41st Symposium on Biotechnology for Fuels and Chemicals

Saturday, April 27

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

Chelais - Room 305, Third Level

Sunday, April 28

8:00 AM - 4:00 PM Exhibit Setup

Columbia Ballroom Foyer, Third level

8:00 AM - 5:00 PM Registration

Columbia Ballroom Foyer, Third level

11:00 AM - 5:00 PM Poster Setup

Columbia A, Third level

1:00 PM - 3:45 PM Session: SO: Student Oral Presentations

Columbia C-D, Third level

1:00 PM SO-1: Developing the thermophilic filamentous fungus *Thermoascus aurantiacus* into a thermostable cellulase production platform

R. Gabriel[*]*, M. Jecmenica, P. Chou, A. Oostlander, L. Matz, C. Hopson, S. Harth, R. Mueller, T. Schuerg and S.W. Singer, Biological Systems and Engineering Division, Lawrence Berkeley National Laboratory, Emeryville, CA, USA*

The thermophilic fungus *Thermoascus aurantiacus* produces highly thermostable and active cellulases, which makes this fungus an interesting platform for the cost-effective conversion of cellulose into renewable fuels and other products. Our goal is to determine the factors that promote high cellulase production in this fungus using -omics technologies and develop genetic transformation tools to improve cellulase production. Initially, we established a minimal growth medium for this fungus and a novel fed-batch system to screen up to 12 CAZyme inducers at a time at 50 ml shake flask scale. Xylose, arabinose and cellobiose were identified as potent inducers of cellulases, while only the pentose sugars induced xylanases. These strong inductive effects were only visible by using the fed-batch cultivation. RNA-Seq data have been obtained from these fed-batch cultivations to study genome-wide gene expression patterns when grown under enzyme secretion conditions compared to no carbon and high glucose medium. In parallel work, we have established an *Agrobacterium tumefaciens*-mediated transformation system (ATMT) for *T. aurantiacus* ascospores with a hygromycin resistance marker. Protocol conditions were optimized for spore production medium, temperature, co-incubation time, filter membranes, pH and acetosyringone concentration. Furthermore, a *ku70* deletion staining was generated to perform efficient gene deletions. With ATMT we overexpressed the xylanase regulator *xlnR*, which increased the xylanase activity of the respective isolates by up to 500 % compared to the wild type.

1:15 PM SO-2: Experimental and Kinetic Modeling Insights into Lignin and Hemicellulose Removal by Low Severity CELF Pretreatment of Corn Stover

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

Pretreatment is a key step for biological conversion of lignocellulosic biomass into renewable fuels. Co-solvent enhanced lignocellulosic fractionation (CELF) that employs tetrahydrofuran (THF) in solution with dilute acid has been shown to effectively fractionate the lignocellulosic matrix and produce solids that are highly digestible by enzymes. In this study, kinetic models

were coupled with experiments to gain insight into factors controlling solubilization of cellulose, xylan, and lignin by CELF pretreatment of corn stover. CELF pretreatments were run at 130°C, 140°C, and 150°C for reaction times of 10, 20, 30, and 40 minutes at 5 wt% solids in a 1:1 THF:water (w/w%) ratio with 0.5 wt% sulfuric acid in the liquid. Afterwards, 15 mg protein of Accellerase 1500/g glucan based on the raw biomass was applied at 5 g/L glucan loading of pretreated solids to understand how CELF pretreatment conditions impacted enzymatic hydrolysis kinetics. Coupling kinetic modeling with experimental results revealed that 1) xylan removal could be accurately modeled assuming fast and slow fractions, 2) lignin removal could also be accurately modeled by treating lignin as bulk and residual fractions, 3) bulk lignin and fast xylan are removed at the same rate, 4) slow xylan and residual lignin are removed at similar rates, 5) CELF reduces or eliminates lignin redeposition that occurs in conventional dilute acid pretreatment, 6) batch CELF operation achieves even higher lignin removal than flowthrough pretreatment, 6) THF catalyzes xylan and lignin removal in the THF/water miscibility regime (>145°C), and 7) the small amount of glucose CELF released into solution appears to be from amorphous cellulose.

1:30 PM SO-3: Techno-economic assessment of C5 fermentation using native and genetically modified strains of *Saccharomyces cerevisiae*

I. Sampaio, C. Sargo, M. Watanabe, A. Bonomi and E. Morais, Brazilian Center for Research in Energy and Materials (CNPEM), Campinas, Brazil*

Second-generation (2G) ethanol is produced through fermentation of sugar monomers obtained from the breakdown of cellulose and hemicellulose. For Brazilian ethanol mills, sugarcane bagasse (and possibly straw) would be the first option considered for the second-generation process, since this material is already available close or in the production site. The composition of sugarcane bagasse found on literature shows that approximately 25% of bagasse fibers are composed by pentosans; this illustrates the importance of optimizing the use of pentoses for ethanol production.

Saccharomyces cerevisiae is the most commonly applied microorganism for first-generation (1G) ethanol production, but this yeast is not able to ferment xylose naturally. Native *Saccharomyces cerevisiae* can, however, ferment xylulose, an isomer of xylose; so, an alternative for C5 fermentation is a two-step process consisting of an *ex-vivo* isomerization of D-xylose to D-xylulose using the enzyme xylose isomerase and subsequent fermentation. Another strategy is the development of genetically modified strains of *S. cerevisiae* with heterologous xylose metabolic pathways. Both arrangements have drawbacks, however; using genetically modified organisms requires special regulations and engineered microorganisms can have problems with genetic instability and present low overall ethanol yields. For the case of xylose isomerization and subsequent xylulose fermentation, the main challenges are the unfavorable equilibrium of xylose and xylulose after isomerization and the slow fermentation of xylulose.

This work compares the techno-economic performance of these two approaches to xylose fermentation, using data from literature. The results indicate the main costs for each alternative and existent bottlenecks to reach 2G ethanol economic feasibility.

1:45 PM SO-4: Prebiotic Xylooligosaccharide Production from Biomass Autohydrolyzate Liquors

D. Corbett, G. Luo, C. Hong, L. Ou, R. Venditti and S. Park, North Carolina State University, Raleigh, NC, USA*

Society for Industrial Microbiology
Spring 2019 Symposium on Biotechnology for Fuels and Chemicals, Seattle, WA
April 28th Ð May 1st
Advances in Conversion Technologies.

Production of Xylooligosaccharide Prebiotics from Biomass Autohydrolyzate Liquors

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Prebiotics are a class of carbohydrates that are indigestible by the enzymes in the human stomach but are metabolized by beneficial bacteria once in the gut. Prebiotics are an important aspect of human health and well-being. In this work, we investigate a commercially viable process for the production of a xylooligosaccharide (XOS) prebiotic product from autohydrolysis liquor. The most common commercial prebiotics are fructooligosaccharides and mannanoligosaccharides, however, xylooligosaccharides are emerging as a promising alternative due to their effectiveness and the potential for their production from lignocellulosic sources. Autohydrolysis is a practical and effective method for the pretreatment of lignocellulosic biomass. However, complete utilization of all fractions produced during pretreatment is essential to the overall economic

success of biorefineries based on autohydrolysis.

In this work, removal of non-carbohydrate components from autohydrolyzate using hydrophobic resin is assessed as well as processes for oligosaccharide isolation and oligomer concentration. Techno-economic analysis was performed on the proposed process utilizing modeling software. Unit operations were identified for further study and optimization in the laboratory, based on the model.

Our results have shown that treatment of autohydrolyzate with hydrophobic resin is effective at removing non-carbohydrate components such as lignin, hydroxymethylfurfural, and furfural (~89%, ~89%, and 95% removed, respectively), but suffers from relatively high removal of XOS as well (> 50% of total unbound XOS). Water usage is a major concern due to costs associated with drying. The estimated overall product yield for the unoptimized process is ~32% (of the total unbound XOS in hardwood autohydrolyzate). The potential economic impact of implementing the prebiotic production process in a biorefinery as well as options for improving the process yield and economics are discussed.

2:00 PM Break

2:15 PM SO-5: Exogeneous efflux pump expression increase robustness against biomass hydrolysates inhibitors on fermentations of the hyperbutanol producer *C. saccharoperbutylacetonicum* N1-4

P. Jimenez-Bonilla, Auburn University / Universidad Nacional de Costa Rica, Auburn, AL, USA and Y. Wang, Auburn University, Auburn, AL, USA*

Butanol has a high potential as a biofuel to replace gasoline in engines for transportation, and as biochemical in the production of solvents, paintings, polymers and others. There is also a big interest in the production of biobutanol from lignocellulosic materials, because they are cheap and widely available, although by products of biomass hydrolyzation, such as, furan aldehydes and phenolic compounds, inhibit bacterial growth. In this work, we probe that *srpB* efflux pump from *Psedumonas putida* increase the tolerance against the biomass hydrolysates inhibitors, when is expressed on *Clostridium saccharoperbutylacetonicum* N1-4. The improved strain is able to grow up in a media containing 17% more furfural and 50% more ferulic acid (respecting a control strain), as model compounds for furan aldehydes and phenolics, respectively. Also, the engineered strain is still able to produce about 14 g/L of butanol at the high concentration of inhibitors. Models shown that the critical parameters are related with the lag phase and the beginning of the fermentation, suggesting that future genetic improvement should target in mechanisms acting on the early stage.

2:30 PM SO-6: Direct conversion of pretreated wheat straw to cellobionic acid without any enzyme addition and the effect of lignin and lignin degradation products on the conversion system

M. Zhou and Z. Fan, University of California, Davis, Davis, CA, USA; T. Kasuga, USDA-ARS, Davis, CA, USA; X. Lü, Northwest A&F University, Yangling, China

In a cellulosic biorefinery, the purchase or on-site production of cellulase is usually needed to produce sugars from pretreated cellulosic biomass and represents a substantial processing cost. When a cellulolytic fungus is used to achieve direct cellulase production and saccharification, the fungus will inevitably consume the resulting sugars during hydrolysis, which results in severe carbon loss. In our prior study, we adopted a novel strategy to engineer a *Neurospora crassa* strain to convert cellulose to cellobionic acid without any enzyme addition at high yields. The fungus naturally produces cellulase and cellobiose dehydrogenase (CDH), and heterologously expresses laccase. When Avicel was used as the carbon source, the engineered fungus HL10 was able to convert Avicel to cellobionic acid at a yield higher than 90% without any enzyme addition. However, the addition of catalytic amount of artificial redox mediators such as 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) was needed to achieve high yield conversion. When a lignocellulosic substrate such as wheat straw or pretreated wheat straw was used as the carbon source, it was found that the addition of exogenous redox mediators was not necessary. Lignin and lignin degradation products were able to serve as redox mediators for CDH-laccase conversion system. However, they inhibit laccase activity at higher concentrations.

2:45 PM SO-7: Mitochondrial compartmentalization confers specificity to recursive alcohol production in *Saccharomyces cerevisiae*

S. Hammer and J. Avalos, Princeton University, Princeton, NJ, USA

Recursive elongation pathways have the potential to produce compounds with increasing carbon-chain length. 2-ketoacids with molecular weights larger than pyruvate are of particular interest due to their role as substrates for the production of higher alcohols, which are valuable renewable fuel compounds. While expression of heterologous enzymes and protein engineering have been successfully employed in *Escherichia coli* to increase the maximum number of iterative elongation cycles, specific production of longer-chain 2-ketoacids remains difficult to achieve. Here, we demonstrate that mitochondrial compartmentalization in the yeast *Saccharomyces cerevisiae* can increase specificity of recursive pathways toward longer-chain products. Using 2-ketoacid elongation as a proof of concept, we show that overexpression of the elongation enzymes in mitochondria of a four-carbon alcohol production strain boosts five-carbon alcohol production more than 13-fold. Total alcohol

production also shifts from a predominately four-carbon alcohol mixture to one with more than 80% five-carbon alcohols. Modifying transport of an intermediate and deregulating transcription of multiple pathway genes in one of our top producing strains achieves the highest five-carbon alcohol titer reported for *S. cerevisiae* in the peer-reviewed literature. This work establishes the ability of mitochondrial compartmentalization of recursive metabolic pathways to enhance product specificity while also achieving high titers, exemplifying the potential benefits of harnessing organelles to engineer recursive pathways for product specificity.

3:00 PM SO-8: Recovery of medium chain carboxylic acids from brewery waste using anaerobic membrane bioreactor integrated with liquid-liquid extraction system

S. Shrestha[*]*, X.F. Almansa and L. Raskin, University of Michigan, Ann Arbor, MI, USA*

The increasing number of breweries in the U.S. is creating considerable challenges for waste management. Brewery waste is rich in biodegradable organics, providing ample opportunities to apply biotechnology to recover these untapped resources. One such process is the production of medium chain carboxylic acids (MCCAs, carboxylic acids with six to 12 carbons [C6-C12]) via chain elongation of short chain carboxylic acids (SCCAs, C2-C5) with a reduced compound, such as ethanol. MCCAs are platform chemicals with several industrial and agricultural applications. The inputs for chain elongation, SCCAs and ethanol, can both be produced from waste streams, reducing the cost and environmental impact of deriving them from non-renewable resources.

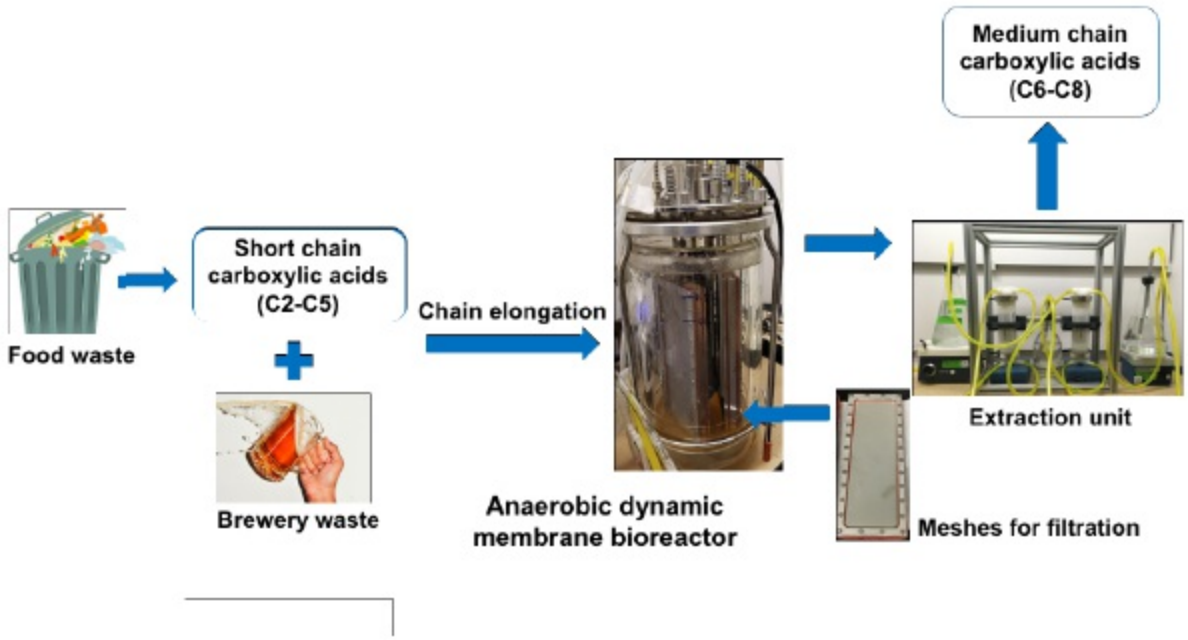

Figure 1. Schematic of the bioreactor system for MCCAs production

A laboratory scale anaerobic dynamic membrane bioreactor (AnDMBR) is being operated with brewery waste and effluent containing SCCAs from an anaerobic, acidogenic bioreactor fed food waste (Figure 1). The AnDMBR is equipped with submerged membrane housings containing stainless steel meshes of 25-mm pore size as dynamic membrane (biofilm) support. A maximum yield of 0.23 g COD MCCAs/g COD_{in} has been achieved during the start-up period. Preliminary results showed that the MCCAs yield is limited by product toxicity necessitating continuous extraction. Thus, a liquid-liquid extraction (LLX) system consisting of two hydrophobic hollow fiber membrane contactors was optimized. The LLX method effectively separated MCCAs from a synthetic mixture of SCCAs and MCCAs. The continuation of this research will include integration of the LLX unit with the AnDMBR for increased MCCAs production and simultaneous recovery.

4:00 PM - 4:45 PM Rapid Fire

Columbia C-D, Third level

5:00 PM - 6:00 PM Exhibits Open

Columbia Ballroom Foyer, Third level

5:00 PM - 6:00 PM Welcome remarks: Steve Van Dien, Persephone Biome, SIMB President; Seema Singh, Sandia National Labs and Claus Felby, Novo Nordisk Foundation, 2019 Program Chairs; Keynote: Jennifer Holmgren, Lanzatech

Columbia C-D, Third level

6:00 PM - 8:00 PM Session: PS1: Poster Session I

Columbia A, Third level

S1 Sulfomethylation of kraft lignin to increase cement fluidity

J.C. Kim, S.Y. Park, S. Yeon, J.H. Choi, J.H. Kim, S.M. Cho, D.S. Lee and I.G. Choi, Seoul National University, Seoul, Korea, Republic of (South)*

In biomass industry, it is very important to use all of the components in wood, which is called biorefinery, due to an economic aspect. For this reason, the use of technical lignin, such as kraft lignin and organosolv lignin, is considered crucial. Especially, kraft lignin, which is obtained from kraft pulping, is the most abundant technical lignin, and the lignin has been used to generate energy by incineration. However, energy efficiency of the incineration is low.

Furthermore, a polycarboxylate ether, which is commonly used as a superplasticizer, has been utilized to increase cement fluidity. However, this material is expensive to use for improvement of cement fluidity, so many studies have been conducted to substitute the material. Therefore, it will be beneficial to substitute polycarboxylate ether to modified kraft lignin by introducing specific functional groups on kraft lignin through a variety of chemical reactions.

In this study, sulfomethylation was conducted to introduce anionic charge on kraft lignin by using sodium sulfite and formaldehyde. As a result, sulfomethyl group, which was attached to kraft lignin, increases repulsion force between cement particles due to anionic charge. Reaction conditions were different depending on reaction temperature, reaction time, final pH and the dosage of reagents. After sulfomethylation of kraft lignin, modified kraft lignin was put into cement paste and cement fluidity was measured by means of mortar flow table test. In conclusion, sulfomethylation was able to attach sulfomethyl group on kraft lignin, which was able to increase cement fluidity when it mixed with cement paste.

S2 Production of xylooligosaccharides in a microchannel reactor

L.B.D. Nascimento, G.M. Zanin, M.D. Souza and F.F. Moraes, State University of Maringa, Maringá, Brazil*

Lignocellulose is the most prominent biomass on the planet. It is composed of numerous organic compounds, and has great potential as a source of raw materials. Xylan is a polysaccharide extracted from lignocellulose and xylanase enzymes can hydrolyze xylan generating xylooligosaccharides of different chain length, which have many uses in the industry. The objective of this work was the production of modified xylooligosaccharides with butyl glycolate in a microchannel reactor that worked at 60 °C with concentrations of xylan and butyl glycolate of 10 g/L and 15 mL/L, respectively. The reaction medium contained one of the three xylanases from Novozymes NS50014, NS50030, or NS22002 with 3 U/mL. The microchannel reactor diameter was 0.6 mm and the length 100 cm. The same reactions and conditions were used in a batch reactor to compare results. HPLC was used to analyze the samples. The results showed that the continuous microchannel reactions produced higher concentrations of longer chain xylooligosaccharides. Using NS22002 xylanase in the batch reactor during 24 h produced 2.7 and 2.9 g/L of xylose and xylotetraose, respectively, whereas the microchannel reactor with a residence time of 15 min produced 0.4 and 4.7 g/L of the same products, respectively. Similar results were found for the other enzymes and residence times, demonstrating that precise control of microchannel conditions can change the fraction of obtained products, and it is possible to select conditions to enhance the production of a xyloside of higher or lower molecular mass.

S3 Continuous enzymatic biodiesel production from *Jatropa curcas* oil in a packed-bed reactor with a glycerol extraction column formed as by-product

L.S. Martin, D. Molinari, C.A. Araki and S.M.P. Marcucci, State University of Maringa, Maringa - PR, Brazil; F.F. Moraes, P.A. Arroyo and G.M. Zanin, State University of Maringa, Maringá, Brazil; H.F.D. Castro, Escola de Engenharia de Lorena - University of São Paulo - USP, Lorena - SP, Brazil*

The present study aimed to investigate the potential of *Jatropha curcas* oil as a raw material for biodiesel production by enzymatic route. To attain this purpose, the transesterification reaction of *Jatropha curcas* oil with ethanol was catalyzed by *Burkholderia cepacia* lipase immobilized on SiO_2-βCD in a packed-bed reactor in continuous mode. The experimental design consisted of a packed-bed reactor incorporating a column with rice husk ash (RHA) to remove the glycerol formed as by-product. Tests were carried out using a reactor with length of 265 mm and diameter of 11 mm and a glycerol extraction column with length of 295 mm and diameter of 6 mm. Runs were performed continuously for 20 days using substrate containing oil to ethanol molar ratio of 1:8 and 1:12 in a solvent-free system and using a space-time of 8 h. Samples were collected before and after the glycerol extraction column. Tests carried out in the reactor using molar ratio of 1:12 and the space-time of 8 h provided ethyl ester formation of 54.27 ± 4.8 wt%, transesterification yield of 80.20 ± 5.5% and productivity of 67.84 ± 5.1 $mg_{éster}.g^{-1}.h^{-1}$. The use of RHA showed a good performance and its application in adsorbing glycerol were determined by FTIR. However, the use of a extraction column did not increase the transesterification yield. Thenceforth a more especific study should be done to verify the potential of the RHA as a glycerol sorbent.

S4 Biocatalytic valorization of furans by *Rhodococcus jostii* RHA1

X. Li, Z. Xu and B. Yang, Washington State University, Richland, WA, USA; J.R. Cort and W. Qian, Pacific Northwest National Laboratory, Richland, WA, USA*

Furfural and 5-hydroxymethylfurfural (5-HMF) are generated from sugars during the pretreatment of lignocellulose in biorefineries. High concentrations of furans may inhibit microbial growth and function, leading to lower product titers in biomass conversion processes, which is what encourages studies on the detoxification of furans in hydrolysates. Meanwhile, furan valorization is attracting an increasing amount of attention because the biogenic furans' chemical functionalization and versatility make them useful building blocks for various value-added compounds (e.g. polyethylene furanoate as an alternative to oil-based polyester). Herein, the growth of *Rhodococcus jostii* RHA1 on furfural or 5-HMF as either the sole carbon source or when supplemented with glucose was investigated. NMR results suggested that the quick accumulation of 2-furoic acid occurred via the oxidation of furfural, while 5-hydroxymethyl-2-furancarboxylic acid and trace of 2,5-furandicarboxylic acid were generated as intermediates from 5-HMF. The catabolism pathway of furfural and 5-HMF in *R. jostii* RHA1 was proposed based on global proteomics analysis. The yields of furanic acids converted from dilute acid pretreated crude hydrolysate were addressed as a potential biocatalytic route.

S5 Non-stationary 13C-metabolic flux analysis to investigate Spathaspora passalidarum metabolism on glucose and xylose

L.E. Biazi, I. J.L. and A.C. Costa, 1School of Chemical Engineering, State University of Campinas – UNICAMP, Campinas, Brazil; C. Bonan, University of Campinas, Campinas, Brazil; F. P., Luxembourg Institute of Science and Technology (LIST), Belvaux, Luxembourg; A. J, Department of Chemical, Biological and Environmental Engineering - Universitat Autonoma de Barcelona, Zip Code 08193 Bellaterra (Cerdanyola del Valles), Barcelona, Spain, Barcelona, Spain; W. A, Department of Biotechnology, Delft, Netherlands*

S6 Enhanced isopropanol-butanol-ethanol (IBE) production using engineered *Clostridium* strain from switchgrass with *Trichoderma* in-house enzymes for biomass hydrolysis

P. Wang and Y. Wang, Auburn University, Auburn, AL, USA; B. Okeke, Auburn University at Montgomery, Montgomery, AL, USA*

The production of solvents including acetone-butanol-ethanol (ABE) using solventogenic clostridia has attracted extensive attention recently. However, due to the corrosive feature of acetone, ABE mixture cannot be used directly as a fuel. Recently, we have developed a *Clostridium saccharoperbutylacetonicum* PW2 strain for high-efficient isopropanol-butanol-ethanol (IBE, which can be used directly as a fuel) production by integrating 'acetone-to-isopropanol' pathway into the chromosome of *C. saccharoperbutylacetonicum* N1-4. Lignocellulose biomass is an abundant renewable carbon source for biosolvent production. However, enzymatic hydrolysis of biomass accounts for a major cost for the bioprocess. Here we deployed a high beta-glucosidase producing wild-type *Trichoderma* species SG2 for cellulase production and *C. saccharoperbutylacetonicum* PW2 for simultaneous biomass saccharification and IBE fermentation using acetic acid pre-treated switchgrass. Crude culture supernatant of *Trichoderma* species SG2 displayed as much as 9.84±1.12, 48.02±2.53, and 30.10±1.11 units mL^{-1} of cellulase, xylanase, and β-glucosidase in 30 min assay, respectively. Filtered and unfiltered *Trichoderma* SG2 cultured on switchgrass biomass supported significant production of IBE from acetic acid pretreated switchgrass by *C. saccharoperbutylacetonicum* PW2. Overall, a total of 6.85 g/L IBE was produced based on 50 g/L dry biomass loading (0.14 g solvent per g dry biomass). Results indicate that simultaneous saccharification and fermentation can be used for economic production of IBE from lignocellulose biomass using in-house enzymes.

S7 Evaluation of the Synergism Degree of Cellulase and Xylanase in the Hydrolysis of Lignocellulosic Biomass

C. Giannini, D. Passos and N. Pereira Jr., Federal University of Rio de Janeiro, Rio de Janeiro, Brazil; L.A.F.D.S. Schlitter, PhD., Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil*

This study proposes to evaluate the synergism between endoglucanases and endoxylanases in order to facilitate the access of cellulolytic enzymes to cellulose which is influenced through the existence of xylans in the biomass. Therefore, the design of an optimal cocktail with endoxylanases e endoglucanases could increase the conversion of the cellulose to glucose in a cellulolytic complex. Two lignocellulosic biomasses were used to evaluate the synergism between these enzymes. One of them came from the cellulose industry - a cellulosic pulp mill residue (PM3); the other one was sugarcane bagasse that was submitted to an alkaline pretreatment (CLD). An assay was first perfomed in the following conditions: solid loading (SL) of 1.0%; proteins loading, (PL) of 10.0mg/$g_{celulose}$; enzymes used were cellulase and xylanase from Sigma-Aldrich®; 50ºC for 24 hours. The hydrolysis of CLD resulted in 31.2% of hydrolytic efficiency (HE) when hydrolyzed only with cellulase; however when a cocktail with both enzymes (1:1) was used, the HE has increased to 43.0%, resulting in a synergism degree (SD) of 1.25. With PM3, HE was 30.5% with cellulase only and 39.8% with both enzymes, providing a SD of 1.13. To confirm this result, other experiment was carried out with CLD, increasing the SL to 2.5%, reaching 75.7% and 94.9% of HE, with only cellulase and with the cocktail containing both enzymes, respectively. Finally, a central composite rotatable design methodology was applied, searching for the optimization of the conditions that can increases glucose release and HE to make the hydrolysis process more feasible.

S8 Fractionation behavior of non-cellulose component in miscanthus by lignin-first extraction coupled with hydrothermal and ball-milling treatment

S.K. Jang, C.D. Jung, J.H. Yu and H. Kim, Korea Research Institute of Chemical Technology, Ulsan, Korea, Republic of (South); J.H. Lee, Seoul National University, Seoul, Korea, Republic of (South); J.W. Choi, Seoul National University, Pyeongchang, Korea, Republic of (South)*

Lignin have been usually recovered as a by-product in the process for obtaining cellulose or hemicellulose fraction. Additionally, most of lignin extraction processes changed the structure of native lignin having more frequent aryl-aryl bonds which causes higher bonding dissociation energy. Although milled-wood lignin can be extracted with conserving the native lignin structure relatively, extraction yield is typically 10% or less in previous studies. Therefore, we designed lignin-first extraction process to improve recovery rate of lignin and prevent alteration lignin bonding properties.

For suitable lignin separation, hydrothermal treatment and ball-milling process were conducted before lignin extraction from miscanthus. A continuous type reactor, which had a processing capacity of 10 kg/h, was employed for the hydrothermal treatment (200°C for 10 min) using high pressure steam only as a solvent. Then the solid residue was air-dried and ground for 6 and 48 h. Three types of aqueous-organic solvent was subjected to lignin extraction as the concentration change of 1,4-dioxane (80, 96, and 100% (v/v)). As a result, more than 50 g gallic acid equivalent/L of total phenolic compound was dissolved into the extract that was detected by Folin-Ciocalteus' assay. Hydrothermal treatment significantly increased extraction yield of lignin than that of untreated samples as approximately 5 times. Meanwhile, residues obtained after the lignin extraction process showed more than 90% of glucose yield after 72 h enzymatic hydrolysis on the Novozyme CTec3 5 FPU/g glucan enzyme loading condition. Characteristics of extracted lignin such as depolymerization behavior and molecular weight distribution will be presented and discussed.

S9 Microwave-assisted direct conversion of biomass sugars to platform chemicals: 5-hydroxymethylfurfural (HMF) and furfural

A. Mittal, H.M. Pilath and D.K. Johnson, National Renewable Energy Laboratory, Golden, CO, USA*

5-hydroxymethylfurfural (HMF) and furfural are promising platform molecules which can be converted to fuel intermediates and hydrocarbon fuels. Herein, one-step, direct conversion of the carbohydrates in untreated biomass to HMF and furfural in a microwave reactor is investigated. The influence of reaction temperature, time, feedstock, addition of Lewis and Brønsted acids, solvent and solvent to aqueous ratio were studied to find the optimum process conditions to maximize the yield of furfurals. The reaction conditions of 200°C for 5 min with 33 mM HCl and 8 mM $AlCl_3$ and using dioxane-water (4:1) miscible solvent system were found to be the most conducive for direct dehydration of glucan and hemicelluloses present in untreated poplar wood resulting in unprecedented high furfural and HMF yields of 91 and 69%, respectively. Furfural could be easily produced in yields greater than 80%, and once formed it was found to be stable under the various process conditions explored. In contrast, HMF yields varied with both reaction time and temperature, as it readily underwent rehydration to levulinic acid in yields approaching 25-30% at increased reaction severity. The addition of both the Brønsted (HCl) and Lewis ($AlCl_3$) acid catalysts in catalytic amounts, i.e., 33 and 8 mM, respectively was required to maximize the yield of furfurals. Interestingly, biomass physical features, such as crystallinity or the presence of other biomass constituents, such as lignin, had little influence on furfurals yields.

