high specific glucose uptake rate, rapid catabolism, and high ethanol yield. *Z. mobilis* has also attracted considerable attention from industry for ethanol production. It has been engineered to efficiently convert the second and third most abundant plant derived sugars, xylose and arabinose, to ethanol at high yield. More recently, we have applied systems biology and genomic tools to investigate and improve its tolerance to the specific inhibitors present in biomass hydrolysate derived from dilute acid pretreatment of corn stover. For these reasons, *Z. mobilis* is one of the top fermentation organisms currently developed for the cellulosic biomass to ethanol conversion process. With its ability to utilize most biomass sugars, even in toxic hydrolysate environments, it is now sensible to enable this microorganism to make other products. Consequently, we introduced a heterologous pathway into *Z. mobilis* for the production of 2,3 butandiol (BDO). However, the strong, native pyruvate decarboxylase (PDC) activity posed a serious challenge for redirecting the carbon to the desired products in high yield and titer. Efforts to knock out the *pdc* gene in *Z. mobilis* strains were reported in literature; however, the results were mixed. Here, we report a successful strategy to enable complete redirection of carbon from ethanol production to BDO. Moreover, high yields and titers of BDO were obtained using engineered *Z. mobilis* for the fermentation of biomass hydrolysates. Initial TEA analysis showed that this approach represents a very promising new pathway to cost effective production of fuels and chemicals.

6:00 PM - 8:00 PM Session: 13: Special Topics 1: Is it Time to Rethink Cellulosic Ethanol?

Conveners: Prof. Bruce Dale, Michigan State University, Lansing, MI, USA

6:00 PM Discussion

6:00 PM - 8:00 PM Session: 14: Special Topics 2: Town Hall Discussion on the DOE Bioenergy Research Centers

Conveners: Dr. Brian Davison, Oak Ridge National Laboratory, Oak Ridge, TN, USA

Grand Ballroom, F-G Lobby Level

6:00 PM 14-1: Sustainable production of fuels and chemicals from dedicated energy crops

T. Donohue, University of Wisconsin-Madison, Madison, WI, USA*

Great Lakes Bioenergy envisions a future in which products derived from dedicated bioenergy crops replace a substantial fraction of the transportation fuels and chemicals that are currently produced from petroleum, while providing substantial environmental benefits and expanding economic opportunities for farmers, refiners, and communities. Our approach to realize this vision is to address major knowledge gaps that currently limit producing a profitable mix of biofuels and products from as much of the plant as possible.

<>*Integration of the Field-to-Product Pipeline.* To reap the benefits of a lignocellulosic industry, it is crucial to understand how to optimize a field-to-product pipeline. We will analyze and model the agricultural, deconstruction, material production systems, and the tradeoffs and synergies of a lignocellulosic industry.

<>*Sustainable Production of Bioenergy Crops with Desirable Traits.* Success of a new lignocellulosic industry will depend greatly on which, where, and how bioenergy crops are produced. We will develop bioenergy crops with traits tailored to enhance their environmental and economic sustainability. We will also investigate how to enhance the productivity of bioenergy crops on marginal land that is not used for food production.

Efficient Conversion of Biomass into Specialty Biofuels and Bioproducts. In the same way that petroleum refineries convert major portions of crude into fuels and chemicals, we predict that lignocellulosic biorefineries will generate multiple products from plant polysaccharides and lignin. We will use microbial, chemical and hybrid systems to produce replacements for petroleum-derived fuels and products from as much of the plant biomass as possible.

6:25 PM 14-2: Center for Advanced Bioenergy and Bioproducts Innovation - CABBI

C. Rao, University of Illinois at Urbana-Champaign, Urbana, IL, USA*

The mission of CABBI is to meet a major challenge facing the world: how to provide sustainable sources of energy for societal needs as the population continues to grow and global change accelerates. CABBI will develop efficient ways to grow, transform, and market biofuels and other bioproducts. The vision of CABBI is to integrate recent advances in genomics, synthetic biology, and computational biology to increase the efficiency, sustainability and value of biomass crops. This holistic approach will help reduce our nationÕs dependence on fossil fuels thereby increasing sustainability and national security.