S10 Toward Complete Lignocellulose Valorization by Fractionating Uncondensed Lignin Using an Acid Hydrotrope at Low Temperatures (≤90 °C)

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

This study demonstrated a potentially economic and sustainable approach for complete lignocellulose valorization. Poplar wood was rapidly fractionated in approximately 5–40 min using aqueous solutions of an acid hydrotrope (AH), *p*-TsOH, at temperatures below 90 ºC, with the resultant lignin fraction in near-native state with well-preserved chemical structure and at excellent yields of up to approximately 80%. ^{13}C-^{1}H 2D nuclear magnetic resonance (NMR) spectroscopic analyses indicated that AH-solubilized lignin (AHL) from a range of fractionation conditions contains very high content of b-aryl ether linkages with minimal condensation, which facilitated subsequent reductive catalytic depolymerization to result in an excellent lignin monomer yield of over 30%. Gel permeation chromatographic (GPC) and differential scanning calorimetric (DSC) analyses showed that minimally condensed AHLs have large molecular weights and low glass transition temperatures *T*g, ideal for direct applications as polymers in composites. AHLs also have excellent optical properties, with brilliant pinkish color, important for applications such as cosmetics and dye dispersant. AH fractionation preserved the cellulose fraction as solid fibers with light pinkish color for the materials market with high value such as dissolving pulp for textile fibers and cellulose nanomaterials. AHF also solubilized up to approximately 90% of xylan, which can be easily converted to furfural using *p*-TsOH in the spent liquor without additional catalyst. The substantial delignification and dissolution of hemicelluloses also facilitated efficient enzymatic saccharification of the cellulosic solids to sugars. *p*-TsOH as a solid catalyst can be reused after lignin precipitation simply through dilution with water to below the minimal hydrotrope concentration (MHC) of 11.5% followed by dehydrating dissolved hemicellulosic sugars into furan. The sheer simplicity of AH fractionation, i.e., using one easily recyclable industrial chemical such as *p*-TsOH in an aqueous system below water boiling temperature, to valorize all three major fractions of lignocelluloses makes it stand out beyond all existing technologies.

S11 Production of pectin, xylooligosaccharide, and fermentable sugars using squeezed mandarin peel by fractionation processes on mild conditions

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Mandarin, which is a special product of Jeju Island in South Korea, is known to have a physiological activity beneficial to human body such as reduction of the blood cholesterol level and prevention of the vascular disease. Additionally, 100,000 tons of mandarin are annually consumed for juice production. However, significant amount of mandarin peel as a waste is generated as much as 50% of the initial mandarin weight during the juice production. The squeezed mandarin peel is managed typically as a landfill, but new treatment methods have been required due to environmental and cost problems of the conventional method. In this study, we conducted separation processes for recovering useful byproducts, and induced biological treatment for reducing the amount of wasted residues.

First, the squeezed mandarin peel was ground gently with washing water using twin-extruder machine to improve storability and processability by diminishing undesirable components such as pectinase, acetyl esterase, and peel oil. Then, solid fraction was separated by filter press until the moisture content reached approximately 60%. Hydrothermal treatment was conducted in a batch-type reactor for recovering the pectin and/or xylooligosaccharide (XOs) fraction from the washed mandarin peel. Mild hydrothermal treatment condition (70°C to 130°C for 30 min) was chosen for selective separation of pectin and XOs from solid fraction and minimization of further degradation of pectin and XOs. After the hydrothermal treatment and separation processes, cellulose-rich and low lignin containing residue was subjected enzymatic hydrolysis and glucose yield was evaluated depending on enzyme loading.

S12 AÂ different view of cell wall diffusion channels suggests pretreatment possibilities

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Biomass pretreatments in addition to size reduction are necessary because of the slow diffusion rates of reactants into, and products out of, cell walls. The literature on transport through biomass cell walls often discusses void channels inside the cell wall. The existence of such channels in unmodified cell walls is unsupported by any experimental evidence or precedent in similar polymers. However the evidence for diffusing agents riding along with mobile, plasticized polymer segments is well supported by experimental data and theory. We will discuss how this view of pore structure of the cell wall suggests new approaches to improving biomass pretreatments.

S13 Lignin First Solvent Pretreatments and Their Influences on Biomass Structural Characteristics

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Lignocellulosic biomass has evolved complex structural and chemical mechanisms to protect itself against microbial attacks,

which are ultimately responsible for its recalcitrance. Pretreatment is a crucial step in biological conversion of biomass as it can render structural changes in biomass to reduce the biomass recalcitrance, thus enhancing its sugar release performance. There have been many efforts to develop an effective solvent system to overcome the limitations of conventional pretreatments, such as biomass dissolution and lignin fractionation. In this study, the effects of two recently developed pretreatment methods, co-solvent-enhanced lignocellulosic fractionation (CELF) and γ-Valerolactone (GVL) pretreatments, on physicochemical properties of biomass were investigated and compared with the influence of conventional ethanol organosolv pretreatment on characteristics of poplar wood. Poplar wood particles were pretreated under their optimal conditions according to the literature. The physicochemical properties of the cellulose and lignins in each pretreated substrate was analyzed and compared using diverse analysis methods. Chemical compositions of cellulose-rich fraction and lignin fraction were analyzed by HPLC. Molecular weights of cellulose and lignin fractions were investigated using GPC. 2D ^{13}C-^{1}H HSQC NMR and ^{31}P NMR analyses were conducted to reveal the structural and chemical transformation of lignin. Crystallinity and accessibility of cellulose were also measured using ^{13}C CP/MAS NMR and Simon's staining technique, respectively. The observation of this study will provide insights on how lignin first organic solvent system affects biomass structural characteristics and recalcitrance.

S14 Valorization of Wheat Straw Using a Recyclable Hydrotrope at Low Temperatures (≤ 90 °C)

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This study evaluated the potential of an acid hydrotrope process at low temperatures for on-farm valorization of wheat straw by producing ligocellulosic nanofibrils (LCNFs), lignin nanoparticles (LNPs), and furfural. *p*-Toluenesulfonic acid (*p*-TsOH) was used to fractionate wheat straw under a range of conditions below 90 °C at low to moderate concentrations, between 15 and 60 wt%, for up to 2 h. *p*-TsOH fractionated wheat straw into a cellulose-rich water-insoluble solid (WIS) fraction and a spent liquor stream that contained dissolved lignin and xylan. Various degrees of delignification and hemicellulose dissolution were obtained and were correlated with a combined delignification factor (CDF) and a combined hydrolysis factor (CHF), respectively. A low *p*-TsOH concentration of 15 wt% can be used to obtain the desired degree of delignification for producing LCNFs directly from wheat straw. Films made of wheat straw LCNFs, with lignin contents of 12–22%, had excellent mechanical properties, with specific tensile strength over 120 kN·m/kg. The dissolved xylan in the spent liquor was directly dehydrated into furfural catalyzed by the *p*-TsOH in the spent liquor without additional catalyst. The dissolved lignin was easily precipitated as LNPs through dilution using water. *p*-TsOH, as a solid catalyst, can be reused after the steps of lignin precipitation, re-concentration, and dehydration of xylose into furfural. The low-temperature fractionation process could substantially reduce capital and operating costs for on-farm applications.

S15 Niobic acid nanoparticles for the solvent-free synthesis of high carbon fuel precursors from biomass-derived furanic compounds

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For a solid acid-catalyzed cross condensation of low carbon furanics into high carbon fuel precursors, a suitable solid acid catalyst having promising textural properties and an optimum acidic density and strength is needed. In this work, mesoporous niobic acid nanoparticles with high surface area (PNA) was prepared by a facile and green sol-gel process with forced hydrolysis and condensation**.** The effects of calcination temperature (100-500˚C) on the textural, acidic, and structural properties of niobic acid with its catalytic performance for the solvent less hydroxyalkylation-alkylation of 2-methylfuran (2-MF) with furfural (Fur) were investigated. Among the investigated catalysts, the resulting niobic acid nanoparticles thermally treated at 400˚C (400-PNA) exhibited the best catalytic performance and demonstrated higher activity over commonly used solid acid catalysts for the upgrade of low carbon biomass furanics to higher carbon fuel precursors. A maximum of 90% yield of C_{15} fuel precursor is obtained via the HAA of 2-MF and Fur. According to the results of characterization, the activity of the niobic acid nanoparticles can be rationalized by its high surface area, large pore diameter, good water resistance, and by the presence of surface Nb-OH groups which acted as Brønsted acidic sites. Stability studies also demonstrated that 400-PNA regains nearly full activity upon regeneration, and thus making it a promising catalyst for C-C coupling reactions.

This work was supported by Korea Institute of Energy Technology Evaluation and Planning (KETEP) funded by the Korea goverenment (MOTIE)(20183030092080).

S16 Sugar production from lignocellulosic biomass using pilot-scale supercritical hydolysis and electrochemical purification method

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In order to improve the efficiency of enzymatic hydrolysis and further downstream process, various pretreatment methods have been researched and are still under development. However, high energy density and process efficiency of pretreatment were considered as major bottlenecks, and therefore a novel way has been required for the production of bioethanol, sugar, or other sugar-derived materials. In this respect, supercritical water hydrolysis of lignocellulosic biomass has demonstrated high potential to decompose the biomass structure with several advantages (short reaction time and sugar production without pretreatment process) compared to other conventional methods. Above all, it is free from burden on enzyme price. In this study, biomass

hydrolysis using the supercritical water was adopted in the pilot-scale reactor. Reaction time for hydrolysis was less than 1 sec, and temperature was ranged from 390 to 400°C at the pressure range of 240-250 bar. For increasing the hydrolysis efficiency, sulfuric acid (0.05% (w/w)) was loaded into the mixture of purified-water and biomass, joined the running supercritical water, and then reacted for extremely short times. After the reaction was terminated, more than half of the sugars in the initial biomass were liberated into the liquid fraction as monosaccharide form (glucose and xylose). Subsequently, the liquid fraction was electrochemically purified and the content of phenolic and furan compounds was reduced without sugar loss. This is considered to be a more suitable process for the sugar production process than the conventional activated carbon treatment method which caused the sugar loss. As a result, considering the process has not been optimized, a relatively high yield of sugar was obtained through these processes.

S17 Effects of drying on cellulose porosity, crystalline structure and enzymatic hydrolysis

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For enzymatic sugar production from biomass, pretreatment must be performed to enhance the enzyme accessibility and storage of the pretreated biomass including drying affects enzymatic hydrolysis. Therefore changes in cellulose properties depending on drying were analyzed and drying effect on enzymatic hydrolysis was investigated to determine the enzymatic hydrolysis kinetics and conversion limit.

The drying effect, difference in enzymatic sugar recovery between never and oven dried pulps, can vary depending on enzyme dosage and the drying effect index (DEI) was suggested to determine the drying effect depending on enzyme dosage. More enzymes caused higher DEI and the DEI was observed at the initial stage of enzymatic hydrolysis. Once DEI reached the highest, the DEI was reduced as the hydrolysis time increased. However, there was no big difference in DEI during enzymatic hydrolysis with less enzyme because a slower hydrolysis caused stronger inhibition due to decrease in enzyme activity. Thus pore formed by biomass degradation during hydrolysis could not enhance enzyme accessibility even after 120 hrs.
The DEI was correlated well with porosity in biomass measured by WRV and Simons' staining. A porosity in biomass was reduced by pore collapse during oven drying and enzyme accessibility decreased as well. Though the pore collapse was reversed partially, there was an irreversible structural change decreasing the upper limit of enzymatic conversion of dried biomass. Crystalline structure was not changed by one time drying and rewetting and thus the decrease in enzymatic sugar recovery by drying had no correlation with crystalline structure in cellulose.

S18 Reduction of CO_2 emission in ethanol plant via dry reforming of methane

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Dry reforming of methane (DRM) has attracted academic and industrial interest, since it utilizes CH_4 and CO_2, two of the main greenhouse gases to produce synthesis gas, which can be used in the production of cleaner and more environmentally friendly fuels and chemicals. However, its commercial application is currently impractical as it has certain limitations, such as the high energy requirementand cost for reaction and CO_2 separation and purification. Besides, the widely used raw material for syngas is natural gas, which is derived from fossil fuels and located far from industrial complexes.

The major waste products from sugar-cane-based bioethanol industry, in addition to CO_2, are sugar-cane bagasse and vinasse, which can be used in energy co-generation and production of CH_4 through anaerobic digestion, respectively. Thus, the bioethanol industry can supply a clean and cheap source of CO_2 and CH_4, as well as the required energy for DRM. Therefore, in order to reduce CO_2 emissions and reduce the processing costs of DRM, this study investigates the valorization of these waste products via DRM using process simulation. Simulations of DRM over Ni-based catalysts were performed using software Aspen Plus® v8.6. When considering also steam reforming and reverse water-gas shift reactions, the sensitivity results demonstrate the potential of this route and the versatility of syngas: the process conditions can be established according to the desired application, such as Fischer-Tropsch synthesis, methanol, dimethyl ether or ethanol production, carbonylation, hydrogenation, hydro-formylation, defined by the H_2/CO ratio of the produced syngas.

S19 Understanding features of CELF pretreated solids that influence deconstruction by *Clostridium thermocellum*

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Co-solvent Enhanced Lignocellulosic Fractionation (CELF) of lignocellulosic biomass produces solids rich in glucan and a hydrolysate stream containing most of the hemicellulose and lignin. Additionally, deconstruction of CELF solids by consolidated bioprocessing (CBP) using *C. thermocellum* rapidly nearly completely deconstructs glucan in these solids without the need to add enzymes. For example, CELF solids were completely solubilized within 24-48 hours, and we have previously shown that

pretreated solids with higher lignin removal than xylan removal achieve greater solubilization. In order to better understand the CELF-CBP synergy, we applied CELF pretreatment at 150 C for reaction times of 5, 15, and 25 minutes over 7 days to poplar to define conditions at which *C. thermocellum* would and would not completely solubilize glucan. Comparing the kinetics of these fermentations, the extent of glucan solubilization, changes in solid compositions, and structural properties of solids residues, key factors will be defined that have the greatest impact on the rate and extent of solubilization.

S20 Elephant grass biorefinery: the potential of byproducts uses in the ethanol production

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Elephant grass (EG) is a C4 plant traditionally used in cattle feeding and has received attention in the second-generation ethanol production. In this work, we aimed the investigation of two scenarios for the improvement of the byproducts utilization in the conversion of EG leaves and stems into ethanol. In the first scenario, supercritical carbon dioxide ($scCO_2$) and pressurized liquid extraction (PLE) – using water and ethanol (50:50) – were used to recovery high value-added products, such as sterols, alcohols and hydrocarbons. The $scCO_2$ extracts were richer on nonpolar components (yield of 0,8% for leaves and 0,2% for stems), while the PLE extracts were richer on polar components (yield of 15% for leaves and 11% for stems); the alkaline pretreatment with optimized conditions (NaOH 4,5% m/v, 85°C and 100 min) led to an increased sugar release of ca. 3 times, besides the possibility of lignin recovery from liquid fraction. In the second scenario, an acid-alkaline treatment was tested to fractionate hemicellulose and lignin in the optimized condition, except by time. For leaves, the best sugar release (925,8 mg of sugar/g of biomass) was achieved using an acid step (H_2SO_4 2% v/v, 121°C and 40 min) followed by alkaline treatment by 20 min. For stems, the best condition (863,9 mg of sugar/g of biomass) was achieved using only an alkaline treatment for 100 min. Therefore, these methods can be used in the enhancing of the EG enzymatic digestibility and in the byproducts recovery, in a biorefinery concept.

S21 A preliminary economic assessment of bionanomaterials and high concentration sugar production

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The pulp and paper industries face a market shift from traditional products to a new generation of forest products driven by the necessity of innovation and guarantee of profit. Nanomaterials from cellulose, such as cellulose nanocrystals (CNC) and cellulose nanofibrils (CNF), are promising products due to their unique properties and for many benefits to the forest biorefineries (ie. revenue enhancement, new sectors achievement in value chain and for stimulating a strong circular bioeconomy). As a contribution to pursue an efficient utilization of cellulose, here, we studied the simultaneous production of CNC, CNF and a sugar stream at high concentration. After high-solids enzymatic hydrolysis of bleached eucalyptus kraft pulp, the sugar stream was recovered by filtration and the solid cellulosic residue (SCR) was used to extract CNC and CNF. The CNC isolation process was previously optimized in relation to CNC yield through an experimental design and response surface methodology that investigated the influence of the cellulose hydrolysis yield. The SCR remained after CNC isolation was defibrillated in a disk ultra-refiner. Gravimetric and Dynamic Light Scattering analyses were applied to determine yields and particle size, respectively. Atomic Force Microscopy was used to confirm isolation of the nanocelluloses, their morphology and to determine their dimensions. This presentation will explore product yields in relation to potential revenue to discuss the best economical proportion between these products. The results obtained indicate the process configuration that integrate bionanomaterials and sugar at high concentration and deliver optimum economic assessment.

Acknowledgements: CAPES, CAPES-PROAP and FAPESP.

S22 Biosensor Applications in Biomanufacturing

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In the drive to shift to a bio-based more sustainable society, microbial production from renewable biomass is an alternative to petroleum-based products. Advancements in synthetic biology has enabled our ability to assemble DNA parts to engineer novel pathways in a host microbe to make virtually any molecule. In an effort to expedite the process for manufacturing of bio-based products, the contemplated Design-Build-Test-Learn (DBTL) cycle provides a suitable process to “learn” from a cycle and enhance the biomanufacturing process in the next DBTL cycle.

The bottleneck in this whole process, is the low-throughput in the “test” step, where the most state-of-the-art technologies often fail to screen all the “designs”, that could be “built” using current molecular biology techniques. Due to insufficient testing, the “learn” step severely suffers, restricting the positive feed into the DBTL cycle.

To alleviate the bottleneck in the “Test” step, we have created a suite of sensor-reporter systems that target industrially relevant molecules (as an input) and fluorescence readout (as an output) providing a simple, quick and superior evaluation technique (with a throughput > 10^6) over traditional methods of chromatography and spectrometry (with a throughput ~10^3) in analyzing the

subtle variations in metabolic designs.
We will present ongoing efforts in our group for development and application of biosensors in synthetic biology. Establishment of a protocatechuate sensor in *Pseudomonas putida* and 4-hydroxybenzoate sensor-enabled alleviation of product inhibition in an enzyme will be discussed. Finally, using novel biosensors for *cis,cis*-muconic acid and b-ketoadipic acid in a growth adapted population, selection of microbial strains for efficient conversion of renewable feedstocks into polymer precursors was achieved. Genome sequencing of the efficient strains (for titer, yield, productivity) showed subtle to dramatic changes in the evolved genome, providing new opportunities for rational design in the subsequent DBTL cycles.

S23 Bioconversion of acrylonitrile to acrylamide using whole cells, lysate, and purified enzyme of *Rhodococcus rhodochrous* DAP 96253

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Acrylamide (AMD) is an important commodity chemical that is used in coagulators, water treatment, soil conditioners, mineral refining, paper treatment, adhesives, paints, petroleum recovering agents, and in certain laboratory procedures. It is often shipped as an aqueous solution (30-50% w/w acrylamide). To bypass the cost which occurs with shipping a solution that is 70-50% w/w water, one can locally produce AMD or poly-AMD using microorganisms to convert acrylonitrile (AN) to AMD. *Rhodococcus rhodochrous* DAP 96253 uses a nitrile degradation pathway involving the enzyme nitrile hydratase. Nitrile hydratase catalyzes hydrolysis of the nitrile to an amide following the equation: RC≡N + H2O à RCONH2. Free whole cells, immobilized whole cells, lysed cells, free purified enzyme, and immobilized purified enzyme of *R. rhodochrous* DAP 96253 were used to compare the production of AMD from AN and to obtain solutions of 40% w/w and 20% w/w to compete with commercially-produced solutions.

AMD was produced by the direct conversion of AN, in a small-scale bioreactor. Acrylonitrile was added into the bioreactor at varying rates. AN conversion to AMD was determined using GLC.
Using catalysts derived from induced cells of *R. rhodochrous* DAP 96253, yields of >40% and >20% by weight AMD are consistently achieved, which meets commercial AMD standards. The varying techniques being used will be compared by its production and cost.

S24 Condensation of Furanic Platform Molecules to C14–C15 Fuel Precursors Over Sulfonic Acid Functionalized Silica Supports

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Furfural has excellent potential to produce versatile furanic platform molecules that can be upgraded to high carbon fuel precursors. 2-Methylfuran is one of the most important derivatives of furfural. The hydrogenation of furfural to 2-methylfuran results in the formation of by-products (n-butanal and 2-pentanone). Implementing all the primary and by-products from the hydrogenation reaction to produce high carbon fuel precursors allows the complete utilization of the lignocellulosic xylose derived furfural. In this work, a self-condensation reaction of 2-methylfuran and its cross-condensation reactions with furfural, n-butanal and 2-pentanone have been implemented to produce C14 and C15 fuel precursors. Sulfonic acid-based catalysts with and without alkyl chain linkers supported on silica nanoparticles (NP), MCM-41, SBA-15, and KCC-1 were synthesized to evaluate the effect of catalyst morphology on activity and selectivity. The correlation between the different support systems and corresponding activity was studied using SEM, TEM, BET, FTIR, and TGA before and after the reaction. Among the synthesized catalysts, sulfonic acid-functionalized KCC-1 (KCC-1SO_3H) and 3-((3-(trimethoxysilyl) propyl) thio) propane-1-oxy-sulfonic acid-functionalized (KCC-1APSO_3H) showed higher conversion and selectivity for the self-condensation and cross-condensation reactions, respectively. The effects of various parameters on the activity and selectivity, such as the reaction time and temperature, were studied. The catalysts have substantial hydrolytic stability in the presence of water and retain their acidity over multiple reaction cycles. The low cost, high activity, and pronounced stability of these fibrous nano silica-based catalysts indicate a promising future application in the biorefinery industries.

This work was supported by the Energy Efficiency & Resources (No. 20163010092210) of the Korea Institute of Energy Technology Evaluation and Planning (KETEP) grant funded by the Korea government Ministry of Trade, Industry & Energy

S25 Enhancing sugar yields from hybrid poplar via alkaline pre-extraction followed by copper catalyzed alkaline hydrogen peroxide post-treatment

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The high recalcitrance of lignocellulosic biomass presents serious challenges in the bioconversion of lignocellulosic feedstocks into sustainable bio-based fuels, chemicals, and materials. In this study, we investigated a two-stage pretreatment process comprising alkaline pre-extraction followed by copper(II) 2,2′-bipyridine (Cu^{II}(bpy))-catalyzed alkaline hydrogen peroxide (Cu-AHP) post-treatment to deconstruct the plant cell wall structure and increase the digestibility of lignocellulosic biomass. Hybrid poplar was first pre-extracted with sodium hydroxide (NaOH) using 10% w/w NaOH at 120 °C for 1 h to remove about 25% of lignin from the biomass. Subsequently, the NaOH-extracted solid substrate was subjected to Cu-AHP post-treatment over

temperatures of 30-95 °C, reaction times of 3-24 h, hydrogen peroxide loadings of 2-10% w/w, and 2,2′-bipyridine concentrations of 0-2 mM. Following Cu-AHP post-treatment, the two-stage pretreated solids were subjected to enzymatic hydrolysis at an enzyme loading of 15 mg protein/g glucan to determine the effect of Cu-AHP post-treatment conditions on the sugar yields. The results showed that the increase of post-treatment severity increased the solubilization of lignin and xylan. After enzymatic hydrolysis, the glucose yields increased with increasing severity of the Cu-AHP post-treatment. By recovering the solubilized polysaccharides in the water-soluble fraction, the yields of glucose and xylose corresponded to approximately 80% and 90% of the two components in the original biomass, respectively. Preliminary techno-economic analysis indicates that the improved yields at increased temperature can allow for a reduction in pretreating raw materials, namely H_2O_2 and bipyridine loadings, thereby potentially reducing the cost of Cu-AHP post-treatment by over $1.00 per gallon biofuel.

S26 Hemicellulose Oligomer Molar Mass Evolution and Kinetics of Softwood Hydrolysis by Population Balance Model

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The heterogeneous composition and structure of hemicellulose makes hemicellulose hydrolysis a complex phenomenon and has stymied efforts to develop broadly applicable conclusions. An important product of hemicellulose hydrolysis is soluble polysaccharides, or oligomers. The resulting oligomers have a wide range of molecular weights and have applications in food, textile, paper, explosives, cosmetic, petroleum and mining industries. Greater understanding of how oligomer size evolves during hydrolysis is needed. A population balance model describing hemicellulose hydrolysis in softwood was posed. The model describes the initial solubilisation to oligomers followed by depolymerization to smaller molecules and ultimate generation of degradation products. The evolution of hemicellulose oligomer molecular weight was measured by size exclusion chromatography; a two-dimensional calibration method enabled simultaneous measurement of oligomer molar mass and concentration. The model provides a lens for identifying and interpreting physical reaction mechanisms. Random bond breaking to form soluble oligomers from the insoluble hemicellulose may be related to the distribution of acidic groups within the solid softwood matrix. The soluble oligomers tended to break at the middle of the chain to produce smaller molar mass oligomers. Transition state theory was applied to analyse the activation energy and pre-exponential factor. An optimum yield and corresponding hydrolysis conditions for targeted molar mass can be determined using the model. This model provides new insights into the relative reactivity of hemicellulose intermediates and opens the door for future downstream sugar conversion and purification technologies.

S27 Application of solid superacid catalyst, sulfated tin (IV) oxide, to coupling reaction of α-pinene

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Alternative fuel synthesis from renewable resources has studied intensively with the concern of sustainable development. Terpene hydrocarbons which are the most abundant secondary metabolites in nature have been considered as one of promising chemicals for the manufacture of alternative fuels. To develop fuels having high energy density, Brønsed acid-catalyzed coupling reaction of α-pinene has been suggested.

In this study, we tried to develop solid superacid catalyst for the synthesis of high density fuel molecules from α-pinene. Among screened catalysts, sulfated tin oxide, SO_4^{2-}/SnO_2, can catalyze coupling reaction of α-pinene, giving product mixture containing both camphene and coupling products.

For the preparation of sulfated tin oxide, SO_4^{2-}/SnO_2, tin oxide powder was obtained by chemical precipitation and subsequent surface promotion was carried out with sulfuric acid. Calcination temperature for catalyst activation was important to catalytic activity. When the calcination temperature was both lower and higher than 550°C, the yield of coupling products was significantly decreased. To determine optimum condition, we screened reaction temperature and time. Like previously reported sulfated zirconium oxide catalyst for camphene production from α-pinene, our sulfated tin oxide catalyst also gave camphene as a co-product. Therefore, isomerization to camphene decreased the yield of coupling products because of the lower catalytic activity of our catalyst toward camphene. However, given the high viscosity of coupling products at low temperature condition, obtained mixture with camphene may be more suitable for fuel application.

S29 Elucidating interactions between lacasse and aqueous ionic liquids to enable lignin valorization

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Lignin makes up 20-30% of the plant biomass and is the most abundant aromatic polymer in nature. Converting lignin into high-value chemicals adds revenue for a biorefinery, improving the economic viability of biofuel production. Ionic liquids (ILs) have received increasing interest because of their ability to fractionate lignocellulosic biomass during pretreatment. Given the unique properties of aqueous ILs for lignin solubility and enzyme biocompatibility, we foresee a great opportunity to develop new strategies for lignin valorization *via* biocatalysis in aqueous ILs. Thermophilic enzymes have exhibited an increased resistance to IL inhibition relative to their mesophilic counterparts. To that end, the stability and activity of thermophilic laccases were evaluated in different concentration of ILs. In the presence of 1-ethyl-3-methylimidazolium acetate ($[C_2C_1Im][OAc]$), the activity of

a thermophilic fungal laccase from Novozymes (NS-22127) increased. Additionally, a plant laccase from *Rhus* vernicifera exhibited increased activity in high concentrations (>10%) of choline lysinate ([Ch][Lys]). Laccase from the hyperthermophilic bacterium *Thermus thermophilus* was recombinantly expressed and screened for activity in ILs. Little to no activity remained in 1% solutions of ILs and NaCl, indicating this laccase is highly sensitive to ionic solutions. The results of docking simulations suggest that the ILs directly compete with the substrate ABTS for access to the Type I copper in this laccase. Enzyme engineering and immobilization strategies with this hyperthermophilic laccase improved activity in ILs, such that it is suitable to valorize IL-extracted lignin *in situ*. Collectively, these results highlight the potential for biocatalytic lignin valorization strategies and the importance of characterizing IL-enzyme interactions in these strategies.

S30 *Zygosaccharomyces bailii* as a new host for metabolic engineering

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Organic acids represent an important class of commodity chemicals which can be produced by microbial fermentation. The intrinsic toxicity of organic acids to microbes requires usage of neutralizing agents to enable optimum growth and production. However, neutralizing agents lead to the formation of organic salts, complicating downstream processing and increasing costs. Exploitation of non-conventional microbes with innate robustness towards organic acids can solve this problem. *Zygosaccharomyces bailii,* a notorious food spoilage yeast found in acidic foods, is able to grow in the presence of various organic acids. We propose that this unique trait of the yeast is useful for production of organic acids. We screened many *Z. bailii* isolates for acetic and lactic acid tolerance at low pH conditions. We have identified one strain which is able to grow in the presence of high acetic or lactic acid concentrations at low pH. We further studied the strain's flask fermentation ability in the presence of acetic or lactic acids. Results showed robust growth and revealed the ability to consume acetic acid in the presence of glucose. It is well-known that genetic manipulation of non-conventional yeasts is challenging. Therefore, we reconstructed and adopted CRISPR/Cas9 system to be functional in *Z. bailii*. As a proof of concept, we were able to successfully induce a loss-of-function mutation in the *Z. bailii ADE2* gene. These findings open new prospects in *Z. bailii* metabolic engineering for organic acid production.

S31 Catalytic hydroprocessing of softwood kraft lignin to jet fuel-range hydrocarbons utilizing non-noble metals

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Catalytic hydroprocessing of softwood kraft lignin to jet fuel-range hydrocarbons utilizing non-noble metals

Matthew Kollman, Hasan Jameel, Hou-min Chang

Government agencies and aviation companies have funded several research initiatives to reduce greenhouse gas emissions of aircraft, including the development of sustainable alternative jet fuels.[1] Lignins are an attractive renewable feedstock for aviation fuel production, having a higher carbon to oxygen ratio than other abundant natural polymers like cellulose and starch. Moreover, aromatic compounds are generated during lignin depolymerization, possibly eliminating the need to add aromatics or blend with conventional fuel.[2]

Biorefinery lignins recovered from dilute-acid or hot water pretreatment processes are of interest for fuel production as they contain a high proportion of etherified linkages that are readily cleaved through acid- or base-catalyzed reactions.[3] Unfortunately, these lignins are not available commercially. Lignin precipitated from spent cooking liquor of the kraft pulping process may be a more economically viable alternative, though characterized by a more condensed structure that could require more energy to upgrade.

The objective of this project is to convert commercially-available softwood kraft lignin to jet-fuel range hydrocarbons through liquid-phase hydroprocessing utilizing solid acids and transition metals, avoiding the use of expensive and rare platinum group metals. The first step of lignin depolymerization converted more than 80% of starting lignin to fragments soluble in ethyl acetate using a novel Co/zeolite catalyst. The resulting bio-oil is upgraded through hydrogenation and hydrodeoxygenation using nickel and/or molybdenum supported on various metal oxides.[4] Carbon yield will be optimized by employing strategies to prevent catalyst deactivation, such as multistep processing.[5] The liquid product will be characterized per ASTM standards for energy content, volatility, oxygen content, and aromatic content.[6]

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(5) Vispute, T. P. et al. *Science* 2010, *330*, 1222–1227.
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S32 Computational fluid dynamics of bioreactors with micro-aeration

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Recent work has probed the potential for using *Zymomonas mobilis* as a flexible platform for the production of advanced fuels and intermediates. One set of recombinant organisms requires a micro-aerobic environment to promote the production of the desired product (2,3-BDO), but the economics and process-control implications of this approach are uncertain at industrial scale. Computational multi-physics models have the potential to describe the fermentation in detail at multiple scales, reducing the risk of scale-up. A two-phase Euler-Euler CFD model is used alongside a simple model of oxygen uptake to describe this bioreaction. A few reactor vessels, including airlift and CSTR bioreactors, are analyzed at multiple scales, regions of oxygen-depletion and over-oxygenation are characterized, and the bioreactors are compared. We show that it is difficult to achieve homogenous oxygen concentrations in industrial-scale CSTR reactors and that advanced bioreactor designs are needed for economical operation.

S33 Fractionation of lignocellulose to biochemicals via sequential hydrothermal liquefaction (SeqHTL)

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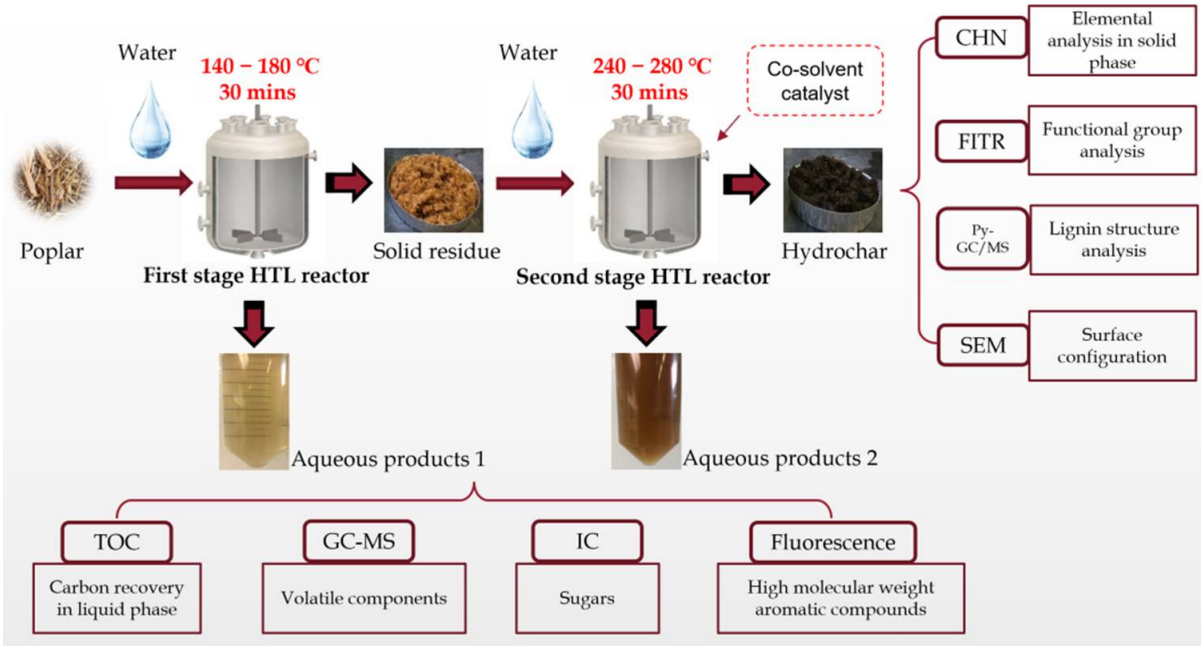
Conventional hydrothermal liquefaction (HTL) of lignocellulose produces low-quality bio-crude at expense of high temperature and pressure. Thus, conversion to bio-based chemicals instead of bio-oil seems promising. However, the lack of practical depolymerization process for producing monomers and derivatives at high yields from lignocellulosic materials has limited the development of HTL technology.

Currently, few studies cover technology that directly converts raw lignocellulose to chemicals with the presence of organic solvent. In absence of these solvent, our newly proposed two-stage sequential hydrothermal liquefaction (SeqHTL) process benefits from (1) no pretreatment or pre-isolation step; (2) simultaneous production of bioactive compounds, C5-C6 sugars and their derivatives, and lignin monomers; (3) moderate operating conditions. In brief, the first stage pretreats the biomass at low temperatures (140-180 °C subcritical water) to break cell walls, release sugars, where solvolysis and hydrolysis are the dominant reactions; subsequently, the second stage operating at moderate temperatures (240-280 °C subcritical water) produces fuel precursors, such as furfural, HMF, and a portion of lignin monomers, and harvests lignin-rich hydrochar as solid product.

In terms of deconstruction performance, SeqHTL achieved biomass degradation rate of 61.1% at 240 °C and 75.6% at 280 °C, while one stage direct HTL (DHTL) was 51.5% and 70.5%, respectively. The highest degradation rate of 91.9% occurred at SeqHTL 280 °C, where water: ethanol (1:1) was deployed at the second stage. GC/MS results showed that SeqHTL and DHTL have similar main products at equivalent conditions: acetic acid, furfural, HMF, phenol, acetol, and S type lignin derived monomers, however, the furfural yield from SeqHTL was doubled compared with DHTL at both 240 and 280 °C. In addition, the missing of S type lignin in solid product was confirmed by NMR spectra due to the its high conversion during HTL. IC results indicated 10% recovery of xylose from SeqHTL first stage at 180 °C, while 140 and 280 °C were not favorable for sugar production. The products were also characterized by SEM and FTIR to demonstrate the differences between SeqHTL and DHTL. In addition, the subcritical water system was also compared with addition of ethanol, acid, and salt system.



S34 Effect of soluble phenolic compounds from steam pretreated wheat straw on cellulose hydrolysis

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Steam pretreatment is widely applied for rendering biomass carbohydrates accessible for enzymatic hydrolysis. The most abundant soluble by-products from steam pretreated wheat straw are mono- and oligosaccharides, acetic acid, furfural, and various phenolic compounds. Hemicellulosic oligosaccharides are known to be strong inhibitors of cellulose hydrolysis, while inhibition by phenolic compounds is still under investigation. Here we fractionate and thoroughly characterize an inhibitor liquor from steam pretreated wheat straw and show its effects on major components of cellulose-degrading cocktail from *T.reesei* along with a state of art commercial cellulosic preparation. The main targeting enzymes for phenolic inhibition are revealed in this study. A screening method for novel inhibitor-tolerant enzymes is proposed based on phenolics inhibition effects on cellulose-degrading enzymes.

S35 Development of a continuous tubular electrosynthesis method for cellulolytic magnetic nanobiocatalyst production

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Enzymatic hydrolysis is a promising option for obtaining monomeric sugars from lignocellulosic biomass for further processing. However, the high price of cellulase enzymes is still a challenge affecting the overall bioprocessing cost. Although the economics of enzymatic hydrolysis could be improved through cellulase recovery and reuse, most recovery methods only capture a fraction of the enzymes either from spent biomass or hydrolysates. Also, the enzyme recovery methods usually require additional costly steps. To overcome these challenges, we are developing magnetic nanobiocatalysts that comprise of polymer enzyme conjugates (PECs) attached on superparamagnetic iron oxide nanoparticles (SPIONs).

Compared to free enzymes, PEC constructs show faster hydrolysis rates in the presence of high glucose concentrations, suggesting that the attachment of enzymes to polymeric chains provides resistance to product inhibition. We found that hydrolysis rates using unattached PECs are slightly dependent on the polymer molecular weight (MW). Lower-MW (60 kg/mol) PECs at high enzyme concentration exhibited higher biomass hydrolysis yield than high-MW (2000 kg/mol) PECs. Higher-MW polymers might have caused steric hindrance between enzyme and substrate during hydrolysis.

Current production methods for magnetic nanobiocatalysts are not scalable at an industrial level. We are developing a continuous tubular electrosynthesis method to produce cellulolytic magnetic nanobiocatalysts. Increasing electrolyte flow rate during continuous tubular electrosynthesis caused a reduction in SPION size which is likely due to shorter exposure time within the reaction zone. The continuous tubular electrosynthesis method will offer advantages of scalability, electrolyte reuse, and improved nanoparticle size control with the ultimate goal of reducing biomass enzymatic hydrolysis costs.

S36 Effects of minerals and salts on biomass conversion processes

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Inorganic minerals and salts are well known to affect biomass thermochemical conversion processes thus feedstocks with low ash content are generally favorable. However, their effects on sugar and lignin yields through various pretreatment approaches and the sequential enzymatic hydrolysis are not very clear. In this study, sugars and lignin yields from corn stover after pretreatment (i.e. using water-only, dilute acid, alkali, ionic liquid, tetrahydrofuran and γ-valerolactone) as well as yields after enzymatic hydrolysis with addition of different amounts of inorganic minerals and salts were investigated. Analytical data of these inorganic minerals and salts, including distribution, adsorption, and potential chemical activities during different pretreatment, will help next generation biorefinery to further reduce operating cost by incorporating cost-effective pretreatment and pre-processing operations in the biomass supply chain.

S37 Improving Sugar Production from Bioenergy Sorghum by Using Continuous Hydrothermal Pretreatment Combined with Disk Milling

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Pretreatment is critical for sugar production from cellulosic feedstocks. Chemical free pretreatments are attracting increased interest due to lower concentration of inhibitors in hydrolysate. In this study, the pilot-scale continuous hydrothermal pretreatment followed by disk milling was evaluated. Bioenergy sorghum (BS) was pretreated at 160 to 190°C for 10 minutes with and without subsequent disk milling. Hydrothermal pretreatment and disk milling synergistically improved glucose and xylose release by 10 to 20% compared to hydrothermal pretreatment alone. The glucose and xylose yields of 82.55% and 70.78%, respectively were achieved, when BS was treated at 190°C and 180°C followed by disk milling. Comparison of continuous hydrothermal pilot-scale pretreatment was also conducted with laboratory-scale batch pretreatment. BS treated with hot water at laboratory-scale has higher sugar yields by 5 to 15% compared to continuous pilot-scale for all pretreatment conditions.

S38 Continuous and homogeneous lignocellulosic fibers from biomass-ionic liquid solutions.

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Natural fibers are environmentally friendly alternatives to substitute glass fibers for composites reinforcement. One driver for such materials is the need for lightweight, energy-efficient, low cost, environmentally sustainable engineered composites in automotive industry, building and construction sectors, and consumer goods applications. However, the inherent natural variability and hygroscopic nature of natural fibers result in compounding difficulties leading to non-uniform dispersion of the fibers within the polymer matrix. Such heterogeneity negatively impacts the composites' properties. Therefore, new product development that capitalizes on the advantages of natural fibers and addresses the variability in mechanical properties and moisture absorption rates of natural fibers is critical for the expansion of lignocellulosic value-added products. In this study, through enhanced solvation of lignocellulosic biomass in ionic liquid (IL) followed by regeneration, we propose a pathway for manufacturing continuous, homogeneous lignocellulosic fibers with controlled chemistry. An initial autohydrolysis is introduced to enhance the dissolution of the biomass in IL and to control the carbohydrates to lignin ratio of the resulting fibers. Lignocellulosic biomass with high lignin and low hemicellulose content is expected to reduce the hydrophilic nature of the regenerated fibers. On the other hand, continuous wet spinning under optimum and controllable conditions will solve the issues related to non-uniformity and variations in dimension and mechanical properties found in natural fibers. This talk will present the properties of such regenerated fibers and discuss the potential of the process to produce biofibers with properties superior to common natural fibers for designing composite products.

S39 Engineering *Saccharomyces cerevisiae* to improve NADPH availability for oleochemical biosynthesis.

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Industrial oleochemicals like fatty alcohols and fatty acids can be produced by microbial cell factories that have been engineered to take advantage of the native lipid biosynthesis pathways. Acyl-CoA synthesis from acetyl-CoA is a highly anabolic process and palmityl-CoA biosynthesis has a high demand for reductant. The fatty acid synthetase in yeast, Fas1 specifically requires NADPH and this can become limiting to acyl-CoA synthesis. Thus engineering redox cofactor availability is essentially to meet the demands of engineered biosynthetic pathways. We have investigated the application of a series of transhydrogenase like shunts to increase NADPH availability in fatty alcohol producing strains. We find that expression of a novel combination of modified forms of Pyc1, Mdh2 and Mae1 resulted in an up to 100% increase in cellular NADPH availability and NADPH/NADP ratio. This modification also produced a near 2 fold increase in fatty alcohol production. Greater effects, but also greater variability were observed when the gene combination was expressed at higher dosages. Overexpression of the NAD kinases Utr1 and Yef1 had little effect on their own but Yef1 had a synergistic effect when combined with the above mentioned transhydrogenase-like shunt. Additionally, we investigated the effect of expressing a truncated cytoplasmic form of the NADH kinase Pos5 on fatty alcohol production. Finally we report on the effect of increasing carbon flux through the oxidative pentose phosphate pathway to increase NADPH synthesis and fatty alcohol production by *S. cerevisiae*.

S40 Engineering *Zymomonas mobilis* as a platform organism for biomass conversion: advances and challenges

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Zymomonas mobilis is known for its high specific glucose uptake rate, rapid catabolism, and high ethanol yield. It has been engineered to efficiently convert the second and third most abundant plant derived sugars, xylose and arabinose, to ethanol at high yield. With its ability to utilize most biomass sugars, even in toxic hydrolysate environments, it is desirable to enable this microorganism to be a platform organism for biomass conversion. It is therefore essential to turn down or knock out the ethanol producing pathway to redirect carbon flow for other chemical synthesis. We recently demonstrated success in redirecting carbon flow by deleting the ethanol producing pathway for 2,3 butandiol (BDO) production. As it is a less toxic product than ethanol, higher titers of BDO can be achieved. However, unlike the ethanol-producing pathway, the 2,3-BDO producing pathway generates a surplus NADH which must be oxidized for redox balance. In the presence of oxygen, the organism is able to oxidize NADH to NAD^+ by the native NADH dehydrogenase. Although minimal air is provided via either sparging or intake to the headspace during fermentation. To drive down the processing cost and mitigate any concerns about oxygen requirement, it is desirable to make the process completely anaerobic by eliminating the use of air. We will present several strategies that can balance the surplus NADH resulting from the 2,3-BDO pathway under anaerobic conditions using stoichiometric analysis, metabolic modeling. We will also discuss the impact of these modifications on the fermentation process.

S41 Co-feeding Lignin Derivatives with Glycerol for Polyhydroxyalkanoate Production by *Pseudomonas putida* KT2440

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In this study, a co-feeding of lignin derivatives with glycerol was demonstrated to increase cell growth and PHA production simultaneously by *Pseudomonas putida* KT2440. Compared to glycerol alone, the co-feeding with lignin derivatives improved the

cell dry weight by 14%-20% after 72 hours of fermentation. PHA production also increased by 69.6%-108.3%. While the feeding with lignin derivatives alone can only reach PHA content about 2%-5% of cell dry weight. $^{1}H/^{13}C$ HSQC and ^{31}P NMR results confirmed the improvement of PHA production and revealed that the accumulation of glycerol-3-P was involved in glycerol degradation and ED pathways. Furthermore, the intracellular NADPH concentration of benzoate with glycerol was significantly increased during the late logarithmic phase in which massive amounts of PHA and biomass were accumulated. Overall, this study provides a co-carbon feeding strategy for higher PHA production, leading to more efficient lignin conversion.

S42 Enhanced bioconversion in herbaceous feedstocks from impregnation of co-catalytic Lewis acid, deacetylation and mechanical refining.

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Improvements in conversion processes are needed to further reduce process complexity, biorefinery capital and operating (CAPEX/OPEX) costs, lower enzyme usage. Previous work has demonstrated that deacetylation and mechanical refining (DMR) process effectively deconstructs herbaceous biomass while producing highly digestible lignocellulosic slurries, with high titers of monomeric sugars, at reduced enzyme loading. Concurrently, highly reactive lignin waste streams were obtained from the DMR process that can be biological and catalytic upgrading into biofuels, bio-based chemicals, and bioproducts. However, further reductions in refining energy, water consumption and enzyme loading are needed for cost-effective conversion. We report on the efficacy of a co-catalytic approaches evaluating three Lewis acids, impregnated into corn stover to enhance biomass deconstruction, Fe^{+2} Fe^{+3}, and Al^{+3} in sulfate form, at 1 mM, 5 mM and 10 mM concentrations. Lewis acids were impregnated into corn stover, followed by deacetylation with sodium hydroxide, and mechanically refined, prior to enzymatic hydrolysis. Increasing concentrations of Lewis acid negatively impacted enzymatic hydrolysis yield, likewise Lewis acid treatment was more effective when combined with deacetylation than with deacetylation or Lewis acid treatment, alone. Comparing the effectiveness of the three Lewis acids identified ferrous iron more effective than ferric or aluminum (Fe^{+2} > Fe^{+3}> Al^{+3}) sulfate. Enzymatic hydrolysis sugar (glucose and xylose) yield was at 80%, with a relevant enzyme loading (15 mg protein/g glucan) from the DMT process. Further increases in yield for monomeric glucose and xylose may be achieve by combining Fenton reactions with peroxide or ozone treatments.

S43 Repurposing newsprint pulp mills as forest biorefineries – Strategies for enzymatic deconstruction of pretreated softwood with high carbohydrate and lignin retention

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A decreasing demand for newsprint has put pressure on the newsprint sector to repurpose redundant mechanical pulping capacity and create novel products for new markets. An attractive option is to reposition existing newsprint mills as forest biorefineries to produce value-added products from biomass via the biochemical route. Mechanical pulping as a pretreatment method has the potential to generate a sugar platform with high yields of carbohydrates, as well as a sulphur-free and reactive lignin. However, the most accessible biomass for Northern American and Northern European pulp mills is softwood, which has a low enzymatic hydrolyzability due to the shielding effect of its complex hemicellulose structure and its hydrophobic lignin.

In the current study the pretreatment of softwood by alkali-oxygen impregnation and mechanical refining was investigated. Lodgepole pine was impregnated with molecular oxygen under various alkaline conditions, varying sodium carbonate concentrations and temperatures, and the resulting material characterized. The hydrolyzability of the resulting pretreated materials was assessed using commercial enzymes in various setups. Endo-mannanase was added to investigate the shielding effect of the softwood hemicelluloses on cellulose conversion and BSA was used to block the hydrophobic enzyme-lignin interaction and assess the extent of enzyme loss due to lignin adsorption. Finally, the pretreated material was used as feedstock for cultivations of the fungal enzyme producer *Trichoderma reesei*, to assess the possibility of further enhancing enzymatic hydrolysis rates and yields using the tailored, in-house produced enzyme mixtures.
This study investigates the potential to use existing pulp mill infrastructure to create an integrated biorefinery process that can convert softwood to a sugar platform at high yields and, concurrently, a sulphur-free and reactive lignin.

S44 Heterologous expression of lignin modifying enzymes in the filamentous fungus *Aspergillus niger*

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Filamentous fungi, on account of their saprophytic lifestyle, have an astounding capacity to secrete digestive enzymes into their extracellular environment. This feature has led filamentous fungi to be exploited for the manufacturing of homologous and heterologous proteins and enzymes for the food, chemical, and biofuel industries. A desire to move away from fossil fuels and towards more sustainable feedstocks has made the hydrolysis of lignocellulosic biomass an exciting and emerging enzyme market. However, the cost of the enzyme cocktails necessary for efficient biomass conversion is a major economic barrier to the commercial viability of using lignocellulosic feedstocks to produce biofuels and other bio-based products. We are developing fungal host systems for the expression and characterization of lignin modifying enzymes (LME) in the filamentous fungus *Aspergillus niger*. More specifically, we are focusing on the post-translational bottlenecks that are known to limit, in terms of

quality and quantity, heterologous protein production. We will present the engineering strategies used to build a fungal expression system for LME, and the plan for genetically engineering key components of the secretory pathway with the aim of enhancing heterologous protein production. Developing an efficient LME expression system in filamentous fungi will both enable us to characterize LME in more detail, and to study enzyme cocktails for the conversion of lignocellulosic biomass.

S45 How can we diminish the influence lignin has on the recalcitrance of biomass after steam pretreatment of various lignocellulosic substrates?

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Steam pretreatment has been shown to be one of the most effective ways of fractionating biomass while enhancing the enzyme mediated hydrolysis of the cellulosic component. However, lignin has proven to be problematic, with the processes used to try to remove lignin typically too expensive to be used routinely. In the work that will be presented, we looked at the possibility of modifying or relocating lignin to try to enhance enzyme mediated hydrolysis of the cellulosic component. Using more severe steam pretreatment conditions, above lignin's glass transition temperature, the lignin in the plant cell wall was partially fluidized and relocated onto the cell surface. However, as will be described, softwood lignin proved more difficult to fluidize and relocate than corn stover lignin. When the isolated lignin was added to filter paper it was apparent that hydrolysis yields were reduced. After steam pretreatment the softwood lignin was plasticized and relocated resulting in improved hydrolysis yields. When the re-condensation reaction of lignin during steam pretreatment was controlled by adding chemicals such as 2-naphthol or dilute acid, it was found that the resulting increase in hydrophobicity suppressed lignin relocation for both softwood and corn stover lignin. It was apparent that, the observed differences in accessibility of the softwood and corn stover lignin to steam greatly impacts lignin fluidization and relocation, consequently influencing the ease of hydrolysis of the cellulosic component.

S46 Alkali–oxygen treatment prior to the mechanical pulping of hardwood enhances enzymatic hydrolysis and carbohydrate recovery through selective lignin modification

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The decline of traditional newsprint market has forced the closure of many mechanical pulp mills. However, this considerable capital investment and established techniques could provide the "front end/pretreatment facilities" for potential biorefinery/biofuels processes. The lignin presents in biomass is known to be a key factor hindering enzymatic hydrolysis and the pulp and paper sector has shown that is difficult and expensive to completely remove this lignin. However, the partial removal and modification of lignin by mild chemical treatment such as alkaline-oxygen have been shown to improve substrate swelling and cellulose accessibility. In the work that will be presented similar processes were incorporated into a chemi-thermomechanical (CTMP) pulping process, followed by subsequent refining which further increased cellulose accessibility through fiber separation and fibrillation.

When aspen chips were impregnated with carbonate and oxygen prior to pre-steaming and mechanical refining this resulted in the partial removal of lignin and the retention of more than half of the hemicellulose in the water-insoluble fraction. The residual lignin had been modified through the addition of carboxylic acid groups, resulting in increased swelling and a decrease in the tendency of the lignin to bind enzymes. Rather than trying to fractionate lignocellulosic substrates into the three separate cellulose, lignin and hemicellulose fractions, a modified thermomechanical pulping (TMP) provided the recovery of much of the hemicellulose with the cellulose fraction and the ready enzymatic hydrolysis of these carbohydrates by enhancing lignin swelling and decreasing unproductive enzyme adsorption by making the lignin more hydrophilic.

S47 The use of cellulose binding modules (CBMs) to better elucidate cellulose deconstruction and functionalization

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Nature has designed cellulose to be a structural material that, through its association with lignin and hemicellulose, is very recalcitrant to degradation. Although the ultimate step in cellulose hydrolysis can be readily measured, by monitoring glucose release, the key limitation that restricts effective enzymatic hydrolysis, i.e. the cellulase enzyme accessibility to the cellulosic substrate, has proven much harder to quantify. A novel method, which has the potential to better elucidate the mechanisms involved, uses specific carbohydrate binding modules (CBMs) as probes. As will be described, CBMs were used to characterize the surface morphology of lignocellulosic substrates to ty to better quantifying and predict cellulose accessibility as a result of their selective and strong binding to specific cellulose regions.

CBM2a (type A CBM) and CBM17 (type B) were primarily employed, as they possess different binding preferences towards specific structures within the cellulose (crystalline vs paracrystalline, respectively). The adsorption profiles and binding kinetics of the CBMs on various cellulosic substrates was assessed to not only increase our understanding of cellulose accessibility with "real" lignocellulosic substrates, but also better determine the influence of the substrate source and the pretreatment method on cellulose accessibility.

When the CBMs were used in parallel with microscopy, fiber analysis (aspect ratio) and water retention values (WRV) it was apparent that the CBM technique better predicated the ease of hydrolysis of some lignocellulosic substrates than did the others

methods.
As well as using CBMs to assess substrate accessibility their potential to modify and functionalize cellulose surfaces will also be described.

S48 Integration of renewable deep eutectic solvent with plant genetic engineering to achieve a closed-loop biorefinery

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The modern lignocellulosic biorefinery strives to develop new processes and products for achieving a sustainable energy future. Although renewable fuels from lignocellulosic biomass have proven to be alternatives for fossil fuels, innovative technologies are still required to build economically viable processes for converting biomass to fuels and chemicals. In this work, we report an integrated biomass processing that uses a renewable deep eutectic solvent synthesized from lignin-derived phenolic aldehydes for the pretreatment of CAD down-regulated biomass. Novel DESs tested in this work showed their potential as solvents for biomass pretreatment in terms of lignin removal and sugar release after enzymatic saccharification. Also, CAD down-regulated biomass has the potential to produce phenolic aldehydes (e.g. vanillin, 4-hydroxybenzaldehyde, etc.), which can be directly used for the DES synthesis. We believe that biomass processes utilizing DESs directly from biomass could lower costs by achieving the closed-loop biorefinery.

S49 Sulfonated Wrinkled Carbon- Silica Nanocomposite: An Efficient Catalyst for the Synthesis of Higher Carbon Fuel Precursors

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Valuable products are obtainable via the catalytic conversion of biomass derived intermediate platforms as renewable carbon sources. Solid acid catalysts show enormous potential for the production of high carbon fuel precursors with environmental benefits. Sulfonic acid functionalized wrinkled carbon/silica spheres (WNSC-SO_3H) with primary mesopores were prepared with KCC-1 as a template and p-toluenesulfonic acid (TsOH) as a carbon precursor and $-SO_3H$ source simultaneously. The amount of p-toluenesulfonic acid was varied to obtain different acidic amount and hydrophobicity. The physical and chemical properties of WS/C-SO_3H were characterized by N2 adsorption, TEM, SEM, XRD, Raman spectrum, element analysis and acid−base titration techniques. Molecular level perceptions into the nature and strength of the acid sites were gained by combining high resolution XPS and ^{1}H-decoupled ^{31}P MAS NMR spectroscopy of adsorbed triethylphosphine oxide. WNSC-SO_3H shows excellent performance in production of high carbon fuel precursors and exhibit a superior intrinsic catalytic activity compared to other commercial solid acids such as Amberlyst-15. The enhanced catalytic activity is attributed to the higher SO_3H acid density, the larger and better communicating pores and the fibrous nature. 100% conversion and 91 % selectivity to target trimer was achieved and no distinct activity drop was observed after 5 recycles.

This work was supported by the Energy Efficiency & Resources (No. 20163010092210) of the Korea Institute of Energy Technology Evaluation and Planning (KETEP) grant funded by the Korea government Ministry of Trade, Industry & Energy

S50 Simultaneous upgrading biomass-derived sugars to HMF/furfural via enzymatically isomerized ketose intermediates

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Current processes to produce 5-hydroxymethyl furfural (HMF) and 2-furaldehyde (furfural) generally involve use of acid catalysts in biphasic systems or solvents such as ionic liquids. However, the yield from transforming glucose to HMF is lower than xylose conversion to furfural. Here, we present an efficient pathway for simultaneously transforming glucose and xylose to HMF and furfural via ketose intermediates, i.e., fructose and xylulose. Mixed sugar feeds were enzymatically isomerized to produce ketoses using a commercial immobilized enzyme without buffer, but with borate added to favor ketose formation. By adding sodium borate to complex with the ketoses, xylose conversion reached equilibrium after 2h with a conversion of 91% and glucose conversion reached 84% after 4h. By enzymatically isomerizing the aldoses to ketoses, the following dehydration reaction to produce HMF and furfural could be performed at low process temperatures (i.e., 110 – 120°C) minimizing the side reactions of the sugars and limiting degradation of furfurals to humins and carboxylic acids. Under the optimum reaction conditions, 120°C for 15 min at pH 0.5, mixed ketose sugars (9 wt% glucose and 6 wt% xylose) were converted to HMF and furfural in yields of 77% and 96%, respectively. In addition, when using biomass hydrolysate, both C5 and C6 sugars were isomerized and then converted to furfural and HMF in good yields. These results demonstrate that this combined biochemical and chemical process could be an effective pathway to simultaneously upgrade glucose and xylose to HMF and furfural, intermediates in the production of hydrocarbons.

S51 The potential to integrate drop-in biofuel upgrading in oil refineries

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Drop-in biofuels that are functionally equivalent to petroleum-based transportation fuels and fully compatible with the existing petroleum infrastructure are gaining significant interests due to their potential to help with climate change mitigation. Currently, so-called “conventional” drop-in biofuels such as renewable diesel are based on oleochemicals/lipids and are almost exclusively produced in dedicated standalone facilities. However, the option of processing biogenic feedstocks along with fossil fuels at existing oil refineries to produce lower carbon fuels (co-processing) could be an alternative way to decarbonise long distance transportation fuels, particularly for the aviation and marine sectors.

In the near term, oleochemicals based on lipids and fats will be the primary biogenic feedstocks that will be processed by oil refineries. However, due to likely variations in the lipid feedstocks, some sort of “pretreatment” will be required to ensure robust catalyst operation. In the longer term “advanced”, or biomass based “biocrudes” will be needed due to the expense and limited supply of oleochemical feedstocks.