CABBI is founded on the Òplants-as-factoriesÓ paradigm, in which biofuels, bioproducts, high-value molecules, and foundation molecules for conversion are synthesized directly in plant stems. CABBI will focus on sorghum, energycane, and Miscanthus, which are high-yielding throughout the rain-fed eastern U.S.

Foundation molecules produced in plants will be efficiently converted using the design-build-test-learn framework to diverse, high-value molecules such as biodiesel, organic acids, jet fuels, lubricants, and alcohols using technologies developed in a versatile, automated biofoundry for rapidly engineering microbial strains.

The Center will employ a data-driven integrated modeling framework to predict which feedstock combinations, regions and land types, market conditions, and bioproducts can support the ecologically and economically sustainable displacement of fossil fuels. The result will be an overarching framework for viewing the research through an environmental and economic lens.

CABBI, a U.S. Department of Energy Bioenergy Research Center, is supported by DOE, Office of Science, Office of Biological and Environmental Research.

6:50 PM 14-3: Developing Science-Based Solutions for the Bioeconomy: the Joint BioEnergy Institute

B.A. Simmons, Joint BioEnergy Institute / Lawrence Berkeley National Laboratory, Berkeley, CA, USA*

The long-term vision of the Joint BioEnergy Institute (JBEI, www.JBEI.org) is that bioenergy crops can be converted into economically-viable, carbon-neutral, specialty biofuels, all of the organic chemicals currently derived from petroleum, and many other bioproducts that cannot be efficiently produced from petroleum. This vision will only be possible when we have sustainable bioenergy crops, biorefinery technologies capable of converting as much carbon in biomass into biofuels as possible, and a vast array of bioproducts that will make biorefineries economically viable. JBEI's mission is to establish the scientific knowledge and new technologies in feedstock development, deconstruction and separation, and conversion needed to transform the maximum amount of carbon available in bioenergy crops into biofuels and bioproducts. Informed and benchmarked by our technoeconomic and life-cycle analyses, JBEI's research program will take a systems-level approach to simultaneously optimize the composition

of our bioenergy crops, the deconstruction and separation process, and the metabolism of our biofuel- and bioproduct-producing microorganisms. The development of an effective and scalable biomass-to-biofuels-and-bioproducts process requires a highly integrated research program that benefits from an embedded technology development program to build tools for all aspects of the research. These advances require continual reassessment of the technoeconomics and life-cycle implications when there are discoveries or roadblocks that require a change in the research program. This presentation will provide an overview of the current and future scientific directions at JBEI and highlight some of the major research accomplishments achieved.

7:15 PM 14-4: The Center for Bioenergy Innovation (CBI): A Biological Approach to Specialty Fuels and Products

G.A. Tuskan[*]*, Oak Ridge National Laboratory, Oak Ridge, TN, USA*

The world, biology (technique and insight), and the bioeconomy (still in its infancy) have experienced profound changes in the past decade. The Center for Bioenergy Innovation (CBI) vision is to accelerate domestication of bioenergy-relevant plants and microbes to enable high-impact, value-added, co-product development at multiple points in the bioenergy supply chain. CBI has identified research targets to overcome key barriers for the current bioeconomy in (1) high-yielding, robust feedstocks, (2) lower capital and processing costs via consolidated bioprocessing (CBP) to specialty biofuels, and (3) methods to create valuable byproducts from the lignin residues. These targets will be achieved through the development and use of accelerated domestication tactics that leverage complex phenotypes in relevant non-model plants and microbes (i.e., poplar, switchgrass, *Clostridium thermocellum* and *Pseudomonas putida*). We are building on strong management, ongoing integrative assessment, institutional capabilities, and a highly experienced research team to implement and realize developments in rapid genetics, bioprocessing, and massive data biocomputing to accelerate these critical advances in an integrated center. Guiding by technoeconomic and life-cycle analyses, CBI will home in on alleviating cost barriers that have thus far prevented the biofuels endeavor from becoming a sustainable and economically viable reality. Key developments and center overview will be presented.

7:40 PM 14-5: BioEnergy Research Center Roundtable

B. Davison[*]*, Oak Ridge National Laboratory, Oak Ridge, TN, USA*

A panel discussion with questions from the audience for any of the previous presenters.