The production of the biocrudes/bio-oils via pyrolysis or hydrothermal liquefaction is at different stages of maturation with some companies, such as Ensyn, operating at a commercial level. However, there have been very limited work on how the various biocrudes that might be produced might be co-processed in an oil refinery as biocrudes are more complex and heterogeneous than oleochemicals. The likely sources and composition of biocrudes and their possible insertion points within existing oil refineries will be discussed.

S52 Manganese-enhanced white rot fungal pretreatment for enhancing enzymatic saccharification of wheat straw and poplar wood

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Lignocellulosic biomass, comprised of agricultural residues and wood waste, could be an abundant feed stock for bioenergy refineries. However, lignin is recalcitrant to degradation and prevents efficient utilization of the sugars in plant cell walls. We propose a manganese-assisted fungal pretreatment (MnAFP) for enhanced degradation and increased cellulose digestibility for wheat straw and poplar wood biomass. Both substrates were treated with *Ceriporiopsis subvermispora* for four weeks; manganese was added to enhance biomass modification and to increase biomass enzymatic digestibility. The effects of manganese supplementation and pretreatment time were evaluated for lignin, hemicellulose, and cellulose degradation. Sugar yields after enzymatic hydrolysis were compared to fungal-only (FS) treated biomass and raw biomass. *C. subvermispora* in combination with 0.01mM Mn resulted in 60.3 % lignin removal in wheat straw and 49.0% lignin removal in poplar, and 19.0% cellulose consumption in wheat straw and 10.3% cellulose consumption in poplar. The correlated glucose yields were 86.0% and 89.1%, and xylose yields were 84.7% and 89.5% for wheat straw and poplar, respectively. Composition analysis and Py-GCMS showed enhanced lignin degradation in manganese-assisted fungal pretreatment (MnAFP) treated wheat straw and poplar compared to FS treated biomass; SEM analysis detected roughened surfaces and decreased crystallinity on MnAFP-treated substrates, resulting in the increased digestibility of wheat straw and poplar wood after pretreatment. This is the first time we report efficient fungal pretreatment of wheat straw and poplar leading to selective lignin removal and high sugar yields but requiring less energy and chemical input than conventional lignocellulosic pretreatment methods.

S53 Sugars and lignin recovery of eucalyptus sawdust for bioethanol production using a phosphoric acid and alkali pretreatments

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Eucalyptus sawdust, a residue from the wood industry, is a promising material to obtain a wide range of bio-based products and biofuels including electricity, heat, chemicals within a biorefinery concept. An appropriate pretreatment should be applied to produce fuel bioethanol from its cellulosic fraction, and to obtain other marketable valuable products from the hemicellulose components and lignin.

A pretreatment in two stages, dilute phosphoric acid treatment followed by an alkaline treatment, was carried out in order to obtain a cellulosic fraction susceptible to enzymatic attack, preserving xylan and lignin fractions for other uses. Different phosphoric acid concentrations (0.5 – 1.5 % w/v), temperatures (170 – 200 °C) and times (10 – 50 min) were evaluated, followed by an alkaline treatment (5 % w/v H_2O_2, 80°C and 40 min). The solid fractions after alkaline treatment were hydrolyzed using 5 % w/v solids, cellulose enzyme complex Cellic Ctec2 15 FPU/g dry solid with sodium citrate buffer (pH 4.8) 0.05 N, 48 °C and 150 rpm.

Xylan recovery showed the best results for 170 °C, 40 min, 1 % w/v H_3PO_4 condition (82 %) while for lignin, the best solubilization was obtained at 185 °C, 30 min, 1 % w/v H_3PO_4 (88 %).

After 96 h, the hydrolysis efficiency reached values near to 100 % for pretreated solids at low temperatures (170 °C), corresponding to an overall efficiency of 0.4 - 0.9 g of glucose per g of theoretical glucose from original glucan in eucalyptus sawdust, depending on the pretreatment condition used.

S54 Improved Biomass Deacetylation and Deconstruction using a Continuous Counter-Current Reactor

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The low severity Deacetylation and Mechanical Refining (DMR) biomass deconstruction process is an emerging technology that has successfully demonstrated capabilities to produce high titer and yield, low toxicity sugar and tractable lignin streams. Previous work has been performed in batch stirred tank reactors with scale up to 100 kg/day. In this work we have adapted a shaftless inclined screw reactor to perform counter-current deacetylation. Continuous counter-current extraction is practiced at the industrial scale in pulping processes to recycle the black liquor at high pressures/temperatures, enabling effective mass and heat transfer to achieve high lignin removal and efficient water/energy usages. Counter-current process steps enable high concentrations of the target compounds to be extracted into the extraction solvent and result in low residual content of the target compound in the extracted residue to increase efficiency and downstream product yields, while keeping the equipment more compact and decreased footprint dimensions compared to batch stirred tank reactors. Preliminary results show improved sugar yields in enzymatic hydrolysis at low enzyme loadings for the continuous counter-current deacetylation/mechanical refining process compared to the currently used batch process, while xylan losses into the black liquor are reduced up to 2/3.

S55 Enhancing Dissolving Pulp Production and Co-product Value

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Unlike the pulps used to make paper and package products, dissolving pulp primarily serves as a chemical feedstock for the manufacture of cellulose derivatives such as cellulose esters and ethers that are used in a host of products such as clothing, cigarette filters, pharmaceuticals, paints, etc. The market for textiles, such as those produced using dissolving pulp is expected to grow faster than the the worlds GDP with dissolving pulp derived fibres accounting for 8 million tonnes of total textile fibre production. Dissolving pulp is generally characterized by a high cellulose, low hemicellulose content and a low, <1% residual lignin, extractives and mineral content. Primarily due to lower fiber strength and chemical recovery challenges when using sulfite pulping, Kraft pulping has superseded the sulfite process as the predominant way to produce dissolving pulps. However, the relatively large amounts of hemicellulose that are typically retained in Kraft pulps are detrimental to dissolving pulp production. Therefore, as will be described, we assessed various ways to develop an effective pre-hydrolysis step to help facilitate hemicellulose removal. Another goal of the work was to better preserve cellulose from peeling by controlling the sudden pH swing from the very acidic pre-hydrolysis to the strong alkalinity used in Kraft pulping. As well as enhancing pulp production there is also considerable potential to utilize the solubilized hemicellulose as a biorefinery feedstock. The presentation will give an overview of how a repurposed Kraft mill can serve as a model biorefinery.

S56 Succinate Production with Metabolically Engineered *Escherichia coli* with Elephant Grass Stalk Hydrolysate as Carbon Source

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Succinic acid can be used as the precursor of various industrial products including pharmaceuticals and biochemicals. The improvement of the succinic acid market depends on strains engineering that is capable of producing succinic acid at high yield and excellent growth rate which could utilize the wide range of carbon sources such as renewable biomass. Here we use counter selection with *cat*A*sac*B for pathway design and strains developments. In this investigation, metabolically engineered *Escherichia coli* M6PM strain was constructed for the synthesis of succinic acid using elephant grass stalk (*Pennisetum purpureum*) as a carbon source. Elephant grass stalk hydrolysate was prepared which comprised of 11.60 ± 0.04 g/L glucose, 27.22 ± 0.04 g/L xylose and 0.65 ± 0.04 g/L arabinose. Metabolically engineered *E. coli* M6PM was constructed and fermentation with pure sugars revealed that it could utilize xylose and glucose efficiently. *E. coli* M6PM produced a final succinate concentration of 30.03 ± 0.02 g/L and a yield of 1.09 mol/mol during 72 h dual-phase fermentation using elephant grass stalk hydrolysate, which resulted in 64% maximum theoretical yield of succinic acid. The high succinate yield from elephant grass stalk demonstrated possible application of renewable biomass as feedstock for the synthesis of succinic acid using recombinant E. coli.

S57 Production of Levulinic Acid Using Alkaline and Acid Hydrothermal Reactions from Lignocellulosic Biomass

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As a promising platform chemical for the biofuels and biochemicals, conversion of levulinic acid from various renewable resources has been studied. Levulinic acid can be further converted to methyltetranhydrofuran (MTHF) and g-valerolactone (GVL) as gasoline additives, ethyl levulinate (EL) as a diesel additive, and 2,5-furandicarboxylic acid (2,5-FDCA) as a substitute for terephthalic acid. For the conversion of levulinic acid from biomass in this study, lignocellulosic biomass were delignified using ammonium hydroxide, which is followed by acid hydrolysis of cellulose into glucose. For the degradation of glucose into levulinic acid, acid hydrothermal reaction conditions (150-190°C).

Conversion yields of glucose, hydroxymethylfurfural (HMF), and levulinic acid from feedstock will be evaluated and reported in this paper. For the increased levulinic acid conversion yield, various reaction conditions, such as catalytic solution

concentrations, reaction temperatures, and reaction times, will be explored to find the optimum conditions.

S58 Characterization of Carboxylic Acid Reductases as Catalysts for Biosynthesis of Industrial Chemicals

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Carboxylic acid reductases (CARs) are of great interest in industrial biocatalysis for their ability to reduce a wide range of carboxylic acid substrates to their corresponding aldehydes, which serve as precursors in biofuel or bio-based chemical syntheses using microbial hosts. CARs are multi-domain enzymes with an N-terminal adenylation domain, a C-terminal reduction domain, and a linkage domain that contains a post-translationally attached phosphopantetheine moiety to transfer covalent intermediates from the N to the C domain. In this work, we identified and characterized CARs for their catalytic activities on short-chain hydroxy acids and dicarboxylic acids, which are common microbial cellular metabolites. All characterized enzymes exhibited broad substrate specificity. Higher catalytic efficiencies were observed on hydroxy acid substrates in comparison to dicarboxylic acid substrates of the same carbon-chain length. In addition, catalytic efficiencies on hydroxy acid and dicarboxylic acid substrates increased as carbon-chain length was increased from C_2 to C_6. CAR activity was coupled with that of an aldehyde reductase in *Escherichia coli* hosts to investigate the whole-cell bioconversion of eleven short-chain carboxylic acid substrates to their corresponding alcohols. Alcohol products were accumulated *in vivo* from short-chain carboxylate substrates at yields ranging from 0.5% and 71%. Engineered *E. coli* strains expressing CAR and aldehyde reductase enzymes were used for the *de novo* stereospecific biosynthesis of 1,2-propanediol (PDO) isomers. Our current research efforts focus on the engineering of CARs to improve the activities on short-chain carboxylate substrates.

S59 "Intramolecular synergy in the multifunctional cellulase CelA from *Caldicellulosiruptor bescii*"

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Caldicellulosiruptor bescii, a hyperthermophilic (T_{opt}, 65-80^{o}C), anaerobic, asporogenous microbe isolated from the hot springs in Kamchatka, Russia, is described as one of the most cellulolytic bacteria in the biosphere. CelA (Athe_1867; 1759 amino acid) is the most prevalent cellulase in the *C. bescii* exoproteome. It has the ability to deconstruct biomass at temperatures up to 90°C and outperform mixtures of commercially available exoglucanases and endoglucanases *in vitro*. CelA consists of an N-terminal GH9-CBM3c processive endoglucanase and a C-terminal GH48 exoglucanase, connected by a highly glycosylated linker region containing two Family 3b carbohydrate-binding domains (CBM3b). Analysis of intra- and intermolecular endo-exo cellulase synergism of this multi-domain cellulase, employing intact and its truncated mutants, has revealed the presence of an intramolecular synergistic effect on the hydrolysis of complex pretreated biomass substrates, for example APCS (Alkaline peroxide pretreated corn stover). However, this is not the case on crystalline cellulose where the combination of its N and C termini outperforms the intact enzyme.

S60 Alteration of lignin biosynthetic pathways in sorghum enhances its deconstruction by adapted microbial consortia

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In natural systems, microbial consortia deconstruct plant biomass, and adapting these consortia to grow with plant biomass as the sole carbon source has enabled the discovery of new microbial and enzymatic activities unobserved in pure culture. Lignin is an important source of recalcitrance for microbial and enzymatic conversion of biomass and cultivating consortia on biomass with altered lignin content and composition may provide unique insights into mechanisms of lignin deconstruction.

Sorghum is a promising bioenergy feedstock for which a number of lignin mutants are available. Here, we designed an experiment to determine the effect of these mutations on the deconstruction of sorghum by microbial consortia grown at elevated temperature. We adapted replicate microbial consortia to grow on sorghum, then compared the growth and extent of biomass solubilization of a wild type (WT) and stacked mutant (SM).

For both WT and SM sorghum, flasks inoculated with sorghum-adapted microbial consortia exhibited greater biomass loss compared to controls. In particular, one consortium was very active in biomass deconstruction with WT and SM sorghum exhibiting 53.2% and 76.6% biomass loss, respectively. Community compositional profiling using metagenomics indicated that the most abundant population in these sorghum-deconstructing communities was related to *Actinotalea fermentans*, a cellulolytic actinomycete. Comparison of high and low-performing communities demonstrated that subtle changes in community composition influenced overall biomass deconstruction. Time series metatranscriptomic data is being analyzed to determine which community members produce enzymes that deconstruct lignin.

S61 Evaluation of ABE and IBE fermentation of enzymatic cellulose hydrolysates from eucalyptus sawdust pretreated by steam explosion

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Butanol is considered an important chemical with wide industrial applications which has shown to have superior fuel properties to ethanol. It can be produced by acetone–butanol–ethanol (ABE) or isopropanol-butanol-ethanol (IBE) fermentation. The production of isopropanol instead of acetone is desirable because of acetone corrosiveness, which allows the produced mixture of solvents to be used directly as biofuel. In this work, ABE and IBE fermentations performance were compared using eucalyptus sawdust as a raw material. The sawdust was subjected to steam explosion pretreatment at 200ºC for 10 min in a continuous type reactor. The pretreated solids were subjected to enzymatic hydrolysis for 72 h with Cellic CTec2 at 50ºC, pH 4.85, solids and enzyme loadings of 16% w/v and 25 FPU/g_{glucan}, respectively. The enzymatic hydrolysate obtained was fermented using *C. beijerinckii* DSM 6422 and *C. beijerinckii* DSM 6423 for ABE and IBE production, respectively. Assays were done in 100 mL bottles at 37ºC, pH 6 and 150 rpm, inoculated with 10% (v/v) highly active cell culture. Both microorganisms were able to ferment the cellulosic hydrolysates. *C. beijerinckii* DSM 6422 reached 5.0 g/L of butanol (8.6 g/L total ABE solvents) at 48 h and *C. beijerinckii* DSM 6423 2.6 g/L of butanol (5.4 g/L total IBE solvents) at 96 h. Complete sugar conversion was not reached (46% and 25% for *C. beijerinckii* DSM 6422 and *C. beijerinckii* DSM 6423, respectively). Even though the IBE mixture has better fuel properties, lower butanol yield and productivity were obtained using *C. beijerinckii* DSM 6423.

S62 In vitro fermentation of xylooligosaccharides in autohydrolyzate from Miscanthus by Lactobacillus brevis

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With an increased awareness of health and wellness, prebiotics have received a great amount of attention recently. In this study, autohydrolyzate from hot water treatment of *Miscanthus* lignocellulosic biomass was evaluated for its prebiotic effect by fermentation of *Lactobacillus brevis* strain. The cell growth on raw autohydrolyzate-supplemented media was comparable to the sample grown on glucose until an incubation time of 24 hrs then gradually declined. Autohydrolyzate contains various inhibitors (25.9% of total dissolved solids) and it was determined that dissolved lignin had a significant inhibitory effect to bacterial growth. Inhibition test with dissolved lignin showed cell density rapidly decreased with increase of dissolved lignin concentration. When the autohydrolyzate was purified using a hydrophobic resin, purified autohydrolyzate exhibited high bacterial growth (cell density (OD 600) of 4.8) and high acid production (7.7 and 3.1 g/L of lactic and acetic acid, respectively), which was comparable with commercial xylooligosaccharides. In conclusion, mixed oligosaccharides in the autohydrolyzate from *Miscanthus* have considerable potential as a prebiotic and are comparable with commercial xylooligosaccharides derived products. This result suggested that biomass autohydrolyzates can be used as prebiotic sources.

S63 Alkali pretreatment of corn stover during storage

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Storage is an important operation in the feedstock logistics supply chain, especially in the case of agricultural residues that are harvested annually. Storage has a relatively long residence time, which offers an opportunity to perform low-severity treatments that can have benefits downstream. Chemical approaches to structural sugar and lignin depolymerization in corn stover were assessed as a means to initiate preprocessing (i.e. biomass deconstruction) during the storage operation. Results of corn stover treated with sodium hydroxide (two concentrations) over a 4-week period in both aerobic and anaerobic conditions are reported for biomass at both 40 and 60% (wet basis) moisture content. Characterization of dry matter preservation, extractives, and structural components was performed. Treatment with the highest level of alkali resulted in a two-fold increase in total extractives and a corresponding reduction of acid insoluble lignin, xylan, and acetate. X-ray diffraction was used to assess changes to cellulose crystallinity. Alkali treatments were shown to increase the crystallinity index of the biomass, likely due to the removal of amorphous material, such as hemicellulose and lignin. Storage treatments were scaled up to 5 kg to provide sufficient biomass to estimate conversion performance. Enzymatic hydrolysis revealed increased carbohydrate release in alkali-treated, stored corn stover. Overall, alkali treatment performed during the storage operation is a promising approach to preserve biomass as well as begin the depolymerization process required for cellulosic biofuel production.

S64 Ethanol production by thermotolerant yeast in extractive batch fermentation

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This work presents the study of ethanol production by a thermotolerant *Kluyveromyces marxianus* strain. Batch experiments were carried out in a bioreactor (2 L) at temperatures ranging from 36 to 44 ^{o}C. A mathematical model using a hybrid Monod-Levenspiel kinetic was proposed to describe the process. Yield coefficients (for cell and ethanol) were calculated from experimental data. The other model parameters were estimated by genetic optimization algorithm. The proposed model predicted fairly well the experimental behavior of cell, substrate, and ethanol concentrations throughout ethanol fermentations. Subsequently, the ethanol and water removal from the fermentation broth was modeled considering a classical first order model (valid for any type of extractant). The first order model constants were obtained from stripping experiments with CO_2 using hydroalcoholic solution in a central composite rotatable design (specific CO_2 flow rate: 1.0 to 4.0 vvm; initial ethanol concentration: 30.0 to 60.0 g/L; temperature: 35 to 45 ^{o}C). Then, extractive batch ethanol fermentation assays were carried with carbon dioxide stripping at 40 ^{o}C and using the following CO_2 specific flow rates: 1.0, 1.5 e 2.5 vvm. The higher ethanol

volumetric productivity (9.02 g_E/L h) was obtained in the experiment performed at 1.0 vvm. This value was 11.2% higher than those value obtained in conventional batch fermentation at the same experimental condition. The proposed mathematical model was able to adequately describe the extractive fermentation process.

Acknowledments: FAPESP (Process 20112/50046-4), PRH-44/ANP, CAPES, and CNPq.

S65 Economics of microaeration for fermentation of lignocellulosic feedstocks

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The dilute acid pre-treatment of lignocellulosic feedstocks produces acetic acid, furfural, and 5-hydroxymethylfurfural (HMF) inhibitors that decrease the specific growth rates and volumetric productivities of downstream fermentations. Previous work with very high gravity feedstocks has shown that microaeration at controlled oxidation-reduction potentials can increase fermentation productivity without significant yield loss when glucose and ethanol are the primary inhibitors. The goal of this study was to apply a microaeration for ORP control strategy to a mock lignocellulose hydrolysate feedstock (LHF) fermentation to determine the effect on rates and yields, and to determine if microaeration has the potential to reduce overall fermentation costs compared to an unaerated fermentation. It was found that microaeration (ORP controlled at -150 mV) increased the specific ethanol productivity from 0.51 to 1.32 g/L/OD/hr with no significant reduction in ethanol yield ($Y_{P/S}$=0.49 g/g); this improved productivity was due in part to enhanced furfural and HMF reduction rates (92% faster for furfural and 200% faster for HMF) with microaeration. When these experimental results were used to design and cost a 5 million gallon per year (MMGPY) LHF to ethanol fermentation, it was estimated that a microaeration design would increase the fermentation equivalent annual operating cost (EAOC) from $113 to $749 thousand/yr. Agitation and compression operating costs accounted for 70% of the total EAOC for the microaeration design. In conclusion, while microaeration has a significant positive impact on LHF fermentation productivity, the additional aeration costs may not be economically justified.

S66 Rice-bran hydrolysate as an inexpensive carbohydrate and protein medium for the production of the beta-glucan lasiodiplodan

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The synthesis of beta-glucans, especially exopolysaccharides (EPS), lacks information concerning renewable feedstock utilization and is traditionally produced using synthetic media. The present work aims to evaluate the production of lasiodiplodan (LAS) by the filamentous fungi *Lasiodiplodia theobromae* CCT 3966 utilizing rice-bran hydrolysates as an inexpensive source of carbohydrates and proteins. Rice-bran hydrolysate (RBH) was prepared in using 20% (w/v) of rice-bran in distilled water and 1% (w/v) of H_2SO_4, autoclaved at 121 °C for 15 minutes. The RBH pH was adjusted to 5.5, centrifuged and autoclaved as previous. Four discs of 5 day grown mycelia, containing the *L. theobromae* CCT 3966, were inoculated in 250 mL Erlenmeyer flasks. Samples were obtained each 24 h. Fermented broth was centrifuged for separation of total cells biomass (TCB) and supernatant. Supernatant was added to cold ethanol (1:4) for LAS precipitation, re-solubilized in water (60 °C) and added to cold ethanol for gravimetric analysis. TCB samples were washed with water (60 °C) and also analyzed gravimetrically. Total sugars (TS), reducing sugars (RS) and total proteins (TP) were evaluated and fermentation steps. Carbohydrate and protein consumption, as well as the LAS and TCB were plotted and the fermentative parameters were calculated. The RBH proved to be an efficient media for beta-glucan lasiodiplodan production, providing both carbohydrate and protein substitution of synthetic media and contributing to the development of low-cost formulations for fungal EPS, integrating biorefinery concepts for production of bio-based products.

Acknowledgements: CAPES, CNPq, FAPESP (Process number 2016/10636-8) and Fundação André Tosello Pesquisa e Tecnologia

S67 Stirred tank reactor production of brewer yeast biomass enriched with selenium using starchy feedstocks

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Yeast biomass is currently used in animal feed to its high protein content and prebiotic properties and selenium is an essential non-metal for animal health, presenting high antioxidant and immunological properties. The use of agroindustrial residues as feedstocks in yeast biomass production is an inexpensive alternative and potential source for animal feed. The present study presents the production of brewer yeast *Saccharomyces cerevisiae* LALVIN ICV D47 using starchy hydrolysate in stirred tank reactor (STR). The bioreactor (Bioengineering, Wald Switzerland, 1.5 L) was filled with 1.0 L working volume, equipped with four removable baffles, one agitator shaft with two standard six-blade flat impellers and one glass condenser. Medium was composed by a mixture of cornmeal and soybean bran hydrolysates (4:1 v:v), obtained from acid hydrolysis with H_2SO_4 1% (w/v), containing 50 g/L of reducing sugars and 12 g/L of proteins. Cells were inoculated in a concentration of $1x10^7$ cel/mL and culture medium was enriched with 10 mg/L of selenium as Na_2SeO_3. Fermentation was carried out at 30 °C, 350 rpm for 72 h. Cells were recovered, and submitted to growth analysis, freeze-drying and selenium content measure. Obtained results presented

8.58 g/L of yeast biomass (dry mass) enriched with high selenium content and 57 % of cells viability. Cornmeal and soy bran residues demonstrated high potential as synthetic media substitutes presenting both carbon and nitrogen sources for the production of enriched yeast biomass, and contributing for studies of sustainable resources and biorefineries application.

Acknowledgments: FAPESP (Process number 2016/10636-8), CAPES, CNPq.

S68 Correlations between methane potential and sugar release in *Salix viminalis*

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Biomass recalcitrance is commonly evaluated using miniaturized high-throughput pretreatment and saccharification (HTP) assays. We have previously demonstrated, in a population of 286 naturally occurring *Salix viminalis* clones, that biomass recalcitrance in this species is a heritable trait which can be used in traditional breeding programs. However, it is not known whether improvements to this trait will also translate into enhanced performance in other conversion systems, such as anaerobic digestion (AD). Therefore, we evaluated 95 natural *S. viminalis* samples, representing the full spectrum of sugar release values seen in the population, using a standard biomethane potential (BMP) test for 94 days. The results show that sugar release, as measured by the HTP assay, is positively correlated with methane production according to the BMP test. However, the association was strongest during the early stage of the anaerobic digestion process, gradually diminishing throughout the assay (R^2= 0.49 at 20 days, R^2= 0.29 at day 94). Weight of the main shoot and lignin S/G ratio, two traits that were previously identified as positively correlated with HTP sugar release, were also positively correlated with methane production. Again, these correlations were strongest during the initial phase of the BMP test. In conclusion, the results of this study indicate that using HTP sugar release as a feedstock improvement trait may also provide benefits to using the feedstock for AD, but that mechanisms determining performance in these two conversion systems are not perfectly overlapping.

S69 Study of pH effect on *Rhodosporidium toruloides* CCT7815 growth and lipogenesis during bioreactor cultivation with xylose-rich medium

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Microbial oil (SCO) has been shown as an alternative to traditional raw material for biodiesel production (vegetal oils) since its production requires less time and agricultural area and do not compete with food. Microorganisms like algae and yeast are two classes of potential sources of SCO. However, yeasts could be more advantageous because they can produce higher amounts of high value molecules like carotenoids. Despite these benefits, microbial oil production is still expensive and require some efforts to overcome this bottleneck. Adequate fermentation parameters like C/N ratio, aeration, temperature, and pH can improve lipid production and helps to make this technology feasible. Fermentation pH is strongly linked with biomass and lipid production since it affects cell physiology and kinetics. Literature showed that each medium for yeast cultivation has its optimum pH weather to produce biomass or lipid. Based on this, the objective of this work was to study the cultivation and lipogenesis of *Rhodosporidium toruloides* CCT7815 in bioreactor under different pH values (4.0 and 5.0) in xylose-rich medium. At pH 5.0 the biomass production was favored achieving after 120 h of cultivation its maximum, 19.0 g/L. Moreover, at pH 5.0 lipogenesis took 24 h to be inducted while at pH 4.0 this phenomenon was observed only after 60 h. At pH 4.0 lipid production was favored: the highest specific lipid production was achieved after 120 h of cultivation, 58% of lipid content in the dried cells, value almost 20% higher than the maximum observed at pH 5.0.

S71 Influence of C/N ratio and medium composition on *Rhodosporidium toruloides* production of oil and carotenoids

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An efficient microbial production of chemicals and fuels is a main requirement for the transition to bioeconomy. *Rhodosporidium toruloides* is a versatile microorganism to produce high value products such as lipids (a substitute for vegetable oils in biodiesel production) and carotenoids (an important antioxidant) from low-cost substrates. The carbon to nitrogen (C/N) ratio has been identified as an important parameter on the production of these compounds. This present study evaluated the influence of different C/N ratios on the lipid and carotenoid production by *R. toruloides* using a synthetic medium (xylose and glucose, 13:1) and renewable carbon sources (sugarcane bagasse hemicellulosic hydrolysate, SBHH; soybean molasses, SM; and glycerol, GLY). In general, lipid and carotenoid production were increased at high C/N ratios lipid and carotenoid production were high at high C/N ratios when synthetic medium were used. At C/N 100, the lipid and carotenoid contents were 5.2 g/L and 33.2 µg/mL, respectively, while at C/N 20 these productions decreased to 1.8 g/L and 7.5 µg/mL, respectively. However, this trend was not so clear when renewable carbon sources were used. When comparing media with different composition of SBHH, SM, and GLY with the C/N ratio increased from 65 to 130 the lipid production was not altered (2.8 g/L). These results indicate that, besides C/N ratio, each carbon source must be considered to be assimilated and metabolized differently by the yeast, being driven to cell metabolism or lipid biosynthesis.

S72 Metabolic engineering of methanotrophs for production of 5-aminolevulinic acid

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Methane is the primary component of shale gas, natural gas, and biogas and is a greenhouse gase (GHG) causing global warming. The bioconversion of methane to platform chemicals provides an attractive opportunity to decrease GHG emissions and to utilize this abundant and inexpensive gas as carbon feedstock. Methanotrophs are considered promising industrial microbes for bioconversion of methane into valuable chemicals. We recently isolated a type I methanotroph, *Methylomonas* sp. DH-1 possessing a relatively rapid growth rate. Genome sequence analysis and development of genetic manipulation techniques have been conducted for metabolic engineering of *Methylomonas* sp. DH-1. In this study, we metabolically engineered *Methylomonas* sp. DH-1 to transform methane directly to 5-aminolevulinic acid (ALA) *via* the C4 pathway. Production of 5-ALA has recently received increasing attention because of its potential clinical applications. Codon-optimized 5-ALA synthase was expressed in *Methylomonas* sp. DH-1 to synthesize 5-ALA. Deletion of genes for the formation of by-products further enhanced 5-ALA synthesis. This is the first study to synthesize ALA, a non-protein amino acid of commercial interest from methane by metabolic engineered methanotroph.

S73 Effect of inoculum load and aeration on the conversion of combined glucose and glycerol into lipids by *Saitoella coloradoensis* at low temperature

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Microbial lipids are an alternative source of polyunsaturated fatty acids such as α- linolenic acid, linoleic acid, eicosapentaenoic acid, arachidonic acid, docosahexaenoic acid, and oleic acid relevant for human nutrition and food application. Oleaginous yeasts can metabolize different substrates to produce lipids, among of which, glycerol represents nowadays a cheap alternative due to its high availability from biodiesel production. This work evaluated the production of lipids by the oleaginous yeast *Saitoella coloradoensis* using combined glucose and glycerol as carbon source. Initially the strain was cultivated in defined medium containing glucose and glycerol at 15 °C. These assays were carried out in shake flasks and the yeast´s performance was compared to the performance of *Leucosporidium scottii,* another yeast strain previously known as lipid´s producer at low temperature. In the sequence, *S. coloradoensis* was cultivated in a 1-L bioreactor keeping the same medium composition and growth temperature, but using different conditions of inoculum load and aeration. *S. coloradoensis* was able to growth, produce lipids, and simultaneously consume glucose and glycerol at low temperature. The increase of inoculum load did not affect the lipids production at limited aeration. However, at surplus of aeration, the increase of inoculum load improved the lipids accumulation.

S74 Metabolic engineering of *Methylomonas* sp. DH-1 for production of succinate from methane

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S75 Direct ethanol production from cellulose through microbial consortium

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Lignocellulosic materials are important for the production of ethanol due to their abundance. Industrial cellulosic ethanol production is still a challenge because of the high processing cost of cellulase for hydrolysis after using lignocellulosic materials as feedstock. In this study, direct ethanol production from cellulose was performed by consortium of *Trichoderma reesei* and yeast (*Candida molischiana* or *Saccharomyces cerevisiae*). Different concentrations of cellulose (Avicel) was hydrolyzed by a fully enzymatic saccharification process using *T. reesei* cellulases. The produced reducing sugar was further utilized by *C. molischiana* or *S. cerevisiae* for ethanol production. *C. molischiana* could utilize both glucose and cellobiose, while *S. cerevisiae* could utilize only glucose. The highest ethanol yield was 0.13 g ethanol/g cellulose by consortium of *T. reesei* and *C. molischiana* from 20 g/L of Avicel as a raw material.

S76 Lactic Acid Production from Aquaponics System Wastes

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The aim of this work was to study the efficiency of using waste streams produced by the aquaponics system as potential feedstocks for lactic acid (LA) production. Fish manure waste collected from the tilapia farming tank and vegetable plant residue from greenhouse were investigated as substrates. Simultaneous Saccharification and Fermentation (SSF) was applied using enzyme and *Lactobacillus pentosus*. Different loadings of fish waste and plant residue were tested to find the optimal conditions. Fish waste contains nitrogen and minerals that could support the growth of lactic acid bacteria (LAB), making it a good candidate as the nutrient source for lactic acid fermentation. The highest lactic acid yield obtained from cellulose fermentation with fish waste was 87% with a corresponding volumetric productivity of 1.006 g/L·h. Due to its low lignin content and relatively simple structure, vegetable plant residue does not require high severity pretreatment which makes it economically attractive as a carbon source for LA fermentation. Autohydrolysis with hot water was used in pretreating vegetable plant residue. Satisfactory LA productivity was achieved by using the mixed feedstock of fish waste and plant residue. The waste streams from the aquaponics system were shown to be potential feedstocks to provide both carbon and nutrients for LA production.

S77 Co-solvent enhanced lignocellulosic fractionation to enable co-production of bioethanol and lignin-based polyurethanes from poplar wood

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Low cost lignocellulosic biomass provides a unique resource to support the production of renewable transportation fuels that can sufficiently contribute to reducing net positive carbon dioxide emissions from the combustion of fossil fuels. High utilization of whole biomass by valorization of both the sugar and lignin fractions into bioethanol and bioplastics, respectively, can be an effective strategy for reducing the cost of producing 2nd generation biofuels. Here, we detail an integrated approach employing Co-solvent Enhanced Lignocellulosic Fractionation (CELF) pretreatment, Simultaneous Saccharification and Fermentation (SSF), and lignin biopolymer synthesis to coproduce ethanol and lignin-based polyurethanes from poplar wood chips. CELF reaction conditions were first optimized to produce highly digestible cellulose-rich material that reduced enzyme dosages by >75% during SSF using *S. cerevisiae* D5A compared to other aqueous pretreatments to achieve batch ethanol titers that far exceed 50 g/L, a critical threshold for economic ethanol recovery. At the same optimal conditions, CELF pretreatment also extracted out a highly pure technical grade lignin product called “CELF lignin” from poplar. CELF lignin exhibited ideal molecular weight range and functionality to be used for the production of flexible lignin-polyurethanes (lignin-PU) by chemical endcapping with di-isocyanate, achieving up to 60 wt% CELF lignin incorporation into the lignin-PU matrix. These results demonstrate how CELF pretreatment of hardwood poplar can be co-optimized to achieve synergistic benefits of both high ethanol titers and improved lignin-PU plastic properties in a biorefinery so that total biomass utilization can be maximized.

S78 Design of biosensor for screening of mevalonate biosynthetic enzymes

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Mevalonate is an intermediate in the isoprenyl pyrophosphate (IPP) biosynthetic pathway. Recent studies have reported that mevalonate can be used as one of monomers for synthesizing biodegradable polymer. To increase titer of mevalonate in heterologous microorganisms, key two biosynthetic enzymes, hydroxylmethylglutaryl (HMG)-CoA synthase and HMG-CoA reductase, must have enhanced catalytic activities. In this study, to efficiently screen HMG-CoA synthase and HMG-CoA reductase with the enhanced activity, GFP-based whole cell biosensor capable of detecting mevalonate *in vivo* was designed and constructed in *Escherichia coli*. The whole cell biosensor was evaluated by treating with varying concentrations of mevalonate in flask scale cultures. The recorded GFP signal intensity was proportional to increasing concentration of mevalonate, proving the functionality of the whole cell biosensor.

S79 Extraction of fermentation inhibitory compounds from switchgrass

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Cost effective and environmentally sustainable sources of energy have been a major research endeavor in recent years. Lignocellulosic biomass has significant potential to meet the growing demands for biofuel production. However, environmental conditions experienced during growth can significantly influence biomass characteristics and biomass conversion to fuels. Previous research showed that drought experienced during switchgrass growth negatively affected fermentation by completely inhibiting yeast growth. In this project, we attempted to identify specific components that were responsible for this inhibition. Untreated and ammonia fiber expansion (AFEX)-treated switchgrass materials that had been harvested in both drought and non-drought years were extracted using various polar, non-polar and neutral solvents. The untreated, extracted samples were pretreated using AFEX to determine the effect of pretreatment. High solids loading enzymatic hydrolysis was conducted on all samples followed by fermentation using *Saccharomyces cerevisiae* Y133. Fermentation performance was evaluated by measuring cell growth, sugar consumption and ethanol production. For extractions that alleviated inhibition, extracts were evaluated using liquid chromatography- mass spectrometry (LC-MS) to try to identify responsible compounds. Compounds then were back-added to non-inhibitory switchgrass hydrolysates in an attempt to replicate inhibition.

S80 Kinetic characterization of phosphofructokinases present in cellulose fermentingÂ *Clostridium thermocellumÂ* ATCC 27405 suggest different metabolic roles

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The efficient production of sustainable biofuels important for the reduction of greenhouse gas emissions. *Clostridium thermocellum* ATCC 27405 is a candidate for ethanol production from lignocellulosic biomass using consolidated bioprocessing. Fermentation of cellulosic biomass goes through an atypical glycolytic pathway in this thermophilic bacterium, with various glycolytic enzymes capable of utilizing different phosphate donors, including GTP and inorganic pyrophosphate(PPi), in addition to or in place of the usual ATP. *C.thermocellum* contains three phosphofructokinases(PFK) genes, the expression of which have all been detected through proteomics and transcriptomics. PFK (Cthe_0347) was previously characterized as pyrophosphate dependent with fructose-6-phosphate as its substrate. We now demonstrate that this enzyme can also phosphorylate seduheptulose-7-phosphate (an intermediate in the pentose phosphate pathway), with the Vmax and Km of fructose-6-phosphate being approximately 13 folds higher and 53 folds lower respectively in comparison to seduheptulose-7-phosphate.

Purified PFK Cthe_1261 shows preference for GTP as phosphate donor over ATP. The Km of GTP and ATP was 0.0676mM and 0.8279mM; while the Vmax was 23.6μmol.min^{-1}mg^{-1}and 38.8μmol.min^{-1}mg^{-1}respectively. The final PFK (Cthe_0389) appears to be ATP dependent based off preliminary result. These data suggest that this organism may coordinate glycolysis and pentose phosphate synthesis at the level of PFK, while regulating ATP, GTP and PPi use in the first stage of glycolysis through the relative flux of these three energy molecules through the 3 PFKs.

S81 Enzymatic basis for C-lignin monomer biosynthesis in the seed coat of *Cleome hassleriana*

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Lignin is a major impediment to bio-based economic feasibility for lignocellulosic feedstocks. C-lignin, which is a linear benzodioxane-linked homopolymer derived from caffeyl alcohol monomers, has favorable properties for the manufacture of carbon fibers and high value chemicals, as well as potentially facilitating processing of biomass for biofuels. However, this unique C-lignin is found only in the seed coats of a number of plant species. In the ornamental plant *Cleome hassleriana*, lignin composition switches from normal G-lignin to C-lignin during the development of the seed coat, and understanding this process might lead to strategies for engineering C-lignin in plants. Transcriptome analysis coupled with kinetic analysis of selected recombinant enzymes showed that the switch to C-lignin formation was accompanied by down-regulation of transcripts encoding caffeoyl CoA- and caffeic acid 3-*O*-methyltransferases (CCoAOMT and COMT) and a normal cinnamyl alcohol dehydrogenase (CAD4) with preference for coniferaldehyde over caffealdehyde, and up-regulation of a form of CAD (CAD5) with kinetic preference for caffealdehyde. In cinnamoyl-CoA reductase (CCR1) and CAD4/5 coupled reactions, the substrate preference of CAD5 for caffealdehyde overcomes the preference of CCR1 for feruloyl CoA. Analysis of transgenic *Cleome* with down-regulated CAD5 supported the functional significance of CAD5 for C-lignin biosynthesis. Structure modeling and mutational analysis revealed important residues for the preference of CAD5 for caffaldehyde. The present data suggest that CAD specificity is important for C-lignin biosynthesis, and provide initial insights into potential structure-based re-design of CADs for C-lignin engineering.

S82 Metabolic engineering of methanotroph for conversion of methane into 2,3-butanediol

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Methane is regarded as a next-generation feedstock, and methanotrophs have the ability to produce a variety of valuable chemicals from methane. For production of 2,3-butanediol from methane, we engineered methanotroph strain based on *in silico* simulation. Metabolic pathway for production of 2,3-butanediol was introduced into methanotroph and triple-mutant strain Δldh Δack Δmdh was also constructed to enhance the titer of 2,3-butanediol. Bioreactor system was also used for production of 2,3-butanediol with the regulation of gas composition.

S84 The challenges in quantifying cellulose accessibility and the potential of enzymes to produce nanofibrillated cellulose as well as enhanced biomass deconstruction

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In addition to sequestering and storing carbon, trees have been used to build centuries old wooden building as well as providing many of the pulp, paper, packaging and renewable energy products that are used by our global society. Unlike starch, nature has designed cellulose as a structural material that has proven difficult to degrade enzymatically. One of the rate-limiting steps in biomass deconstruction involves the limited accessibility of the cellulase enzymes to the cellulosic substrate. Our group, as well as others, have assessed and developed various ways to try to quantify cellulose accessibility, both after pretreatment/pulping and after enzyme mediated amorphogenesis. As will be described in the presentation, various established pulp and paper methods (e.g. Water Retention Value, aspect ratio, fibre quality analysis, etc.) were combined with methods such as Simons' stain and selective Cellulose Binding Module (CBM) adsorption to get a better understanding of the mode of deconstruction/fibre modification that occurs with different enzyme cocktails on various pretreated biomass substrates. Typically, nanofibrillated cellulose (NFC) is made by the mechanical refining of softwood derived Kraft pulps. This retains the longer, stronger fibers while encouraging increase fibrillation that enhances the fiber networks properties. In the work that will be presented, we have assessed various chemical, mechanical and enzyme mediated ways of enhancing cellulose fibrillation as well as better understanding the mechanisms involved in achieving effective deconstruction of biomass. Potential applications of modified NFC, such as increasing their hydrophobicity to aid as reinforcement agents will also be discussed.

S83 Influence of non-enzymatic proteins and air-liquid interface on adsorption of cellulase on sugarcane bagasse lignin.

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The focus of this work was to study the influence of non-enzymatic proteins (BSA and Soy Protein) and air-liquid interface in the adsorption of endoglucanase from a commercial preparation (Celluclast 1.5) on lignin derived from acid or enzyme hydrolyzed liquid hot water pretreated sugarcane bagasse (SBL - 190°C, 20 min). The enzymatic adsorption experiments were carried out through three different experimental designs (2^3) during 48 hours in Erlenmeyer flasks at 50°C and 150 rpm. The variables were the air-liquid interfacial area (measured by working with 25 or 250 mL flasks), the presence or not of acid or enzymatic lignin and the presence or not of a non-enzymatic protein. The response was endoglucanase activity measured using 1% (w/v) carboxymethyl cellulose. The results showed the acid isolated lignin is very reactive and quickly adsorbed almost all endoglucanase present in 25 or 250 mL flasks. In the case of acid lignin, the presence of BSA reduced by 80% the enzyme adsorption, and the air-liquid interfacial area was not a significant factor. In the presence of enzymatic isolated lignin, the endoglucanase adsorption was lower (30%) over a period of 48h using the 25 mL flask. The air-liquid interfacial area had an effect in this case, and more endoglucanase was adsorbed (75%) using higher volume flasks. The presence of BSA or soy protein reduced absorption by 70 and 40%, respectively, for the 25 and 250 mL flasks.

S85 Optimization and characterization freeze dried fish protein hydrolysate production

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Fish waste is a good source of protein; however, a large amount of fish by-product is currently disposed from processing and used for low-value products. This research is to study the optimization for fish hydrolysate production from 5 fish waste strains. Five strains of fish as *Rastrelliger brachysoma* (short-bodied mackerel), *Rastrelliger kanagurta* (Indian mackerel), *Leiognathidae* (Ponyfish), *Amblygaster leiogaster* (Smooth belly sardinella), and *Selaroides leptolepis* (yellow-stripe scad) were investigated for the degree of hydrolysis using Alcalase™ through Central Composite Design. Response surface methodology was employed to optimize for hydrolysis conditions, including the effects of time, temperature, pH and enzyme to substrate ratio (E/S) on the degree of hydrolysis. The optimized condition of 89.42% degree of hydrolysis was obtained from 2.85% v/w of E/S ratio at 61°C, pH 8.5 for 27 min. Mathematical model from response surface methodology can be predicted degree of hydrolysis quite well because of detecting 11% error from validation. Lower than 10 kDa of molecular weight were detected and showed significantly antioxidant activity and the sequence of peptides lower than 3 kDa were identified by MALDI-TOF mass spectrometry and LC/MS-MS analysis. Freeze dried fish protein hydrolysate revealed that histidine and lysine were two predominant amino acids with respect to human requirement while triple arginine ratio gained from the optimization condition. Based on amino acid compositions, the waste fish hydrolysate showed nutritional value and high potential for applications as the supplement in animal feeds.

S86 Impact of feedstock attributes on the performance of processing and conversion in cellulosic ethanol applications

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The feedstock supply system predominately utilized in the production of cellulosic biofuels, is typically designed around a single biomass type available within a small supply radius. While the limited supply area reduces the cost of transporting the biomass, there is little opportunity to buffer against the wide variability of physical and chemical properties that may be encountered during processing and conversion. Feedstock variability is a major contributor to delays and disruptions in the preprocessing and conversion systems. The use of discrete event simulation (DES) was chosen to improve upon classical approaches that utilize a sequential approach to the analysis production systems and considering interactions across the system. To examine the impact of feedstock variability on processing and conversion performance, DES models were developed for preprocessing operations of a herbaceous feedstock supply system, and included feedstock property based equipment performance, equipment failure modes (biomass properties causing equipment failures), and the expected time to repair or correct these failures. Initial models were developed to represent a commercial scale biorefinery (focusing on preprocessing and infeed to conversion reactor), with failure and repair information based on industrial operational experience. Initial runs of the models resulted in a capacity utilization of 29.1% of the design capacity. After the initial models, data on conversion performance was also included to integrate aspects of both chemical and physical performance into the model. The performance of the physical system was based on actual data from a pilot processing facilty, which produced similar results as the initial simulations and had a capacity utilization factor of 28.9%. Based on the feedstock property chemical variation, resulted in a yield factor of 88.1%. The methodology of combining chemical and physical performance of individual units of variable feedstock material, has provided a way to examine biorefinery operational performance in relation to variability; and allows for the definition of a new metric, termed "Operational Reliability". Operational reliability combines capacity utilization with feedstock yield performance to provide an overall picture of how well the system operates. Ultimately the final operational reliability of was found to be 25.5%.

S87 Surface Energy Characterization of Air Fractionated Corn Stover

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Despite decades of research, Integrated Biorefineries (IBRs) are still not commercially viable because of the long-standing, unresolved challenges related to biomass solids handling and transport (e.g., flowability & fouling), and pretreatment e.g., wettability & conversion). The compendium of challenges facing IBRs lead to operational reliability and time-on-stream estimates of 30%, yet economically viable IBRs require greater than 90% operational reliabilities. The performance of commercial solids handling operations is largely dependent on the fundamental thermodynamic property, surface energy. Combining surface area

and inverse gas chromatography analyses, we show the observed differences in surface energy (work of cohesion) for anatomically air fractionated corn stover. This study highlights the importance of understanding and tuning biomass surface energy to improve IBR solids handling and transport, and pretreatment operations.

S88 Techno-economic-environmental analysis of vinasse biogas on different sugarcane biorefinery configurations

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Anaerobic digestion (AD) of effluents in sugarcane biorefineries may improve the energy balance in the bioethanol production process and the environmental suitability of wastes disposal. This study analyses the inclusion of AD of vinasse (stillage) into different configurations of sugarcane biorefineries, using technical, economic and environmental metrics. The three base-case scenarios are a first generation (1G) ethanol plant, an integrated first and second generation (1G2G) ethanol plant with alcoholic fermentation of the hemicellulose (C5) fraction, and an 1G2G ethanol plant with biodigestion of the C5 fraction. These three base scenarios are compared with the similar ones including the biodigestion of vinasse. The implementation of vinasse biodigestion had positive impact on the productivity and sustainability of sugarcane biorefineries. The use of biogas from vinasse for a 1G ethanol plant increased in 9.20% the surplus of electric energy yielded to the grid. This scenario had a positive net present value, NPV: $+11.5 \cdot 10^6$ USD, assuming minimum acceptable rate of return, MARR=11%. For the 1G2G+C5 fermentation scenario the NPV was $+4.63 \cdot 10^6$ USD. Assuming NPV=0, the IRR for 1G becomes 19.7% and for 1G2G+C5, 13.6%, an IRR higher than the MARR. The inclusion of AD of vinasse when the C5 fraction is biodigested was not an economically feasible option. In all scenarios, vinasse biodigestion reduced environmental impacts, and the 1G2G processes exhibit better results than the consolidated 1G for almost all environmental impact categories. The AD of vinasse can be considered an environmental-friendly and economical-feasible technology to improve the process in sugarcane biorefineries.

S89 CO_2 driven acetoin production by a synthetic acetogenic bacterium

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Acetogenic bacteria are considered to be the most efficient microorganism for fixing C1 compound as they gain energy from operating the pathway, in contrast to the other C1 compound-fixing bacteria that spend energy during the uptake. Acetogenic bacteria are present in 23 different genera with over a hundred strains isolated from diverse habitats. The Wood-Ljungdahl pathway in the microorganism converts C1 into acetyl-CoA, which is an important cellular precursor that is converted into biochemicals. Despite the potential to reduce C1 compound in the atmosphere and industrial waste gases, lack of a systemic understanding, complex layers of regulation system, and inefficient electron delivery has limited the construction of a cellular factory optimized for producing the desired chemical. To overcome the limitation, molecular level insight has been obtained via multi-layered genome-scale analyses. The results indicate that majority of genes associated with autotrophic growth including the Wood-Ljungdahl pathway, the reduction of electron carriers, the energy conservation system, and gluconeogenesis were transcriptionally upregulated. The translation efficiency of genes in cellular respiration and electron bifurcation was also highly enhanced. In contrast, the transcriptionally abundant genes involved in the carbonyl branch of the Wood-Ljungdahl pathway, as well as the ion-translocating complex and ATP synthase complex in the energy conservation system, showed decreased translation efficiency. The translation efficiencies of genes were regulated by 5′UTR secondary structure under the autotrophic growth condition. The results illustrated that the acetogenic bacteria reallocate protein synthesis, focusing more on the translation of genes for the generation of reduced electron carriers via electron bifurcation, rather than on those for carbon metabolism under autotrophic growth. Finally, integration of the information-rich data types with synthetic biology tools facilitates the construction of optimal C1 fixing and biochemical-producing cellular factories.

S90 Physicochemical characterization and performance of immobilized cells systems based on alginate for production of propionic acid from xylose by *Propionibacterium Acidipropionicii*

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The dependence from petroleum by several sectors of economy has shown itself economical and environmentally unsustainable. Alternative sources of energy and chemicals, besides new processes to generate them, has prompted academic and industrial scientific activity. Biotechnological production of chemicals from lignocellulosic biomass allied with other bioprocesses for production of first and second generation bioproducts and energy recycling integrates the concept of biorefinaries. On that context, the biotechnological production propionic acid, a substance largely applied on food and chemical industries, from lignocellulosic materials, has been focused on some scientific works. On that study, the performance of immobilized *Propionibacterium acidipropionicii* on production of propionic acid from comercial xylose was evaluated, as well as, the physicochemical properties of the alginate beads, employed as immobilization medium. Batch fermentation was runned along 72 h, on 300.0 mL TSB medium enriched with 40.0 $g.L^{-1}$ of xylose. Organic acids production besides xylose consumption was monitored by HPLC analysis. Physicochemical properties of alginate beads, contenting cells or not, were also analyzed. Beads size and shape, density and porosity were determined. Beads size and density was about 4.7 mm and 1.03 $g.cm^{-3}$. After 72 h,

it was obtained an end title of 21.8 $g.L^{-1}$ of propionic acid, 9.1 $g.L^{-1}$ of succinic acid and 11.3 $g.L^{-1}$ of acetic acid Residual xylose concentration was about 0.27 $g.L^{-1}$. The results showed that immobilized *P. acidipropionicii* strain is capable of producing propionic acid at reasonable concentrations. Mass transfer of components, as the pH, were major issues of concerning on application of that fermentation.

S91 A review of biofuels polices in the key biofuels producing countries

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This study compares and contrasts the biofuels polices in 15 major biofuels producing countries in the Americas (North and South), Europe Union and Asia Pacific. This study examines biofuel policies being implemented in these countries and the extent to which they have been effective. These polices include biofuels obligations and mandates, excise duty reductions, fiscal incentives, investment subsidies and other measures stimulating the implementation of biofuels. It also assesses the measures being taken by these countries to develop or stimulate their respective biofuels industries, including incentives and investment in research, development and commercialization. The data for this study were collected from these countries through a questionnaire. The questionnaire for each country was completed by the representative of the IEA Bioenergy Task 39 member country. The completed questionnaires were complied to identify the strengths and weaknesses of the existing biofuel polices in each country. The results of this study show that policy is the still the primary driver for the production and use of biofuels in these countries. Biofuel blending mandates are still the primary biofuels policy tool. However, the success of low carbon fuel standards in California, USA and British Columbia, Canada has led to many states and even other countries looking to adopt similar programs. As a result, international, national and regional GHG reduction goals has shifted the attention from blending mandates to the carbon intensity of biofuels.

S92 Accelerating lignocellulosic anaerobic digestion using cotreatment by a ball mill

A. Bharadwaj and T. Richard, The Pennsylvania State University, University Park, PA, USA; E. Holwerda and L.R. Lynd, Dartmouth College, Hanover, NH, USA*

Lignocellulose recalcitrance has been a long-standing challenge in its use as a feedstock to produce value-added chemicals. Cotreatment, described as the mechanical disruption of lignocellulose during fermentation, has been demonstrated as an effective method to enhance the degradation of lignocellulose. Studies by Paye et al., 2016 and Balch et al., 2017 have shown enhanced biomass solubilization using cotreatment in pure culture systems (with Clostridium thermocellum to produce ethanol).

In this study, the cotreatment strategy is applied on a mixed culture system. Partially digested lignocellulosic anaerobic digestate (from a continuously running digester) is subjected to mechanical disruption using a ball mill. The material was milled for different time periods (0, 0.5, 2, 5 and 10 min), followed by a second fermentation in a batch reactor set up.

Results include measurement of the extent of biomass consumed using biogas production and sugar consumption as the metrics. Secondary measurements include particle size reduction and energy consumed by milling to provide perspective on the efficiency of the milling process.

S93 Acetylation of galactomannans affects their biodegradation by endo-mannanases

S. Malgas and B. Pletschke, Rhodes University, Grahamstown, South Africa*

Softwood hemicelluloses, particularly acetyl-galactoglucomannans, are reported to be acetylated. The biological functions of acetylation on hemicelluloses are not completely understood but their suggested function is to decrease the microbial degradability of plant cell walls. The aim of this study was to evaluate and compare the mode of action of an endo-mannanase from *Aspergillus niger* (AnMan26A) on unmodified galactomannan and galactomannan which was chemically acetylated. The unmodified and acetylated galactomannans were characterised by NMR, XRD, FTIR and for dispersibility in water. The unmodified galactomannan was more readily dispersible in water compared to the acetylated galactomannan. Chemical acetylation resulted in decreased hydrolysability of galactomannan with activity reduction as high as 94%, and we suggest that the reduced substrate solubility was responsible for the reported reduction in enzyme activity. We suspect that this then led to decreased substrate accessibility. As a result, we evaluated the adsorption of AnMan26A onto the unmodified and acetylated galactomannans. Treating the acetylated galactomannan with higher enzyme loadings of AnMan26A or with a combination of AnMan26A and acetyl xylan esterases (BhAxe12A from *Bacillus halodurans* or AxeA from *Orpinomyces* sp. (strain PC-2)) did not improve its hydrolysability. The biotechnological significance of acetylated hemicelluloses could be their use as internal plasticisers to produce slow/controlled biodegradability in biodegradable materials which are generally prone to moulding.

S94 Ternary deep eutectic solvents enable rapid high-solid biomass deconstruction

C. Wan[*] and C. Zhu, University of Missouri, Columbia, MO, USA

Ternary deep eutectic solvents (DESs) were developed to enable rapid and high-solid biomass pretreatment as well as concentrated sugar hydrolysate production. Ternary DES constituted choline chloride (ChCl)/guanidine hydrochloride (GH) as a hydrogen bond acceptor, ethylene glycol (EG)/propylene glycol (PG)/glycerin (Gly) as a poly-based hydrogen bond donor, and *p*-toluenesulfonic acid (PTSA) as an acidic proton donor. Among six combinations, GH-EG-PTSA was the most effective, which was evidenced by 79% xylan and 82% lignin removal in only 6 min at 120 ℃ and 10 wt% solid loading. Its excellent performance were further proved by high solid loading pretreatment. At a solid loading as high as 35%, both GH-EG-PTSA and ChCl-EG-PTSA removed more than 60% xylan and 60% lignin at 120 ℃ in 30 min. The pretreated switchgrass was readily hydrolysable with a low enzyme loading of 5 mg protein/g solid. Even at 20% solid loading, a glucose yield as high as 78.4% was obtained, which corresponded to 148 g/L major monomeric sugar (128 g/L glucose and 20 g/L xylose). Overall, this study demonstrated novel and high-performance ternary DESs for cost-effective lignocellulose deconstruction.

S95 Adipic acid production in yeast – addressing the challenges of the lysine pathway

L. Olsson[*], E. Skoog, J.H. Shin, V.S. Jimenez and V. Mapelli, Department of Biology and Biological Engineering, Gothenburg, Sweden

Microbial conversion of sugars to adipic acid, a platform chemical used to produce nylon, may substitute the current production from fossil raw material. A microorganism naturally producing adipic acid is not known, therefore strain engineering is required. From a careful evaluation of suggested metabolic pathways towards adipic acid, redox imbalance and conversion steps not described so far by any enzyme are key challenges.

We are focusing on the conversion pathway via lysine to adipic acid, where the original pathway harbors three enzymatic steps with non-described enzymes. Rearrangement of the enzymatic reactions for lysine conversion reduced the number of unknown enzymes to two. First, strategies for the reduction of unsaturated α,β bonds of 6-amino-*trans*-2-hexenoic acid and *trans*-2-hexenedioic acid were studied. Secondly, the deamination of lysine, and 2-aminoadipic acid was studied. In both cases our strategies focused on identifying potential enzymes that could perform the targetted reactions. A combined approach of *in vitro* and *in silico* analysis was used to understand the reactions and to form the basis for suggesting protein engineering strategies. A successful cell factory for adipic acid production also requires a carefully suggested host organism. We have screened among bacteria, yeasts and filamentous fungi to select a suitable host. After selecting *Saccharomyces cerevisiae* and *Candida viswanathii,* more in depth studies were performed to understand the basis for their different ability to handle high concentrations of adipic acid.

S96 Technoeconomic analysis of distillation strategies for dilute cellulosic ethanol produced as a bolt-on colocated at a first generation host plant

X. Liang[*] and L.R. Lynd, Thayer School of Engineering, Dartmouth College; M. Laser, Center for Bioenergy Innovation, Oak Ridge, TN, USA

When introducing new technology for cellulosic ethanol production, it is natural to consider co-location at larger host plants processing 1G feedstocks. For such “bolt-on” applications, the ethanol titer produced from cellulosic feedstocks will generally be lower than for the host plant due to solids-handling constraints, and/or the technology employed. Use of thermophilic bacteria is a case in point. Here we analyze distillation of dilute cellulosic ethanol from a bolt-on facility at a host plant processing either corn or sugar cane. Three configurations are evaluated: stand-alone distillation of the dilute stream, mixing the dilute and concentrated streams prior to distillation, and an integrated scheme in which vapor from stripping the dilute stream is introduced as a second feed to the column receiving the concentrated stream.

The incremental cost (operating plus annualized capital per gallon cellulosic ethanol) is substantially lower for the integrated configuration than for the other two configurations. For a bolt-on cellulosic ethanol plant producing 20 g/L ethanol at a corn ethanol host plant, the incremental distillation costs are 9.8, 27, and 38 cents/gallon for the integrated, mixed, and stand-alone configurations respectively. If the cellulosic ethanol titer is 35 g/L, the incremental distillation costs compared to a corn ethanol host plant are 5.0, 14, and 22 cents/gallon. Relative to a sugar cane host plant, the incremental distillation cost for the integrated configuration is 8 cents per gallon for cellulosic ethanol produced at 20 g/L and 1.7 cents/gallon for cellulosic ethanol produced at 35 g/L. For the stand-alone and mixed configurations, the incremental steam requirements per cellulosic ethanol distillate are high (> 50 % of the ethanol heating value). For the integrated configuration, however, the incremental steam requirement for separating cellulosic ethanol is *lower* than for separating concentrated ethanol at the host plant for most of the combinations of ethanol titer, flow rate, and host plant considered. This surprising result is explained in terms of heat integration benefits and reduced internal irreversibility.

S97 Improving the economics of Isopropanol-Butanol-Ethanol (IBE) production using continuous flash fermentation

E.R. Silva Dantas, R. Maciel Filho[*] and A.P. Mariano, University of Campinas, Campinas, Brazil
Production of n-butanol via the IBE route has market advantages in comparison to the more conventional ABE (acetone-butanol-ethanol) fermentation process. Isopropanol has a higher market price than acetone and can also be used as an automotive fuel. However, the IBE route suffers more severely from product inhibition. Consequently, reactor productivity is an important issue. As such, in this work, we investigated whether continuous fermentation combined with product recovery in an external vessel under partial vacuum (flash fermentation) can increase the economics of IBE production. We simulated an IBE plant that produces 30,000 ton butanol from sugarcane bagasse during a crop season of 174 days. Energy and mass balance of the plant was implemented in Excel considering data from (i) the literature (steam explosion pretreatment followed by enzymatic hydrolysis), and (ii) process simulation (fermentation and distillation). Since the product recovery allows the processing of more concentrated sugar solutions (106 against 40 g/L), IBE concentration in the beer increased by ~3 times (from 11 to 30 g/L) in relation to batch fermentors. Similarly, the stillage footprint decreased by half (from 64 to 32 m^3 stillage/m^3 IBE). Moreover, continuous operation improved productivity from 0.2 to 2.0 g/L·h. Consequently, 3 continuous fermentors connected to flash tanks can substitute for 20 batch fermentation tanks (3780 m^3 each). The investment cost of the IBE plant decreased from 57 (batch technology) to 39 (flash technology) MMUSD, and the net present value (NPV at 10% discount rate) improved from −0.2 to 25 MMUSD. Therefore, the flash fermentation technology can offer economic gains along with a remarkable decrease in equipment and stillage footprint.

S97 Techno-economic analysis and exergo-environmental performance of integrated first- and second-generation bioethanol production plants through biochemical and thermochemical conversion pathways

P. Silva Ortiz, University of Campinas-UNICAMP, Campinas, Brazil, A.P. Mariano, University of Campinas, Campinas, Brazil and R. Maciel Filho[*], State University of Campinas - UNICAMP, Campinas, Brazil
Driven by a range of bioenergy sustainability challenges, advanced conversion technologies are required to reduce costs, environmental impacts, and increase the productivity efficiency to continue the transition of lignocellulosic biofuel production from pilot scales to industrial implementation. Thus, biorefinery technologies could play an important role to produce a comprehensive range of marketable products in a sustainable way from widely available lignocellulosic residues. This study analyses the integrated first (1G) and second-generation (2G) ethanol production plants via biochemical and thermochemical pathways to improve sustainability-related indexes of sugarcane-based biorefineries in Brazil. The integrated 1G + 2G process designs (combining biochemical and thermochemical pathways) for bioethanol production from sugarcane bagasse aiming to develop a thermodynamic-based approach for integrating large resources use efficiency with advanced conversion technologies from a technical, economic and environmental perspective. Thus, several techno-economic and environmental performance parameters are using in the assessment: *i). Energy and exergy efficiency, ii). Average unitary exergy cost (AUEC), iii). Irreversibility/Exergy products ratio, iv). Global CO2 emissions, v). CAPEX and OPEX (capital and operational expenditure)*. Results regarding the technical conversion of these systems indicated that the higher exergy efficiency (37%) was presented in the integrated 1G + 2G biochemical process and consequently a lower average unitary exergy cost (AUEC=2.7 kJ/kJ). Furthermore, the global CO2 emissions was 4.04 kgCO2equiv./kg ethanol and the CO2 equivalent index in exergetic base was 149 gCO2/MJ ethanol for the biochemical process. Lastly, this process shows a reduction of 20 % on the capital investment cost in comparison with the thermochemical pathway.

S98 Modeling biological degradation and moisture migration in baled biomass feedstock storage

W.A. Smith[*], C. Quiroz-Arita, M. Plummer and L.M. Wendt, Idaho National Laboratory, Idaho Falls, ID, USA
The U.S. Department of Energy Bioenergy Technologies Office (DOE-BETO) is committed to the development of sustainable, nationwide, commercial biofuel production to displace petroleum-derived fuels, increase domestic energy production, and encourage the creation of a domestic bioenergy and bio-products industry. Reliable and robust biomass supply systems are necessary to realize this vision. Research at Idaho National Laboratory (INL) focuses on improvements to supply chain logistics operations—harvesting, storing, milling, handling, and feeding—that enable efficient conversion processes. Biomass variability, in the form of moisture content, composition, and conversion yield, poses a significant challenge to industrial scale biomass utilization. Exposure to moisture while harvesting and storing biomass results in varying extents of physical and chemical degradation over time. Research to date indicates that aerobic biological degradation leads to internal heating (> 65°C), carbohydrate loss (>20%), and moisture migration in stored corn stover bales. The rates and extents of degradation, moisture migration, and dry matter loss have been measured under specific field conditions and under a range of controlled conditions in the laboratory. These data have been used to create 1-D and 2-D numerical simulations describing airflow, microbial respiration, dry matter loss, self-heating, and moisture migration in baled corn stover. These coupled computational models provide a means of examining moisture loss and dry matter stability in a variety of simulated storage configurations and environmental conditions and evaluate how these conditions may be modified to improve storage stability and enhance moisture loss over time. Preliminary results indicate that modest energy inputs such as biological heating, ambient wind loading, and diurnal temperature changes have the potential to enhance moisture loss in baled corn stover. The moisture loss over time serves to improve biological stability and reduce the moisture content of bales exiting storage from 30% to <20% over 180d in storage with < 5% dry matter loss in the current simulations. Results will show how the models perform under four simulated climate conditions within the U.S. Corn Belt.

Monday, April 29

7:00 AM - 8:00 AM Speaker Breakfast-Speakers and Conveners on date of your session

Duwamish - Room 306, Third Level

7:00 AM - 5:00 PM Registration

Columbia Ballroom Foyer, Third level

Registration

8:00 AM - 11:25 AM Session: 1: Lignocellulosic Feedstocks

Conveners: Janet Westpheling, University of Georgia, Athens, GA, USA and **Troy Semelsberger**, Los Alamos National Laboratory, Los Alamos, NM, USA

Columbia C, Third level

8:00 AM 1-1: Structural insights into low and high recalcitrant natural poplars with neutron and X-ray scattering:

R. Shah and S. Bhagia, University of Tennessee, Knoxville, Knoxville, TN, USA; S.V. Pingali, A. Ragauskas, B. Davison and H. O'Neill, Oak Ridge National Laboratory, Oak Ridge, TN, USA*

Structural changes leading to biomass recalcitrance is crucial to study for future development of biofuels and bioproducts. A genome wide association study identified two naturally occurring poplars, BESC-316 and GW-11012, that had 22% and 18% lignin content, respectively. The lower lignin content genotype showed greater sugar release before and after hot water pretreatment compared to the lower lignin counterpart. We used small-angle neutron scattering (SANS) and wide-angle x-ray scattering (WAXS) to investigate the structural changes in the BESC-316 and GW-11012 genotypes that were subjected to hot water pretreatment (160˚C 70min, 180˚C 18min and 180˚C 45min) to obtain information about cellulose microfibril organization, the lignin and hemicellulose network, and overall morphology of the plant cell walls. Cellulose microfibril arrangement in GW-11012 is consistent with aggregated microfibrils and differed significantly from the well-ordered cellulose microfibrils in BESC-316 before pretreatment. Post-pretreatment, little change was seen in cellulose arrangement for GW-11012 whereas BESC-316 showed aggregation of microfibrils. SANS showed that GW-11012 had increased scattering intensity in the mid Q region compared to BESC-316 which may be due to pores. After pretreatment, both genotypes have very similar scattering patterns indicative of similar structural changes occurring in the pretreated cell walls. Cellulose accessibility measured using the modified Simons' stain before and post pretreatment was similar for GW-11012 and BESC-316. No significant difference in the amount of 5-hydroxymethyl furfural, furfural and acetic acid for BESC-316 and GW-11012 was observed in the pretreatment liquor. We will discuss differences in sugar release of the native and pretreated BESC-316 and GW-11012 due to the variations in the cellulose microfibril arrangement, fibrillar orientation, porosity and lignin content.

8:25 AM 1-2: A method for in-liquid, label-free, chemical imaging of lignocellulosic substrates using Coherent Anti-stokes Raman Scattering

S. Vilms Pedersen, M.A.B. Hedegaard and E.C. Arnspang, University of Southern Denmark, Odense M, Denmark; S.D. Hafner, Aarhus University, Aarhus, Denmark*

Rate limitations related to enzymatic deconstruction of cellulose in lignocellulosic matrices are a major challenge in the production of chemical commodities from lignocellulosic substrates. A significant body of literature points towards not only an intrinsic cellulose recalcitrance, but also recalcitrance of the entire lignocellulosic matrix. Nonetheless, only little research has gone into studying molecular-level mechanisms of enzymatic deconstruction in lignocellulosic substrates. Conducting such studies using conventional fluorescence approaches is complicated. Visualizing the various biochemistries in lignocellulosic substrates will rely on auto-fluorescence or applying chemically specific fluorescent stains, both of which are susceptible to photobleaching. Another issue is that dyes, such as Congo Red, adsorbs to the surface of cellulose, and how this mechanistically affects surface-active and processive enzymes in the lignocellulosic matrix, is unclear.

In this work we have used Coherent Anti-stokes Raman Scattering (CARS) to collect hyperspectral images of *B. napus*, a model lignocellulosic substrate, to develop a chemical imaging protocol allowing label-free determination of major biochemical constituents in lignocellulosic matrices. An inherent drawback of chemical imaging methods is that during in-liquid imaging, some buffers will yield Raman signals in the same spectral regions as many polysaccharides and lignin. During this oral

presentation we will present our work on the label-free imaging methodology, unmixing of hyperspectral information correlating to different biochemistries, image reconstruction, and of utmost importance, methods for removing the buffer background from hyperspectral data cubes. We hope the method will inspire more substrate deconstruction studies with canonical cellulases on lignocellulosic matrices, free from substrate labelling.

8:50 AM 1-3: Increasing sustainability and resilience of bioenergy feedstocks using the plant microbiome

S. Doty, A. Sher, A. Firrincieli, H. Rho, Z. Khan, P. Joubert, M. Aghai, G. Ettl and S.H. Kim, University of Washington, Seattle, WA, USA*

The plant microbiome can profoundly impact plant growth, health, and resilience to abiotic and biotic stresses. Endophytes, the microorganisms within plants, can increase nutrient acquisition, produce antimicrobial compounds against pathogens, improve water use efficiency and drought tolerance, and detoxify environmental pollutants. We demonstrated N-fixation in the important bioenergy plant species, poplar (Populus). A consortium of the N-fixing strains was added to hybrid poplar, increasing growth and N-fixation under greenhouse conditions. Not only did the microbes impact this important bioenergy plant species, they also increased growth, health, and yields of an exceptionally broad range of plant species, including grasses and conifers under nutrient-limited conditions. Inoculation of plants with the endophytes also improved water use efficiency, drought tolerance, and rooting. Increased health, growth, and phytoremediation success was achieved through inoculation of poplar with a TCE-degrading endophyte at a contaminated site. By using natural plant-microbe interactions to reduce the need for chemical fertilizers and fresh water, and to detoxify pollutants, the environmental range of biomass production can be increased with fewer inputs, potentially improving the economics of feedstock production.

9:15 AM Break

9:45 AM 1-4: Field testing of engineered switchgrass with enhanced biomass saccharification and improved yield

M.Y. Lee, A. Eudes, T. Scavuzzo-Duggan, C.Y. Lin, J. Ortega, J. Mortimer and H.V. Scheller, Joint BioEnergy Institute, Lawrence Berkeley National Laboratory, Emeryville, CA, USA; G. Li and P. Ronald, Joint BioEnergy Institute, Emeryville, CA, USA; B. Perez, University of California Davis, Davis, CA, USA; C. Scown and D. Putnam, Joint BioEnergy Institute, Berkeley, CA, USA*

Switchgrass (Panicum virgatum L.) is a promising bioenergy feedstock because it can be grown on marginal land and produces abundant biomass. Recalcitrance of the lignocellulosic components of the switchgrass cell wall to enzymatic degradation into simple sugars impedes efficient biofuel production. We conducted the first year field study using two independently transformed lines of switchgrass overexpressing OsAT10, a BAHD acyltransferase affecting ferulic acid esters in biomass, and three independent transformant lines expressing QsuB, a dehydroshikimate dehydratase from Corynebacterium glutamicum. These engineering strategies result in increased saccharification efficiency and low lignin and ferulic acid esters according to our previous studies in switchgrass and other plant species. The plants grown in the field showed changes in biomass composition in general agreement with previous results, although the effects were smaller. Surprisingly, the plants expressing QsuB had higher biomass yield than control plants. Arabidopsis plant expressing QsuB have been shown to exhibit substantial drought tolerance. The drought response of the corresponding switchgrass plants will be reported.

10:10 AM 1-5: Genetic targets for lignin engineering and valorization in lignocellulosic feedstocks

R.A. Dixon, J. Barros-Rios, C. Zhuo, C. Man Ha and C. Liu, BioDiscovery Institute; L. Gallego-Giraldo, Center for Bioenergy Innovation*

High lignin content correlates with the recalcitrance of biomass to enzymatic saccharification during biofuel production and with reduced in-rumen digestibility of forages. As a result of many years work on the biosynthesis of lignin and its genetic controls in forage legumes, collaboration with a commercial partner has resulted in the release of low lignin alfalfa with superior forage quality and management characteristics. However, economic considerations support valorization, rather than simple removal, of lignin for biorefining. To this end, we have been working on the biosynthesis and engineering of novel lignins, such a C-lignin, with favorable properties for conversion to bioproducts, and these studies are revealing new twists to the previously accepted pathways for biosynthesis of lignin. Unfortunately, lignin modification often results in negative growth phenotypes, and we will describe recent studies that ascribe such effects to ectopic activation of defense responses through signaling initiated by cell wall remodeling.

10:35 AM 1-6: Genetic and genomic improvement of *Populus* feedstocks for biofuels and bioproduct production

J. Zhang, M. Xie, K. Feng, S. Jawdy, L. Gunter, W. Muchero and J.G. Chen, Oak Ridge National Laboratory, Oak Ridge, TN, USA; G. Tuskan, Center for Bioenergy Innovation, Oak Ridge, TN, USA*

Lignocellulosic biomass is a renewable source for conversion into biofuels and bioproducts. *Populus* is the primary perennial woody species that is of special interest as a biofuel feedstock. Current grand challenge is to make *Populus* economically

competitive to achieve cost-effective, sustainable production and conversion of *Populus* biomass into biofuels and bioproducts. To address this challenge, we take several different approaches to select or generate *Populus* feedstocks with desired characteristics including biomass yield and cell wall chemistry. We screened a population of 1,100 *Populus trichocarpa* natural variants for enhanced biofuels conversion with increased biomass productivity and have selected several superior natural variants with these desired traits. Through Genome-Wide Association Studies (GWAS), we have identified genetic loci that are highly associated with these phonotypic traits. We further applied genome-editing tools to modify these genetic loci or generated *Populus* transgenic plants to genetically manipulate the expression of these loci to both validate the GWAS findings and to create new *Populus* feedstocks with desired traits. Transcriptomics, metabolomics, expression Quantitative Trait Locus mapping, and molecular and biochemical analyses are being used to gain mechanistic understanding of the linkages between genetic loci and associated phenotypic traits.

11:00 AM 1-7: Fine-tuning feedstock quality and fundamental understanding of pectin structure-function relationships – a yin and yang story

M.A. Atmodjo, A.K. Biswal, S.S. Mohanty, D. Ryno, R.A. Amos and K.A. Engle, Complex Carbohydrate Research Center, Athens, GA, USA; D. Mohnen, Center for Bioenergy Innovation, Athens, GA, USA*

Lignocellulosic biomass, a renewable resource for the production of fuels, chemicals, and other bio-based products, is made up primarily of plant cell walls. Engineering improved lignocellulosic feedstock requires a fundamental understanding of the plant cell walls, which are complex composites of multiple types of polysaccharides, proteins, and polyphenolics present in different amounts and architectural arrangements in different cell types and plant species. The main components of lignocellulosic biomass, i.e. cellulose, hemicellulose, and lignin, have been the main targets of feedstock improvement efforts. However, more and more evidence shows that pectin, the most complex polysaccharide of cell walls, can also be a major target for biofeedstock improvement. The question addressed in this talk is how the modification of a relatively minor component in biofeedstocks, pectin, can lead to both increases in plant growth and wall deconstruction potential. This talk will show how cell wall structural analyses of improved feedstock reveal that: (1) desired improvement of growth and cell wall integrity requires precise regulation of pectin content; (2) not all pectins are equal – i.e. even within one type of pectin there are different subclasses of pectic polymers; and (3) these different pectic subclasses appear to be synthesized by different members of the same gene family. The presentation will illustrate how a fundamental understanding of pectin structure-function relationships is critical for fine-tuning feedstock quality.

This project was supported by the Center for Bioenergy Innovation, which is a US Department of Energy Bioenergy Research Center supported by the Office of Biological and Environmental Research in the Department of Energy's Office of Science.

8:00 AM - 11:25 AM Session: 2: Biopolymers and Biomaterials

Conveners: Tao Dong, NREL and **Jason Hallett**, Imperial College

Columbia D, Third level

8:00 AM 2-1: Circular economy towards improved sustainability of bioplastics and biomaterials - Green packaging to ecofriendly light-weight auto-parts

A. Mohanty, University of Guelph, Guelph, ON, Canada*

Renewable resource-based materials at one of the cornerstones of addressing climate change and increasing greenhouse gas (GHG) emissions. Sustainable materials and products today target not only bio-based, but also waste-based resources, becoming a part of the global race that can give manufacturers a competitive advantage. Plastic and food wastes are our new feedstocks. Researchers at University of Guelph's Bioproducts Discovery and Development Centre (BDDC) are developing several innovative biomaterials for uses in green packaging, consumer products and light-weight auto-parts. We have engineered hybrid biocomposites using plastics (from bio/petro-based or recycled plastics) in combination with food wastes, biofuel co-products, natural fibres, perennial grasses and pyrolyzed biomass. Our strategy is to engineer new matrix system of polymer blends, with reinforcement through mixing two or more fiber/filler to optimize the performance requirements. Our process of biomass and waste pyrolysis created a new biocarbon resource for novel composite materials well suited for use as a light-weight filler, compared to denser mineral fillers like talc and short synthetic glass fibers. These light-weight biocomposites are well suited for automotive applications. The material chemistry, filler-matrix adhesion and process engineering were key principles in the development of a number of biocomposites for industrial applications, some already available commercially.

ACKNOWLEDGEMENTS

Ontario Ministry of Agriculture, Food, and Rural Affairs (OMAFRA); Agriculture and Agri-Food Canada (AAFC) and Competitive Green Technologies through AgriInnovation Program; Natural Sciences and Engineering Research Council (NSERC) of Canada - Discovery Grants; Ontario Ministry of Research and Innovation (MRI) through the Ontario Research Fund, Research Excellence Program (ORF-RE07).

8:25 AM 2-2: Studying suitability of different lignins as bisphenol-A replacement in epoxy resins

M. Nejad, N. Chen and A. Gondaliya, Michigan State University, East Lansing, MI, USA*
The phenolic structure of lignin makes it a good candidate for replacing bisphenol-A (BPA) in epoxy resin formulation. However, it is not known how different lignin properties will affect its reactivity toward epichlorohydrin (ECH). We measured physical, chemical, and thermal properties more than twelve different lignin samples from various sources (hardwood, softwood, bagasse, and wheat straw), and isolated through different extraction processes (kraft, soda and organosolv). Then the reactivity of these lignins with a biobased epichlorohydrin were measured following a modified version of ASTM D1652 test method. Additionally, the curing kinetic of epoxidized lignins with a commercially available curing agent were studied using differential scanning calorimetry (DSC). The measured amount of unreacted ECH will be used as a function of lignin reactivity to study correlation between lignin properties and its reactivity with ECH. The developed partial least square regression model will help us to predict the reactivity of any new lignin sample with ECH, thus its suitability for epoxy resin applications.

8:50 AM 2-3: Tailoring lignin chemistry to enable value-added products for sustainable and viable biorefienry

Q. Li, Texas A&M University, College Station, TX, USA, Z. Liu, Texas A&M University, College station, TX, USA, N. Hao, University of Tennessee, Knoxville, TN, USA, B. Yang, Washington State University, Richland, WA, USA, A. Ragauskas, Oak Ridge National Laboratory, Oak Ridge, TN, USA and J.S. Yuan, Synthetic and Systems Biology Innovation Hub, Texas A&M university, College Station, TX, USA*
The success of a modern biorefinery heavily depends on the availability of value-added product streams from lignin. Nevertheless, the quality and yield of lignin-derived products heavily depends on the fundamental understanding of lignin chemistry. In particular, there is an imperative need to define the relationship between lignin chemical characteristics and performance of products like carbon fiber, asphalt binder, and nanoparticles. In the past several years, our multidisciplinary team has significantly advanced the understanding of how to tailor lignin chemistry to improve the performance of lignin-based products. For carbon fiber, we have revealed that carbon fiber mechanical and conductive performance heavily depends on the molecular weight, uniformity, chemical linkages, and functional group profile of lignin. The chemical characteristics determines the miscibility of lignin with guest polymer and thus impacts the crystallite content and size, resulting in carbon fibers with various performance. For nanoparticles, we have shown that the functional groups, chemical linkages, molecular weight, S/G ratio, and lignin condensation all could impact the hydrogen bond networks and electron double layers, which could in turn define the size, uniformity, and stability of lignin nanoparticles. For asphalt binder modifiers, the functional groups and molecular weight also impact the interaction with asphaltene, thus defining the high temperature and low temperature performance of asphalt binder. Overall, lignin chemistry is crucial for the performance of various high value products. The fundamental understanding transformed the biorefinery design, where new pretreatment, fractionation, and feedstock development strategies are being developed to tailor lignin chemistry toward best-performing lignin-based products.

9:15 AM Break

9:45 AM 2-4: Fully renewable non-isocyanate polyurethane polymers produced from bio-based Llpids and amines

T. Dong, E. Dheressa, L.M.L. Laurens and P.T. Pienkos, National Renewable Energy Laboratory, Golden, CO, USA; A. Prates-Pereira, University of Bath, Bath, United Kingdom*
The plastic market has seen a growing interest for polyurethane materials with the global polyurethane market size expected to grow up to 24 million tons by 2022. Traditionally, polyurethanes have been produced from petroleum-based products via polyaddition of polyols with isocyanates. The latter are directly produced from the corresponding amines and phosgene, and both phosgene and the isocyanates are highly toxic. Therefore, alternative pathways for PUs production have become increasingly attractive. Recently, numerous efforts have focused on the development of green chemistry approaches for replacing isocyanates in polyurethane manufacturing. The reaction of cyclic carbonates with amines has been identified as a practical alternative route to produce non-isocyanate polyurethanes (NIPUs). The additional benefit of the carbonate route is the utilization of carbon dioxide, allowing for carbon capture, sequestration, and reuse to further reduce the greenhouse gas footprint. In this research we will present the production of several fully renewable NIPUs, derived mainly from plant/algal lipids and bio-based diamines. The synthesis processes and the performance of the produced NIPUs will be compared and discussed, aiming to provide more insights to facilitate the commercialization of the NIPUs.

10:10 AM 2-5: A combinatorial and scalable industrial platform for diverse fatty acid-derived bioproducts

A. Schirmer, REG Life Sciences, LLC, South San Francisco, CA, USA*
Built on the highly efficient microbial catalyst engineered to produce over 150g/L of fatty acid methyl esters and detergent alcohols developed by LS9, Inc, REG Life Sciences has developed an expanded palette of efficient and exchangeable biosynthetic modules that provide access to over 15,000 compounds, each defined by a unique and logical combination of genetic parts. We have most recently focused this platform for the selective production of difficult to access commercial products, high-value performance compounds, and as an innovation platform for the discovery and development of novel materials. This talk will focus on the technical development of the platform and its tuning for the selective production of C8 and

C10 compounds, C12 and C14 performance molecules, and a platform for flavor and fragrance innovation. From ideation to tons, a combinatorial biosynthesis platform built on microbial fatty acid metabolism is delivering.

10:35 AM 2-6: LPMO-assisted preparation of oxidized nanocellulose with high carboxyl content from tunicate biomass

A. Karnaouri, L. Matsakas, 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; B.J. Sánchez and A. Mathew, Stockholm University,, Stockholm, Sweden; P. Moritz, O. Hoefft and W. Maus-Friedrichs, Technical University Clausthal, Clausthal-Zellerfeld, Germany; G. Sourkouni - Argirusi, Clausthal Centre of Material Technology, Clausthal-Zellerfeld, Germany*

The tunicate species *Ciona intestinalis* is a fast-growing marine invertebrate animal which contains cellulose in its outer part – the tunic. The high crystallinity and the high microfibrils aspect ratio of tunicate cellulose indicate its excellent chemical and material applications. Oxidized nanocelluloses have gained much attention in the biomedical field due to their antimicrobial properties, but also as scaffolds for the grafting of various molecules to produce modified materials. Lytic polysaccharide monooxygenases (LPMOs) are biocatalysts that are able to oxidatively cleave the glycosidic bonds of a polysaccharide substrate by introducing carboxylic groups. In the present work, tunic from *C. intestinalis* was used as a feedstock for the isolation of nanocrystals, either with or without treatment with an LPMO of AA9 family from *Thermothelomyces thermophila*. Tunic was subjected to organosolv pretreatment, followed by bleaching and acid-hydrolysis steps for the isolation of nanocrystals. Non-bleached pulps could not be processed whereas non-bleached pulps treated with LPMOs allowed the production of good quality nanocrystals. Structural modifications were investigated by scanning electron microscopy (SEM), atomic force microscopy (AFM) and dynamic light scattering (DLS). X-ray photoelectron spectroscopy (XPS) was used to evaluate the presence of oxidized cellulose following the LPMO treatment, demonstrating a significant increase in the atomic percentage of the C=O/ O–C–O and O–C=O bonds. The results prove that LPMOs are promising candidates for enzymatic modification of cellulose fibers including the preparation of nanocelluloses and offer great perspectives for the production of novel bio-based functionalized nanomaterials.

11:00 AM 2-7: Novel tunable nano-structured biodegradable plastic blends: From research to application

M. Misra, F. Wu and A. Mohanty, University of Guelph, Guelph, ON, Canada*

With the short lifetime of disposable plastics and their low recycling rate there are growing concerns on their environmental impacts. In addition, the continued growth in production volumes and unsustainable fossil resources to generate these plastics augment these issues. We argue that bio-based polymers derived from renewable resources and 'biodegradable' polymers can provide a platform as "green" plastics to reduce the environmental burden. Targeting to fabricate high-performance biodegradable plastics with properties comparable to the "conventional" plastic, such as acrylonitrile butadiene styrene (ABS) via low-cost and versatile polymer blending, novel tunable super-tough binary, ternary and quaternary blends from polylactide (PLA), polybutylene succinate (PBS), polybutylene adipate terephthalate (PBAT) and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) were successfully engineered. Different morphologies such as nano-structuring, core-shell dispersion and co-continuous are structured in the final blends via composing the biodegradable polymers with different surface tensions. Interfacial adhesion was improved by in-situ compatibilization technology in reactive extrusion. The developed biodegradable blends show properties comparable to convention plastics such as polypropylene (PP) and ABS and are expected to substitute them. The blends are suitable for varies applications from packaging to automobile. In-situ reaction, morphologies and interfacial compatibility play key roles in developing such high-performance biodegradable blends for industrial applications.

Acknowledgements:
Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA)/University of Guelph; Agriculture and Agri-Food Canada (AAFC) and Competitive Green Technologies through AgriInnovation Program project; Ontario Ministry of Research and Innovation (MRI) through the Ontario Research Fund, Research Excellence Program (ORF-RE07).

11:25 AM - 1:00 PM Lunch-on your own

1:00 PM - 4:25 PM Session: 3: Physical, Thermal, and Chemical Deconstruction

Conveners: Venkatesh Balan, University of Houston, Houston, TX, USA and **Prof. Bin Yang**, Washington State University, Richland, WA, USA

Columbia C, Third level

1:00 PM 3-1: Previously unknown nanoporous celluloses as the best primary feedstocks for energy, chemical and agricultural feed production.

R. Atalla, Cellulose Sciences International & University of Wisconsin - Madison, Madison, WI, USA*
We describe a novel process developed by CSI for pretreating native celluloses. It transforms them into previously unknown nanoporous forms that are much more accessible to polysaccharide hydrolases than celluloses isolated at elevated temperatures. Simultaneously, it removes the biologically active inhibitors of the enzymes of pathogens that can break down the native tissues. The process is carried out at ambient temperature and pressure using only water, ethanol, sodium hydroxide and carbon dioxide. It is commercially attractive for nonwoody biomass, particularly tissues of the *Poaceae* family, which includes major agricultural residues such as corn stover, wheat straw and sugar cane bagasse, as well as other agricultural residues and grasses. The process also allows isolation of the hydroxycinnamic acids and their oligomers, which represent an important coproduct stream. Both capital and operating costs are far less than those of traditional high-temperature and high-pressure biomass pretreatment processes. The treated biomass is easily converted to monosaccharides that can be used as feedstocks for biosynthetic processes, for fermentation to fuels or for other organic synthetic processes. Enzyme doses required are an order of magnitude lower than those necessary for celluloses isolated at elevated temperature. The process can also convert agricultural residues into nutritious feeds for ruminant livestock. Digestibility by dairy cows has been shown to increase from 30% to 90%. The enhanced value of the treated biomass is complemented by the high value of the biologically active hydroxycinnamic acids and their oligomers that are extracted in the early stages of the treatment.

1:25 PM 3-2: Anti-microbial strategies in the enzymatic hydrolysis of a waste-derived pulp into monomeric sugars

F. Climent Barba, A.J. Blacker, R.A. Bourne and N. Kapur, University of Leeds, Leeds, United Kingdom*
Organic pulps produced from municipal solid waste (MSW) are useful sources of sugars and related chemicals. These are commercially attractive, non-food competing commodities that can be used in a variety of sugar-platform applications. A considerable problem is microbial infection of the source material which lowers process yields that generates lignocellulosic inhibitors. Sterilisation of the equipment and feedstock is not possible due to the high energy requirements and associated costs of auto-claving. Also ineffective are acid hydrolysis pulp pretreatments that release further inhibitory compounds, leading to failure to control infection and generation of problematic waste streams. In this context, four anti-microbial agents (sodium azide, benzisothiazolinone, hydrogen peroxide and tetracycline) were each tested *in situ*. The saccharification tests were carried out in a 1-L stirred tank reactor and 250-ml Erlenmeyer flask at 8 % total solids with 7 mg protein/g substrate of an enzymatic cocktail (CTec3). Key indicators of microbial growth (pH and dissolved oxygen) were monitored during the hydrolysis. The results showed that whilst 0.1 % of tetracycline was the optimum agent to tackle microbial contamination, its cost and socio-environmental impact make it the least desirable. On the other hand H_2O_2 performed well and was easily degraded to be traceless within the product. This work aimed to evaluate the techno-economic aspects of using various anti-microbial agents in the enzymatic hydrolysis of a waste-derived pulp into monomeric sugars.

1:50 PM 3-3: The production of isoprene from lignocellulose by *Pleurotus ostreatus*

S. Nakagame, Kanagawa Institute of Technology, Atsugi, Japan*
Isoprene, which is widely used for chemicals such as synthetic rubber, is currently produced mainly from petroleum refining process. Thus, the development of isoprene production process from lignocelluloses is expected to reduce CO_2 emission. Using white-rot fungi as a host strain for producing isoprene from lignocelluloses seems to be a promising compared with using other microorganisms, because white rot fungi highly degrade lignocelluloses. The high degradability of lignocelluloses by white-rot fungi would be a beneficial for simplifying the isoprene producing process from lignocelluloses, because only modest pretreatment of lignocellulose would be required. In addition, volatile property of isoprene enables the isoprene purification process to be simple, because the heating of the lignocelluloses that was inoculated with isoprene producible white-rot fungi can easily separate isoprene and lignocellulose residues. In this study, the codon-optimized isoprene synthase gene for *Pleurotus ostreatus* was chemically synthesized based on the isoprene synthase gene from *Pueraria montana* (kudzu vine). The codon-optimized isoprene synthase gene was ligated with the promotor of glyceraldehyde-3-phosphate dehydrogenase gene, which could express isoprene synthase constitutively. The constructed plasmid for producing isoprene was introduced into *P. ostreatus* host strain. Five transformants harboring the codon-optimized isoprene synthase gene were selected and the productions of isoprene were compared with the host strain by GC-MS. It was shown that the transformants produced isoprene in the presence of lignocelluloses such as wood meal and crystalline cellulose, while the host did not produce isoprene. This result suggests that using isoprene producible white-rot fungi have a high potential to produce isoprene from lignocellulose.

2:15 PM Break

2:45 PM 3-4: Developing *Hypocrea jecorina* into a protein expression factory

V. Subramanian, K.T. Moore, L.A. Schuster, M. Himmel and S.R. Decker, National Renewable Energy Laboratory, Golden, CO, USA; S.J. Farmer, W. Sun, Y.B. Chaudhari, A. Ho and D. Hu, National Renewable Energy Laboratory, Biosciences Center, Golden, CO, USA; E. Bredeweg and G. Orr, Pacific Northwest National Laboratory, Richland, WA, USA*
Despite its excellent reputation for expressing cellulolytic enzymes, *Hypocrea jecorina* has not developed as a model expression host due to its frequent inability to produce high titers of heterologous proteins. Along with an incomplete understanding of the

protein synthesis- and transport- machinery, a lack of ready screening methodology for difficult-to-assay or detect proteins has also restricted development of *H. jecorina* as a model microbe. Routinely used detection techniques such as PCR, biochemical assays, and western blotting all involve a series of processing steps, making protein expression screening cumbersome. Using the foot-and-mouth-disease-virus (FMDV) 2A peptide-based multi-gene expression system, we have developed an enhanced Green Fluorescent Protein (eGFP)-based screening method to detect expression of additional heterologous proteins. The 2A peptide system expresses multiple genes within a single transcript while generating independent proteins through a "ribosomal skipping" mechanism. We have successfully demonstrated co-expression of multiple heterologous proteins with ~100 % separation efficiency using this approach; although we have observed that expression level depends heavily on the length of its transcript. We have experienced challenges in the use of eGFP as a marker and as a model test protein within the 2A context. Specifically, the position of eGFP, source of carbon in the growth medium, and different secretion signals have been shown to differentially impact its expression and/or secretion, thereby providing interesting insights into the protein expression challenges in this fungus. Overall, demonstration of a fully functional multi-cistronic protein expression- and-detection platform along with factors influencing protein expression in *H. jecorina* will be presented.

3:10 PM 3-5: Novel gas fermenting organisms for the production of food, feed and chemicals from CO_2 and renewable energy

L. Ojala, D. Ercili-Cura, L. Salusjärvi, G. Peddinti, S. Castillo, A. Tamminen, M. Toivari, M. Penttilä and M. Lienemann, VTT Technical Research Centre of Finland Ltd, Espoo, Finland; M. Wuokko, Neste Engineering Solutions, Porvoo, Finland*

The continued release of CO_2 from fossil carbon sources and a growing global population have created the urgent need for technologies with which chemicals and food can be produced in a more sustainable manner. In this context, hydrogen oxidizing bacteria (HOB) are attractive biosynthetic platforms because they can convert CO_2 into organic compounds using renewable energy at a more than four times higher efficiency than photosynthetic plants. This is facilitated by hydrogen as an energy carrier, which can be produced either outside or inside of a bioreactor using water electrolysis. So far, production of mainly natural organic molecules, such as proteins, lipids, and storage polymers has been reported in gram negative species, which pose certain risks to food applications. Here, we report on the CO_2 assimilation by a novel gram positive HOB species and its natural product spectrum as well as its genetic setup with relevance to metabolic pathway engineering.

3:35 PM 3-6: Commercializing methanotrophs for the production of value added products

J. Scholten, Intrexon, South San Francisco, CA, USA*

Natural gas is currently one of the most economical sources of carbon in terms of high carbon abundance and low carbon cost. Methanotroph bacteria use methane in natural gas as the sole carbon source to support cellular metabolism and growth. Therefore, natural gas is an attractive potential feedstock for microbial bioconversion efforts because, unlike sugar, it is a highly reduced form of carbon, allowing conversion of methane into reduced products with high stoichiometric yields.

Intrexon has developed an advanced suite of tools that enables rapid genetic engineering of methanotroph bacteria. These tools include plasmid-based expression systems, promoter systems, methods for gene knock in/out, direct transformation/ electroporation, and advanced genome editing. Using these tools, intrexon has generated methanotrophic strains, which enable industrial-scale bioconversion of natural gas to higher value lubricants, fuels and chemicals. Furthermore, Intrexon has used different –omics approaches like transcript- and proteomics to understand and improve product formation (2,3 BDO) in natural gas fermentations.

4:00 PM 3-7: Closing the sequence to function gap

N. Mouncey, DOE Joint Genome Institute, Walnut Creek, CA, USA*

As a result of the tremendous advances in DNA sequencing, we have accumulated vast amounts of sequence information from all types of organisms and environments. However, the rate of functional protein characterization has lagged behind, resulting in a pressing need for scalable functional annotation methods. At the JGI, we seek to close this gap through deploying integrative genome science from sequencing, synthetic biology, functional genomics, transcriptomics, metabolomics to large computational analyses and modeling. Furthermore, we have also developed new testbeds using fabricated ecosystems and model plants, along with natural and synthetic microbial communities. Here, I will showcase some of the recent developments and their applications in biomass deconstruction and conversion.

1:00 PM - 4:25 PM Session: 4: Lipid Production and Processing

Conveners: Prof. Sergei Markov, Austin Peay State University, Clarksville, TN, USA and **Dr. Taraka Dale**, Los Alamos National Laboratory, Los Alamos, NM, USA

Columbia D, Third level

1:00 PM 4-1: Phosphate addition strategies for enhancing the co-production of biofuelÂ lipids andÂ N-

acetyl glucosamineÂ nanofibers by the diatom *Cyclotella*

O. Chiriboga and G. Rorrer, Oregon State University, Corvallis, OR, USA*

Diatoms, which are single-celled algae that make cell walls of biogenic silica, are gaining considerable interest as a platform organism for algal biorefineries. Diatoms can provide high lipid yield and productivity under diverse environmental conditions, and make unique and valuable co-products. In particular, the diatom *Cyclotella* accumulates lipids at levels exceeding 50 wt% within the biomass for biofuel or food applications, and extrudes pure N-acetyl glucosamine biopolymer nanofibers that have advanced biomaterial applications. Phosphorus is a limited resource in the earth's crust, but is a critical nutrient for algae cultivation at large scale. The goal of this study was to develop phosphate addition strategies for reducing phosphorus consumption and enhancing the co-production of lipids and biopolymer nanofibers by *Cyclotella*. Experiments were carried out under silicon-delivery limited division within fed-batch photobioreactor culture. The outcomes of this study suggested two options for phosphate delivery. In the first option, pulse delivery of phosphate under continuous silicon delivery elicited lipid levels exceeding 60 wt% during the last cell division cycle when all intracellular phosphate was consumed. Lipidomic analysis showed that phospholipids remodeled to neutral lipids. In the second option, continuous delivery of phosphate during at a rate near the minimum phosphate requirement for cell division sustained biomass, lipid, and biopolymer production at high volumetric rates, but at the expense of lowered biomass lipid content. Overall, this study showed the potential for controlled phosphate and silicon delivery to enhance the rate and selectivity of lipid production by diatom algae while concurrently minimizing phosphate consumption.

1:25 PM 4-2: Microbial production of biodiesel from cellulosic hydrolysates

B. da Costa, REG Life Sciences, South San Francisco, CA, USA*

REG Life Sciences, LLC is a subsidiary of REG, the largest producer of biomass based diesel in North America. The company is commercializing an industrial biotechnology platform for the conversion of renewable carbohydrate to a pipeline of products that will feed into diverse industries from fragrances and personal care to polymers and fuels. In this presentation, we will discuss (1) the microbial fatty acid platform that supports this diverse product portfolio; (2) provide an overview of our joint development program with ExxonMobil Research and Engineering for its application to the production of biodiesel from non-food biomass feedstocks.

1:50 PM 4-3: Microbial oils as advanced feedstock for Hydrogenated Vegetable Oil (HVO) based biorefineries

D. Bianchi, Eni spa, Novara, Italy*

Eni, the major Italian oil company, has invested in the conversion of two traditional refineries into biorefineries based on the EcofiningTM technology to produce paraffinic Green Diesel (Hydrogenated Vegetable Oil) with a total capacity up to 1 million tons per year. Paraffinic bio-fuels possess several advantages with respect to oxygenated ones (e.g. biodiesel), such as excellent cold properties, high heating value, high cetane number and high miscibility with traditional fuels.

A key point of this technology is the feedstock selection in order to improve the process sustainability.
Microbial oil, produced by fermentation of cellulosic sugar, is one of the most promising alternative to vegetable oils.
The presentation will describe the whole process, developed by Eni, to produce microbial oils using oleaginous yeasts, including the strain selection and improvement, the sugar feed specifications, the fermentation and the oil downstream optimization, the lipids characterization by lipidomics, the purification treatment to meet the refinery requirements, and finally the production of diesel fuels by hydrotreatment (HVO).

2:15 PM Break

2:45 PM 4-4: Effects of impurities in two-step vs. one-step hydroprocessing of algae oils

J. Kruger, E. Christensen, T. Dong, G. Fioroni, P.T. Pienkos and R. McCormick, National Renewable Energy Laboratory, Golden, CO, USA*

Microbial lipids are a promising precursor to renewable diesel fuels, but catalytic hydroprocessing of the crude lipid extracts is an under-studied operation. In particular, impurities in the crude extracts can lead to catalyst deactivation. Hydroprocessing of these lipids to green diesel fuel typically comprises deoxygenation (DO) and hydroisomerization (HI) chemistry, and can be conducted in a two-step or one-step configuration. We have shown that in a two-step configuration using Pd/C for DO and Pt/SAPO-11 for HI, a fuel with acceptable cold weather and boiling range properties can be produced without lipid cleanup steps but under severe conditions. In contrast, a Pt/SAPO-11 catalyst is deactivated in one-step DO/HI. However, multiple oil bleaching approaches can remove nitrogen-containing impurities that may be causing the deactivation.

3:10 PM 4-5: Improvement of lipid-based chemicals production by overcoming metabolism overflow in oleaginous yeast *Yarrowia lipolytica*

X. Xiong, Y. Zhang, B. Zhao and S. Chen, Washington State University, Pullman, WA, USA*
The oleaginous yeast *Yarrowia lipolytica* with “generally recognized as safe” (GRAS) status has attracted growing attention due to its capacity to accumulate lipids. Lipid metabolism in *Y. lipolytica* can be tailored to produce advanced biofuels and oleochemicals with wide industrial applications. However, secretion of a large amount of citric acid to the supernatant of the culture, which indicates the presence of overflow metabolism and represented a substantial carbon loss, was observed in both wild-type and recombinant *Y. lipolytica* strains. After developing a comprehensive molecular toolbox, we carried out intensive genetic modifications including construction of functional pathways for targeted product formation to successfully biosynthesize a group of lipid-based chemicals including free fatty acids, fatty alcohols, wax esters, and long-chain dicarboxylic acid (DCA). The recombinant produced 0.6 g/L of fatty alcohols in flask culture. Long-chain DCA (C16-C18) at titer of 3.4 g/L could be produced from glycerol in a 1-liter bioreactor fermentation, while 39 g/L of citric acid was secreted to the supernatant. A flux balance analysis model indicated that energy metabolism was a rate-limiting factor for fatty acid precursor generation in *Y. lipolytica,* and insufficient energy supply could result in citric acid overflow. Inspired by this finding, we improved the fatty alcohol titer by 33% to 18.2 g/L in the recombinant strain, and citric acid accumulation was eliminated by boosting cellular energy generation. Our study has opened a new avenue to overcome metabolism overflow, which would remove one of the major hurdles for higher yield and improve oleochemical production through metabolic engineering.

3:35 PM 4-6: Conversion of lignin monomers to glycolipids by oleaginous yeasts

K.L. Boundy-Mills, I. Sitepu, D. Wong, P. Hernes and T. Jeoh, University of California, Davis, Davis, CA, USA*
Current lignocellulose conversion technologies focus on the cellulose and hemicellulose fractions, with little attention paid to converting the lignin component to value-added products. Technologies to convert lignin and lignin monomers to renewable, sustainable biobased co-products such as chemicals, lubricants, films and industrial polymers are receiving new attention. In this study, several abundant feedstocks including sorghum biomass, and almond hulls, shells, and prunings were fractionated to produce a soluble, lignin- and hemicellulose-rich fraction for use as a yeast growth medium. Oleaginous yeasts in the taxonomic order Sporidiobolales were previously shown to secrete a new class of amphiphilic glycolipids called polyol esters of fatty acids (PEFA), which are environmentally-friendly biosurfactants. Strains of these yeasts from the Phaff Yeast Culture Collection at UC Davis were tested for ability to tolerate the inhibitors or consume the nutrients found in the hydrolysates, including lignin monomers, using laboratory media. PEFA-secreting yeasts including *Rhodotorula babjevae, R.* aff. *paludigena, R. graminis, R. diobovata, Rhodosporidiobolus ruineniae* and/or *R. bacarum* does indeed grow when supplied with vanillic acid, p-hydroxybenzoic acid (PHBA) or coumaric acid up to 0.5% as the sole carbon source. *Starmerella bombicola*, a sophorolipid-secreting yeast, grew very weakly on these media. When grown in lab media with PHBA at pH 6.5, *R.* aff. *paludigena* UCDFST 81-84 produced visible PEFA globules. The most promising PEFA-secreting yeast candidates were then cultivated in the authentic hydrolysates to determine compatible growth conditions, and growth on hydrolysates was confirmed.

4:00 PM 4-7: Lipid production increase through optimization of the medium composition for the oleaginous yeast *Rhodosporidium toruloides*

E.A. Miranda, A. Ikeda Francisco and L. Pires Vaz, School of Chemical Engineering, State University of Campinas, Campinas-SP, Brazil*
Microbial oils (SCO, single cell oil) produced by oleaginous yeasts are an option for replacing vegetable oils in biodiesel production. *Rhodosporidium toruloides* is a potential microorganism to produce SCO from low-cost substrates such as agro-industrial wastes due to its ability to consume multiple sources of carbon and nitrogen. In this present study, microbial oil production by the yeast strain *Rhodosporidium toruloides* CCT7815 was evaluated in a two-stage statistical modeling approach to maximize lipid production and content. The experiments were conducted in 250 mL Erlenmeyer flasks incubated at 28 °C for 120 h at 200 rpm using low-cost substrates: sugarcane bagasse hemicellulosic hydrolysate (SCBHH) and simulated crude glycerol (60% glycerol). According to the response surface methodology, the optimized medium contained 71.5 g/L of crude glycerol, 2.5 g/L of yeast extract, 40 g/L of sugars contained in the SCBHH. It resulted in 14.0 g/L of dried cellular weight (DCW), 4.7 g/L of lipids, and 33.7% of lipid content. The addition of glycerol caused an increase in lipid production, but it decreased when glycerol concentration exceeded 100 g/L. This decrease is possibly explained by the high osmotic stress inhibition of metabolic activities in the yeast. Moreover, the presence of yeast extract reflected directly and proportionally on the DCW. When comparing the SCBHH medium with the optimized medium increases of 3.5- and 2.7-fold in lipid production and DCW, respectively, were detected. This shows that the supplementation with glycerol is a simple way to improve lipid production by *R. toruloides* from SCBHH.

4:45 PM - 5:45 PM Biotechnology for Biofuels Editorial Board Meeting

Duwamish - Room 306, Third Level

5:00 PM - 6:00 PM Exhibits Open

Columbia Ballroom Foyer, Third level

6:00 PM - 8:00 PM Session: PS2: Poster Session II

Columbia A, Third level

M1 The role of non-structural components on biomass preprocessing efficacy, and the impacts of preprocessing pH on ethanol yield and overall process economics

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

It has been proven that biomass preprocessing can increase hydrolysis and fermentation yields while using a feedstock with a high content of non-structural components (NSCs), i.e. ash and organic extractives. This improvement is usually associated with the removal of NSCs from the biomass, resulting in a more efficient pretreatment, decreased buffering capacity of biomass, and higher monomeric sugar recovery. In the present study, we assessed the efficacy of preprocessing using two different feedstocks: heterogeneous 3-year-old poplar with high NSCs content, and homogeneous 12-year-old poplar with low NSCs content. Our goal is to determine if preprocessing is still effective while using a biomass with low NSCs content. In addition, we discuss the effects of preprocessing pH on every step of ethanol production. Both feedstocks were preprocessed by acidic, alkaline, or neutral washing prior to steam pretreatment, followed by enzymatic hydrolysis and fermentation. Acidic preprocessing was more effective in removing ash and extractives from both feedstocks. On the other hand, the alkaline wash was able to significantly remove acetic acid to an extent of 48% and 73% from high and low NSCs poplar, respectively. The total sugar recovery after steam pretreatment was higher for alkaline preprocessed biomass, mostly due to a higher solids recovery. When compared to untreated biomass, the high NSCs poplar had a sugar recovery increase of 69 kg/tonne, and low NSCs poplar had an increase of 130 kg/tonne. Enzymatic hydrolysis conversion, fermentation yield, as well as a techno-economic analysis of the overall process will be discussed.

M2 New observed intermediate in the mechanism of copper containing lytic polysaccharide monooxygenase

M.J. Bjerrum, University of Copenhagen, Copenhagen, Denmark*

M3 Kinetics modeling for design of continuous enzymatic hydrolysis

J. Lischeske, N. Grundl, D.A. Sievers and J.J. Stickel, National Renewable Energy Laboratory, Golden, CO, USA; J.D. McMillan, NREL, Golden, CO, USA*

Enzymatic hydrolysis of cellulose to monomeric sugars continues to be a limiting step in cost-effectively producing sugar and fermentation-based biofuels from biomass. In particular, the high cost of enzymes coupled with the long time-scale of reaction pose challenges to economic viability. Performing enzymatic hydrolysis in a continuous mode with enzyme recycle may provide a path towards substantially reduced costs for sugar production, but design and analysis are complicated by a lack of suitable kinetics models. Computationally attainable models, such as fractal-based models, require knowledge of the reaction-history of the biomass, and are thus only suitable for describing the batch reactions from which they are derived. Fundamental models, while potentially more generalizable, are often too computationally intensive to use in process or reactor modeling. In this work, a phenomenological rate model is proposed based on a two-phase substrate representation. Good agreement is seen between batch and continuous enzymatic hydrolysis (CEH) experiment data, which validates the model and enables us to solve for reactor design parameters, such as CEH reactor size and stream flow rates, based on process variables like yield. This model is integrated with techno-economic analysis software to explore economic sensitivities. Important design optimizations and tradeoffs are identified and quantified, including the relative cost imposed by rate slowdown from sugar inhibition versus the cost to remove and concentrate sugars at a lower concentration. It also identifies, high-leverage avenues for further exploration, such as increasing the maximum feasible solids concentration, and sustaining high membrane flux and reliability.

M4 Exploring microbial biodiversity for novel pathways to catabolize lignin-derived aromatic compounds

G.N. Presley, O.N. Cannon, J.K. Michener and J.G. Elkins, Oak Ridge National Laboratory, Oak Ridge, TN, USA*

Lignin-rich waste streams from plant biomass-based fuel production can be biologically upgraded to valuable chemicals, making the overall process more economically viable. This conversion requires the expression of several heterologous aromatic compound-degrading pathways in a microbial production host. The known toolkit for lignin catabolism is limited to a small set of well-studied microbes, leaving abundant diversity in microbial lignin catabolism uncharacterized and thus unexploitable. This work describes efforts to isolate novel aromatic-degrading microbes and characterize their catabolic capabilities. To date we have isolated ~90 strains from soil samples with the capacity to degrade model lignin monomers and dimers. Screening these isolates has identified several strains with the capacity to degrade lignin β-O-4, 5-5, and/or β-1 bonds. These include several strains of *Pseudomonas*, *Methylorubrum*, *Sphingomonas*, *Novosphingobium*, *Chryseobacterium*, and *Streptomyces* that are being submitted for whole genome sequencing. Rapid genetic characterization of lignin catabolic pathways using Tnseq/Barseq will be performed on select organisms as we have accomplished with the lignin monomer-degrading strain *Pseudomonas fluorescens* A1. Genetic characterization of lignin monomer decomposition by *P. fluorescens* A1 has simultaneously identified

several genes involved in ferulate, *p*-coumarate, and syringate metabolism. This strain lacks homologs to several well-characterized monomer-degrading genes in other *Pseudomonas* species and may decompose monomers using novel enzymes. Confirmation of gene function is being performed by generating clean knockouts of essential genes identified using Barseq. We aim to apply this characterization pipeline to a variety of lignin-based aromatic compound-degrading organisms to rapidly expand the molecular toolkit available for microbial lignin valorization.

M5 Metal Chloride Deep Eutectic Solvents for Biomass Fractionation: Experimental and Computational Approach

L. Das[*] and A. George, Sandia National Labrotories/Joint BioEnergy Institute, emeryville, CA, USA; A. Landera, Sandia National Labrotories, emeryville, CA, USA

M6 Techno-economic analysis of Compacted Biomass with Recycled Ammonia (COBRA) pretreatment process

V. Balan[*], University of Houston, Houston, TX, USA, D. Hodge, Montana State University, Bozeman, MT, USA, J. Zhang, East China University of Science and Technology, Shanghai, China and S. Leonardo da Costa, Michigan State University, Lansing, MI, USA

Pretreating lignocellulosic biomass helps to open up the cell wall and aid enzymes to hydrolyze the substrate more efficiently. Since milled biomass occupies large volume in a reactor due to its low bulk density and fibrous nature, the amount of biomass that could be pretreated in a given reactor volume is very limited. The newly developed pretreatment process called COmpacted Biomass with Recycled Ammonia (COBRA) gave higher sugar conversion (>90%) in a short period of time (24 h). The advantage of using the new COBRA process compared to previously reported extractive ammonia pretreatment include: (1) less ammonia requirement for pretreatment (1:1 vs 3:1 to 6:1); (2) can operate at lower pressures (~300 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 has helped to reduce both operating and capital costs requirement for pretreating biomass. A techno-economic analysis (TEA) has been carried out for COBRA process resulting in lowest minimum ethanol selling price (MESP) < $2.00/gal, primarily due to pretreating the biomass with high density and due to higher rate of sugar conversion due to formation of cellulose III. Details about logistics of biomass densification near the fields, transporting to centralized biorefinery where they will be converted to biofuels will be discussed.

M7 Recovery of medium chain carboxylic acids from brewery waste using anaerobic membrane bioreactor integrated with liquid-liquid extraction system_

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The increasing number of breweries in the U.S. is creating considerable challenges for waste management. Brewery waste is rich in biodegradable organics, providing ample opportunities to apply biotechnology to recover these untapped resources. One such process is the production of medium chain carboxylic acids (MCCAs, carboxylic acids with six to 12 carbons [C6-C12]) via chain elongation of short chain carboxylic acids (SCCAs, C2-C5) with a reduced compound, such as ethanol. MCCAs are platform chemicals with several industrial and agricultural applications. The inputs for chain elongation, SCCAs and ethanol, can both be produced from waste streams, reducing the cost and environmental impact of deriving them from non-renewable resources.

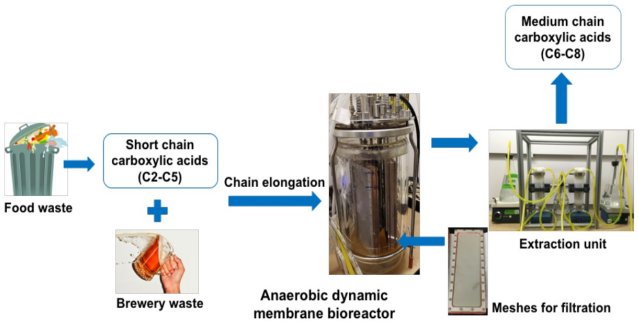

Figure 1. Schematic of the bioreactor system for MCCAs production

A laboratory scale anaerobic dynamic membrane bioreactor (AnDMBR) is being operated with brewery waste and effluent containing SCCAs from an anaerobic, acidogenic bioreactor fed food waste (Figure 1). The AnDMBR is equipped with submerged membrane housings containing stainless steel meshes of 25-mm pore size as dynamic membrane (biofilm) support. A maximum yield of 0.23 g COD MCCAs/g COD_{in} has been achieved during the start-up period. Preliminary results showed that the MCCAs yield is limited by product toxicity necessitating continuous extraction. Thus, a liquid-liquid extraction (LLX) system

consisting of two hydrophobic hollow fiber membrane contactors was optimized. The LLX method effectively separated MCCAs from a synthetic mixture of SCCAs and MCCAs. The continuation of this research will include integration of the LLX unit with the AnDMBR for increased MCCAs production and simultaneous recovery.

M8 Cell-free biomanufacturing: Synthetic proteomes for biochemical production

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Cell-free biomanufacturing offers a powerful alternative to microbial biochemical conversion platforms due to advantages in achieving higher titer and yield, simplifying separations and dramatically driving down production costs as a result. But recycling of enzymes and cofactors is key for the viability of such systems. The synthetic proteome technology, developed at NREL in recent years, offers a method for immobilizing enzymatic pathways using a protein scaffold, providing a platform for the production of many molecules too toxic or otherwise untenable for cost-effective microbial production. This technology leverages discoveries over the past two decades in cellulosome research to offer a highly modular platform technology for the production of biochemicals, biofuels, and high-value compounds using rationally designed, immobilized enzymatic pathways.

M9 Optimization of immobilization conditions of *Burkholderia cepacia* lipase on bentonite clays, SPECTROGEL TYPE C, using experimental design

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To lower the costs of using free enzymes in products and processes, the enzyme immobilization should bring a number of benefits such as reuse and improved operational stability. The *Burkholderia cepacia* lipase enzyme was immobilized by adsorption on commercial Bentonite type C spectrogel clay. A central composite rotatable design (CCRD) was used with the aid of Statistical 7® software to investigate and optimize immobilization conditions in order to obtain a biocatalyst with high recovered enzymatic activity. The studied variables were the offered activity for immobilization (98 U, 200 U, 350 U, 500 U, 602 U), buffer pH (3.6, 5.0, 7.0, 9.0, 10.6) and buffer molar concentration (0.01M, 0.05M, 0.1M, 0.16M, 0.19M). Reactions occurred for 24 hours at 25ºC in a refrigerated incubator. To analyze the immobilization efficiency, analyzes were performed by the hydrolysis activity test of olive oil. The results indicated that the three variables had a significant influence on the immobilization process on the recovered activity, with the pH being the most influential, followed by the concentration and offered.activity The optimum reaction point was at pH 7.15, offered activity of 365 U and buffer concentration 0.1056 M, with predicted activity yield above 100%. The immobilization validated the model, the obtained yield was 102.56%, with hyperactivation, in which a microenvironment, that maximizes the catalytic efficiency of the lipase, is formed.

M10 Does de-aromatization of lignin increase its bioavailability?

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Lignin is typically 15-28% of the total dry weight of biomass, making it a significant potential source of revenue, yet it is currently a waste stream in biofuel production that is usually burned to generate power. Efficient conversion of lignin to useful molecular building blocks has been elusive. One promising approach is partial de-polymerization of lignin and subsequent bioconversion of the mono, di, and poly-aromatic breakdown products into value-added compounds. Work to date using this approach has examined growth of individual organisms and also microbial communities on partially depolymerized lignin streams. Various processes have been explored to depolymerize lignin, such as acid- or base-catalysis, and reductive and oxidative processes. Much of the prior work has focused on processes that leave the aromatic rings largely intact, with the goal of utilizing aromatic catabolic pathways in soil microbes to funnel the carbon into central metabolism. However, conversion is generally low. With *P. putida*, a conversion host that has been extensively explored for lignin bioconversion, conversion is limited largely to monomeric aromatic species, yet most depolymerization processes result in only a small fraction of monomeric products. In this work, we consider whether de-aromatization of lignin might lead to higher biological conversions of the carbon. In particular, we wish to determine whether de-aromatization leads to more facile conversion of oligomeric or polymeric fragments by microbial communities. In initial studies, we extensively de-aromatized lignin from corn stover using an oxidative process involving chelator-mediated Fenton chemistry. We measured the consumption of the de-aromatized lignin by several microbial communities and compared the results to consumption of base-catalyzed depolymerized lignin from corn stover by the same communities.

M11 Transport of lignin-breakdown products into bacteria and fungi

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While much lignin breakdown occurs extracellularly, breakdown products, a substantial fraction of which are mono- and di-aromatics, are known to be taken up by lignolytic fungi and bacteria and metabolized intracellularly. Assimilation of these breakdown products is an important part of lignin conversion, yet little is known about transporters that shuttle these compounds

into lignolytic organisms, the substrate range, or the kinetics of transport. We performed a study of native transport of 4-hydroxybenzoic acid (HBA), vanillic acid (VA), *p*-coumaric acid (CA), syringic acid (SA) and the model dimer guaiacylglycerol beta-guaiacyl ether (GGE) in *Pseudomonas putida*, *Enterobacter lignolyticus*, *Escherichia coli, Phanerochaete chrysosporium,* and *Saccharomyces cerevisiae*. Internalization and accumulation of each compound was determined via LC-MS of the cell lysate. Additionally, click-chemistry-enabled fluorescence microscopy was performed for HBA, VA and GGE analogues to determine single cell uptake efficiency. For *Pseudomonas putida* the monomeric compounds were internalized and subsequently metabolized whereas GGE was internalized but was not metabolized. Other notable results include very high accumulation of vanillic acid in *Escherichia coli* and of coumaric acid in *Saccharomyces cerevisiae*. In addition to the native transport study, we genetically engineered putative lignin transporters into the non-lignolytic organism, *Escherichia coli*. We expressed the lignin transporters in cultures grown in minimal media supplemented with individual lignin breakdown products of interest. Mass spectrometry analysis of cell lysate and fluorescence imaging both showed increased uptake of GGE for several engineered transporters. This work shows that uptake of lignin breakdown products can be enhanced through expression of exogenous transporters.

M12 Optimization of enzyme cocktails and process conditions for efficient saccharification of Norway spruce

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Efficient and sustainable use of biomass as renewable feedstock for heat, power and transportation is key to achieving the 2^{o}C climate goal proposed by UNFCCC1. These issues are addressed in the Norwegian research center Bio4Fuels where the development of new technology for production of biofuels and value-added chemicals from lignocellulosic biomass is the main goal.

Enzymatic hydrolysis of lignocellulosic biomass is a limiting step for making bioconversion processes economically feasible. Therefore, an important topic in Bio4Fuels is process development to improve saccharification of softwood at high dry matter, assessing enzyme tools, reactor technologies and reaction conditions.
Lytic polysaccharide monooxygenases (LPMOs) use H_2O_2 as a co-substrate, which may be supplied directly to the reaction or generated *in situ* through reduction of O_2 by reductants present in the reaction mixture, possibly assisted by LPMOs.[1] Addition of H_2O_2 at different rates has been shown to proportionally boost the formation of LPMO products in reactions with Avicel and sulfite pulped spruce[2]. We have studied the effect of different inclusion levels of LPMOs in cellulase cocktails and different H_2O_2 supply schemes on saccharification of cellulosic substrates. Our results indicate that depending on the lignin content of the substrate both direct addition of H_2O_2 and *in situ* generation of H_2O_2 may be efficient means of activating LPMOs. Here we present recent findings related to H_2O_2 activation of LPMOs in reactions with industrially relevant substrates and discuss how these findings may affect both process design and the composition of future enzyme cocktails.
[1] Bissaro, B. et al. (2017). Oxidative cleavage of polysaccharides by monocopper enzymes depends on H2O2. *Nature Chemical Biology*,*13*(10),1123–1128.
[2] Müller, G. et al. (2018). The impact of hydrogen peroxide supply on LPMO activity and overall saccharification efficiency of a commercial cellulase cocktail. *Biotechnology for Biofuels*,*11*(1),209.

M13 The production of lipids using 5-HMF tolerance *Rhodotorula graminis* on the hydrolyzates of steam pretreated biomass substrates

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The 5-hydroxymetyl furfural (5-HMF) tolerance of *Rhodotorula graminis* was improved to produce lipid from the water soluble fraction of the SO_2 catalyzed steam pretreated biomass using corn steep liquor (CSL) for nitrogen source. After the SO_2 catalyzed steam pretreatment of biomass, the water soluble fraction contained about 0.2% of 5-HMF and 0.1% of furfural, which negatively affected the growth of *R. graminis*. To improve 5-HMF tolerance of *R. graminis*, wild type of *R. graminis* was incubated in the medium containing 5-HMF that was gradually increased by 0.5%. As the result, *R. graminis* strain that grew well in the presence of 5-HMF (0.5%) was obtained. Evaporation of the water soluble fraction of the SO_2 catalyzed steam pretreatment was effective to increase the productivity of lipid using the strain, because the evaporation increased the sugar concentration in the medium and removed furfural easily from the water soluble fraction due to the formation of azeotrope between furfural and water.

M14 Production of 2-furaldehyde and 5-hydroxymethyl-2-furaldehyde from biomass hydrolysates as intermediates in the production of hydrocarbons from biomass sugars

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Carbohydrates from lignocellulosic biomass represent an abundant source of carbon that can be transformed into transportation fuel precursors through cellulose and hemicellulose depolymerization and hydrolysis to pentoses and hexoses. Dehydration of the sugars under acidic conditions results in formation of 2-furfuraldehyde (2-F) and 5-hydroxymethyl-2-furfuraldehyde (HMF),

which can then be used as intermediates in production of hydrocarbons for blending into jet and diesel fuels. The fuel products are paraffins with excellent properties for blending into jet and diesel fuels (cetane number >60, boiling point 150 - 350 ^{o}C, and a freezing point <-60 ^{o}C giving them very good Cloud Point properties). This research, therefore, directly supports the DOE Bioenergy Technology Office's goal to demonstrate conversion of biomass-derived intermediates into hydrocarbons for use as advanced drop-in biofuels.

Simultaneous conversion of both pentose and hexose sugars is required for efficient production of biofuel intermediates from biomass hydrolysates. Using Lewis acid catalysts and 1,4-dioxane as a co-solvent, 2-F and HMF have been produced from pure sugars and the pentoses and hexoses present in corn stover hydrolysates in combined yields up to 80 mol%, in 2 minutes at 180 ^{o}C using microwave heating. The effects of different Lewis acids and co-solvents have been studied and hypotheses to explain their varying effectiveness have been developed. Catalyst inhibition has also been observed from hydrolysate components. The furfural mixture has been converted into an intermediate by aldol condensation with 2-butanone, and then converted into a mixture of paraffins, predominately in the C12 to C16 range, by hydrodeoxygenation.

M15 Gas Fermentation of *Cupriavidus necator* Variants 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, with applications targeting both bio fuels and bio alcohols. Operating at a range of fermenter volumes from typically 100ml upwards and working pressures of up to 150psi (~10bar), production rates for CO2/H2 feed comparable to that with fructose will be demonstrated.

M16 Wax off, wax on: Developing a bacterial biocatalyst for wax ester production

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Rhodococci, among the best-studied oleaginous bacteria, have considerable potential for the sustainable production of lipid-based chemicals. We harnessed the biosynthetic capabilities of rhodococci for the production of wax esters (WEs), a class of high-value neutral lipids. Initially, we established that *Rhodococcus jostii* RHA1 (RHA1 hereafter) produces WEs and identified a fatty acyl-CoA reductase, FcrA, involved in this production. We then developed genetic tools to facilitate the creation of rhodococcal biocatalysts. Briefly, we constructed pSYN, a modular integrative-vector, and employed it to identify and characterize strong constitutive rhodococcal promoters. We used these tools to develop a biocatalyst for WE production. First, RHA1 transformed with a single, integrated copy of *fcrA* was used to screen wax synthases for the ability to increase WE production, identifying WS2 of *Marinobacter hydrocarbonoclasticus* DSM 8798 as an effective wax synthase in RHA1. Co-expression of chromosomally integrated *fcrA* and *ws2* resulted in a biocatalyst that accumulated WEs to >15% CDW, at yields of 0.05 g/g glucose. Accumulated WEs were 29 to 38 carbon atoms in length, with unsaturated, mono- and di-unsaturated species being present in an approximate ratio of 1:2:1. This biocatalyst was scaled from shake flasks to bench-top fermenters, and high-density fermentation strategies were developed for WE production. At scale, the biocatalyst reached cellular densities of >50 g/L CDW, while maintaining the WE yields seen in shake flasks. Overall, this study provides insight into the biosynthesis of WEs in rhodococci and provides tools to develop the biocatalytic potential of rhodococci.

M17 a

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M18 The bacterial catabolism of lignin monomers generated via reductive catalytic fractionation

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Lignin is a major component of lignocellulosic biomass and the most abundant aromatic biopolymer on earth. Due in part to its heterogeneous intersubunit linkages, lignin is exceptionally recalcitrant. Nevertheless, its valorization is essential for sustainability of biorefineries. One effective means to break down lignin is reductive catalytic fractionation (RCF), which depolymerizes lignin into low molecular weight fragments. We have isolated a bacterium, *Rhodococcus* EP4, based on its ability

to grow on the two major RCF monomers of corn stover: 4-ethylphenol and 4-propylguaiacol. Transcriptomic analysis of cells grown on each monomer revealed the up-regulation of genes encoding a *meta* cleavage pathway. Bioinformatic analyses further suggested that 4-ethylphenol and 4-propylguaiacol are converted to their respective 4-alkylcatechols by a cytochrome P450 and a phenol hydroxylase (HpaB), respectively. Consistent with the P450 catalyzing *O*-demethylation, the purified hemoprotein had high affinity for 4-substituted phenolics, and was shown to demethylate 4-propylguaiacol *in vitro*. Similarly, HpaB was shown to hydroxylate 4-ethylphenol. However, deletion of the ortholog in *Rhodococcus jostii* RHA1 did not abolish growth on 4-ethylphenol. RT-qPCR revealed the up-regulation of several *hpaB* paralogs in the mutant, but not WT RHA1, highlighting the adaptability of this strain. Finally, EP4 was able to grow on corn stover RCF. Taken together, the data suggest that 4-ethylphenol and 4-propylguaiacol are catabolized via a convergent pathway in which ring-cleavage precedes side chain degradation. More generally, these data improve our understanding of the bacterial catabolism of lignin-derived aromatic compounds, and expand the suite of genes available for engineering biocatalysts to valorize lignin.

M19 High yield production of muconic acid from glucose by supplementing the carbon source from xylose for cell growth.

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Shikimate pathway is one of the most important metabolic pathways in bacteria for synthesizing various aromatic compounds including aromatic amino acid. The first reaction in the shikimate pathway is the stereospecific condensation of erythrose-4-phosphate (E4P) and phosphoenolpyruvate (PEP) to generate 3-deoxy-D-heptulosonate-7-phosphate (DAHP), catalyzed by DAHP synthase. Chorismate, a terminal metabolite of shikimate pathway, is synthesized via several step reactions from DAHP in the shikimate pathway. Intermediates of the shikimate pathway including chorismate are candidates for starting compounds in the bio-production of various chemicals (e.g. aromatic compounds, dicarboxylic acids, and aniline derivatives).

Previous our study, we designed the metabolic pathway of *Escherichia coli* to enhance shikimate pathway by accumulating the intracellular PEP concentration and to produce chorismate derivatives in high yields. In order to avoid consuming PEP when uptake of glucose, we have generated a recombinant strain with replacing the endogenous phosphotransferase system (PTS) with GalP/Glk system (GGS) (PTS- GGS+ strain). In order to prevent leakage of carbon flux to the TCA cycle, we also disrupted two genes encoding pyruvate kinase (pykA, pykF), from PTS- GGS+ strain.
In this study, we further modified the metabolism in *E. coli* to be confined the use of the glucose to the production of a target compound, MA. After that, to regain the capacity of cell growth, we introduced an exogenous Dahms pathway that supplements the metabolites necessary for cell growth with xylose into the strain.

M20 Algae biomass pretreatment and bioconversion into D-lactate by a genetically modified *Corynebacterium*

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Algae biomass has significant potential as a feedstock for producing a variety of biobased chemicals. The state-of-the-art for algae-based commodities have primarily focused on biodiesel production through prompting high algal lipid yields under the nutrient stress conditions. Filamentous algae *Tribonema sp.* is a potential candidate for use in biodiesel and bioethanol production due to its high lipid and carbohydrate content which are governed by growth conditions and harvesting time. It has also advantages in relatively easy harvesting and resistance to grazers in mass cultivation due to its filamentous morphology. We explored the potential of using this feedstock as a carbohydrate and protein source for fermentative production of a high-value bioproduct, D-lactate. Through this work we demonstrated one-pot conversion of algae biomass hydrolysate into D-lactate using engineered microbial strain, *Corynebacterium*. In this study, dilute acid pretreatment for *Tribonea* sp. algal biomass was optimized. Dilute acid pretreatment has been demonstrated as an efficient approach to efficiently utilize the major biochemical constituents of algal biomass, by hydrolyzing microalgal carbohydrates into fermentable sugars, while making the lipids more extractable. The effect of different pretreatment severity on *Tribonema sp.* was investigated by varying the acid concentration, temperature and reaction time, with emphasis on increasing the solids loading to make the process more economical. Hydrolyzed algal biomass was subsequently utilized in fermentation trial to produce D-lactate and fermentation process parameters were optimized to increase D-lactate production rate, yield, and titer.

M21 SANS study of structures and deuterium incorporation into vegetative leaf stalks of deuterated Kale (*Brassica oleracea*)

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The unique capabilities of small angle neutron scattering (SANS) assisted by NMR and FTIR have been applied to reveal the detailed molecular structure of both natural lignocellulosic biomass and model cellulosic composites. In this study, these techniques are applied to examine the stem structure of a deuterated herbaceous dicotyledonous plant, kale, which exhibits a hierarchical structure that resembles that of wood in vascular architecture with. In this study, partial deuteration was achieved

through cultivating the kale plant in deuterated media to enhance contrast between component plant biopolymers. Cellulose microfibrils that solely contributes to SANS data in high-*Q* region ($Q > 0.08$ Å^{-1}) is determined to scatter similar to 65% D_2O solvent (SLD ~ 3.94 E-06 Å^{-2}), indicating that approximately 50% of the available covalently bonded H atoms would be substituted by D atoms. In the low-*Q* region, the features responsible for these power-law decay show a neutron sensitivity that matches a solvent of 48% D_2O (SLD ~ 2.77 E-06 Å^{-2}). To result in an overall SLD that is lower than cellulose, the SLD of lignin and hemicellulose needs to be lower to off-set the high SLD of cellulose within cell wall. To confirm the deuterium incorporation in the cellulose component of deuterated kale stem, contrast variation SANS experiments has been carried out on extracted cellulose. The results demonstrated that the contrast matching points exhibit a nearly constant value of ~ 70% D_2O over the whole *Q* range, which is comparable to 65% D_2O obtained for the cellulose component in the deuterated kale stem. Finally, this study revealed the hierarchical structures of deuterated kale stems and different deuterium incorporation is found at different cell wall component polymers.

M22 A multi-scale study to elucidate the role of cellulose physicochemical properties in productive binding of cellulases

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The role of the insoluble cellulosic substrate in the mechanisms of cellulose hydrolysis by cellulases remains largely unexplained. While short term cellulose hydrolysis rate limitations can be attributed to the efficiency of cellulase-cellulose interactions, cellulose is often treated as unchanging in these models. Cellulases must bind productively to cellulose, i.e. adsorb and complex with an accessible cellulose surface chains, before hydrolysis can proceed. We have shown substrate processing history impacts the productive binding capacity (i.e. the maximum number of productive binding sites/mass of substrate) for the exocellulase *Trichoderma reesei* Cel7A (*Tr*Cel7A), which limits initial cellulose hydrolysis rates. Furthermore, the depletion of productive binding sites on cellulosic substrates limits extended-time hydrolysis rates. To understand the physicochemical implication of how enzyme action depletes productive binding sites on cellulose, we are studying the evolving properties of cellulose from varying sources and processing histories during enzymatic hydrolysis by *Tr*Cel7A using synchrotron infrared nanospectroscopy (SINS) in conjunction with far field synchrotron FTIR (sFTIR). The combination of a variety of experimental scales (SINS: ~20nm resolution, sFTIR: ~2 µm resolution, and productive binding capacity: bulk biochemical assay) allows us to identify the physicochemical properties of cellulose which promotes hydrolysis by cellulases throughout hydrolysis and evaluate their molecular level origins to inform a more concrete definition of the physical meaning of a productive binding site.

M23 Ethanol production from coffee mucilage fermentation by *S. cerevisiae* immobilized in calcium-alginate beads

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One of the main challenges in the coffee industry is the generation of residual by-products, which are commonly released during coffee bean processing. The green coffee bean is ordinarily extracted for brewed coffee while un-used components are removed and discarded, estimating a generation of 15 million tons of residues per year, which leads to environmental pollution. Our recent study reported an optimization and scale-up of coffee mucilage fermentation that enables it to be directly fermented into ethanol. In order to follow up on our previous work, all fermentation runs were consecutively carried out with the aim to convert sugars to ethanol in recyclable systems with calcium alginate-entrapped *S. cerevisiae* cells. Several tests were performed via alginate-entrapped cells in different bead sizes (3 or 7 mm) combined to alginate concentrations (2-4%). The highest yield of 0.33 g ethanol/g sugar was achieved at 18 h using alginate-entrapped cells in 3 mm diameter and 2% (w/v) alginate, which corresponded to 64% of the theoretically achievable yield of ethanol. Tests with 7 mm beads took 6 h longer to reach maximum ethanol productions. The reduction of bead size increased mass transfer of substrates from the liquid to the immobilized cells, accelerating sugar consumption and ethanol production. Entrapment, enabled re-use of the cells for up to 3 consecutive batches with stable ethanol production (up to 72 h). Continuous fermentation using immobilized cells highlights a potential application for effective ethanol production, confirming the alcohol fermentation of coffee mucilage is feasible without any pretreatment and nutrition supplement.

M24 Identification of laboratory-evolved *Saccharomyces cerevisiae* HJ7-14 with efficient ability of algae-based ethanol production

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The goal of this work is the characterization of laboratory-evolved *Saccharomyces cerevisiae* HJ7-14 by multi-omic approaches. Genome sequencing of the HJ7-14 showed a point mutation in the *GAL83* gene (G703A) involved in the catabolite repression as well as the galactose metabolism. Cultural and transcriptional analyses of a *S. cerevisiae* mutant with chromosomal *GAL83* (G703A) indicated that the catabolite repression onto the galactose metabolism was relieved in all cell growth phases.

Untargeted metabolomic approach revealed that metabolic phenotypes between the control D452-2 and HJ7-14 strains were clearly separated in time-dependent manner. Especially in early growth stage at 6 h, the HJ7-14 showed dramatic and coordinated changes in central carbon and amino acid metabolisms. Through metabolomic re-organization, fold changes in fatty acid metabolism and metabolites related to stress response system were also found upon glucose depletion and active galactose utilization. Multi-omic characterization using genome sequencing, transcription, and metabolome profiling clearly unveiled that the *GAL83* gene mutation partially relieved glucose-dependent catabolite repression and allowed the evolved HJ7-14 to efficiently convert algal sugars to ethanol. We demonstrated conclusively that multi-omic approach could be a promising tool for engineering of *S. cerevisiae* able to covert red algal biomass to other biofuels and biochemicals.

M25 Enhanced bioconversion of (*E*)-11-(heptanoyloxy) undec-9-enoic acid from ricinoleic acid by recombinant *Escherichia coli* through continuous supply of glucose and glycerol

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The aim of study is the development of bioransformation strategies with supplying energy sources for bioconversion of ricinoleic acid to (*E*)-11-(heptanoyloxy) undec-9-enoic acid (11-HOUA), a key intermediate of brassylic acid, by recombinant *Escherichia coli* overexpressing an alcohol dehydrogenase from *Micrococcus luteus* and a Baeyer-Villiger monooxygenase from *Pseudomonas putida* KT2440.Supplying glucose or glycerol facilitated both the preparation of high-density cell biocatalyst and supply of the NAD^+ and NADPH cofactors. By the glucose supply strategy, 30.8 g/L of the engineered *E. coli* cells produced 29.7 mM of 11-HOUA with 1.9 mM/h of productivity, which were 1.8 and 1.6 times higher than the same biotransformation without supplying glucose, respectively. Intermittent addition of glycerol increased 11-HOUA productivity by 16% compared to that by the glucose feeding. Finally, 34.5 mM of 11-HOUA concentration, 77% conversion and 2.2 mM/h productivity were acquired using 31.6 g/L of cell biocatalyst along with the glycerol supplement. This result suggested that supplementation of additional carbon sources in biotransformation process would be a valuable strategy to increase the performance of fatty acid biotransformation.

M26 A Quantification Method for C1-specific Cellulose Active LPMOs

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Background: Lytic polysaccharide monooxygenases (LPMOs) have received much interest among other due to their ability to boost the degradation of lignocellulose when combined with canonical cellulases. LPMOs produce an array of oxidized products, both soluble and insoluble, from their polymeric substrates [1]. The detection of these products is often based on MS/HPLC-methods, however, the heterogeneity of the products, the lack of commercial standards, and the insolubility of the substratescomplicate the identification and quantification [2].A number of assays for the quantification of LPMO products have been developed to circumvent some of these challenges, however, most assays can only measure LPMO activity on model substrates.

Results: In the current work we investigate the applicability of a β-glucosidase-assisted method to quantify the release of C1-oxidized products from cellulose and lignocellulose substrates incubated with *Tt*LPMO9E, by quantification of gluconic acidwith an already established UV-based method. We demonstrate that the method can be used to quantify the LPMO reaction on a wide range of substrates.

Conclusions: We describe a high-throughput assay for the quantification of cellulose active C1-oxidizing LPMO products. The versatility of the assay can make it useful in a wide range of experiments in biomass saccharification research, such as comparing the activity of different C1-oxidizing cellulose active LPMOs, comparing the LPMO activity on different substrates, testing promoters or inhibitors of LPMO etc.

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M27 The effect of high dry matter content and enzyme family on fibre modification

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Cellulose is the most abundant renewable biomass resource on Earth and thus a more widespread use of cellulose in material and chemical applications is appealing. However, the dense and hierarchical structure of cellulose in wood pulp needs to be unwound using pretreatments to enable subsequent cellulose modifications. A fibre pretreatment, combining mechanical and enzymatic action was studied using softwood kraft pulp. The role of enzyme family in fibre modification at high consistency

(20% w/w) was assessed using endoglucanases from three structurally different glycoside hydrolase families. After the enzymatic treatment, cellulose reactivity and accessibility was evaluated by several methods that measure accessibility or the tendency of cellulose to dissolve. Endoglucanase from the glycoside hydrolase family 45 was found superior in targeted modification of the fibres.

M28 Synthesis of kanosamine from D-glucose by E. coli

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Kanosamine (3-amino-3-deoxy-D-glucose) is exported as a monomer by several species of *Bacillus* and displays activity against oomycetes and various fungal species. Kanosamine is also a subunit of aminoglycoside antibiotics and the nitrogen atom source in the mC_7N subunit of ansamycins and mitomycins, including rifamycin. Two pathways for kanosamine biosynthesis have been previously described: one begins from D-glucose-6-phosphate and one proceeds from UDP-D-glucose. In this study we report that heterologous expression of glucose-6-phosphate 3-dehydrogenase (EC 1.1.1.361), 3-oxo-glucose-6-phosphate:glutamate aminotransferase (EC 2.6.1.104), and kanosamine-6-phosphate phosphatase (EC 3.1.3.92) from *B. subtilis*and separately from *B. pumilus*enables synthesis of kanosamine by *Escherichia coli*, an organism that does not naturally synthesize kanosamine. This research validates kanosamine biosynthesis in these *Bacillus* strains proceeds via the D-glucose-6-phosphate pathway and could have a significant impact on biocatalytic synthesis of antifungal, antibacterial, and antiviral agents.

M29 Cellulosome plasticity - size, shape, and subunit functionality imaged by transmission electron microscopy

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The highly efficient biomass deconstruction by *C. thermocellum* is believed to be due to its powerful cellulosomal system, the multi-enzyme system that utilize scaffolding proteins to assemble multiple functional CAZyme subunits into a biomass degrading macromolecular complex. Proposed as the cellulosome plasticity theory, the dynamics in both composition and structure of the cellulosomes and the ternary cell-cellulosome-substrate complex may explain the high deconstruction efficiency of *C. thermocellum* cells vs free enzymes. Cellulosomes appear as protuberances on the *C. thermocellum* cell surface and as extensive protracted protuberances connecting cells to substrate surfaces. Cellulosomes can also become detached from the cell surface and act on biomass away from the microbe. Transmission electron microscopy (TEM) has revealed that more cellulosomes are produced as protuberances on cell surface when *C. thermocellum* was grown on biomass instead of cellobiose. TEM also revealed drastic size and shape variations of the cellulosomal system. Single cellulosome consisting ~ 10 subunits were observed in folded and extended forms. Poly-cellulosomes consisting of multiple single cellulosomes can have size up to a few microns. Although TEM images showed possible single cellulosome and poly-cellulosome still attached to cell surfaces, the catalytical subunits appeared to act away from the scaffolding proteins. Immuno-EM labeling of CBM3a, Cel8a, Cel48s and OlpB subunits suggests that these components might not always be attached to the microbe cell. It is possible for maximum substrate accessibility that subunits can be released from (poly-)cellulosomes and separated to better diffuse into the substrate which are usually not accessible for large complexes or microbes.

M30 “Plug and Play" Cell-Free Systems to Compartmentalize Production of Biochemicals

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Several key factors negatively impact the production yield, and thus, cost of biochemicals from renewable sources. Common hindrances in the biological production of chemicals are 1) microbial toxicity of end-products (or intermediates), 2) loss of carbon to microbial biomass formation, 3) co-production of undesired byproducts, and 4) costly or complex product separation steps. Therefore, a particularly attractive alternative is to eliminate the biocatalyst entirely and instead operate the desired metabolic pathway in isolation, thus circumventing these roadblocks. However, traditional cell-free technology (CFT) using free enzymes *in vitro* suffers from low product productivities and titers, owing in part to limitations from modest (cytosolic-like) enzyme concentrations, diffusion of intermediates within metabolic pathways, lack of long term enzyme stability, cofactor cost or inefficient recycling rates, product separation issues, and finally, the cost of enzyme production/purification. We propose that the second generation biochemicals industry requires cost competitive and tractable cell free approaches as versatile platforms that combine flexibility and robustness and offer opportunities for significant reduction in processing costs. Moreover, production of a broad range of chemicals could eventually be possible using CFT. To achieve this, it is essential to develop a new platform that relies on a cost effective enzyme production/arraying technology that demonstrates high specificity, long enzyme lifetime, and can self-assemble to facilitate protein purification and pathway production. Here we present an enzyme immobilization/arraying method with *in vivo* self-assembly to an engineered protein scaffold that allows for easy purification, improved yield and consequently more efficient cell free conversion of feedstocks to biochemicals.

M31 Digestion of variously recalcitrant unpretreated corn stover by *Clostridium Thermocellum*

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The thermophilic, anaerobic bacterium *Clostridium Thermocellum* has been increasingly investigated not just for its basic cellulosic properties, but as a front runner for consolidated bio-processing (CBP) and co-treatment (CoT). It's high optimum temperature (55-60°C) coupled with the energy-saving lack of aeration and ability to digest unpretreated biomass means process kinetics can be high, while process costs low. However, for either of these routes to be useful at industrial scale, any prospective organism's ability to digest a variety of biomass compositions must be known. Considering corn stover's variation between both geographical and seasonal harvests, any large scale feedstock may diverge largely between lots. This study compares *C. Thermocellum*'s ability to digest unpretreated corn stover of differing recalcitrance, compared to traditional enzymatic hydrolysis.

M32 Organosolv pretreatment of eucalyptus sawdust to enhance enzymatic cellulose hydrolysis and lignin recovery

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The growing interest for bio-based material from lignocellulosic biomass has promoted an increased use of lignin in various applications. Organosolv has shown to be an interesting pretreatment to produce high purity and low molecular weight lignin, while recovering a solid residue rich in cellulose.

Organosolv pretreatment was used for selectively extract lignin from eucalyptus sawdust, obtaining a pretreated solid suitable for enzymatic hydrolysis. Organosolv pretreatments with ethanol were performed using a batch type reactor under the following conditions: temperature of 170 and 180ºC, reaction times of 15 to 90 min, ethanol concentration of 50 and 75%, and liquid to solid ratio of 8 g/g. Lignin was precipitated from the organosolv spent liquors under four different conditions (pH 2 and 5; temperature 25ºC and 60ºC), selecting the one that presented the highest yield (pH 2, 60ºC). Characteristics of precipitated lignins were evaluated by performing phenolic hydroxyl groups content determination, molecular size distribution by GPC, Syringyl/Guaiacyl (S/G) ratio determination by oxidation with nitrobenzene and ATR-FTIR spectra. Organosolv lignin resulted in low content of phenolic hydroxyl groups, high average molecular weight with high polydispersity and low S/G ratio, in comparison with those values observed in eucalyptus lignin from the kraft process commonly used in the cellulose pulp production industry. Pretreatment liquors were also characterized for other value-added products (xylose, xylo-oligomers, acetic acid). Finally, pretreated solids were subjected to enzymatic hydrolysis (pH 4.8, 16% solids, 25 FPU/$g_{cellulose}$, 48ºC, 72 h) achieving cellulose to glucose conversions of 20% to 82%.

M33 Comparison of base-catalyzed depolymerization of lignin-enriched residues from multiple biochemical conversion processes, feedstock processing processes and biomass sources

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To realize the promise of industrial-scale lignocellulosic biorefineries, effective strategies for lignin valorization are required. Lignin quantity and quality can vary significantly across feedstocks and the type of process applied to biomass, most often to extract polysaccharides. 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). To assess lignin susceptibility to BCD, we determined the carbohydrate yields resulting from deconstruction steps and the yields of solid, aqueous, and gas fraction from base-catalyzed depolymerization (BCD) of the post-EH solid residues, and chemical analyses were subjected to each BCD fraction.

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 blend feedstock has higher sugar and lower lignin contents than CS/DMR-EH. This suggests that sugar content effects more on the yield of aqueous fraction than lignin content. There was no significant difference between the pelleted and non-pelleted samples. Based on GPC analysis, BCD solid residues from non-pelleted/pelleted DAP-EHs have higher molecular weight than those from DMR-EHs. In this work, detail effects of biochemical conversion processes, biomass sources, and feedstock processing processes on BCD results are discussed.

M34 Preparation of biopolyol from lignin residue for biopolyurethane production

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Due to its high abundance and low price, lignin is considered as a potential chemical feedstock for chemicals production, especially for the replacement of petroleum-based polyol. In this study, two step acid-base catalyzed liquefaction process was conducted for the preparation of biopolyol with low acid number, and its application to biopolyurethane production. From low grade lignin from EFB, biopolyol with molecular weight more than 4000 g/mol and hydroxyl number higher than 800 mg KOH/g was prepared. The biopolyol was used to produce biopolyurethane elastomer and foam with different isocyanates. Thermal

stability, compressive strength and density of biopolyurethane were analyzed to evaluate the technological feasibility of using biopolyol for biopolyurethane production.

M35 Protein-protein interactions sequester monounsaturated acyl-CoAs from fatty acyl-CoA reductase in fatty alcohol production

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Fatty alcohols are used industrially, amounting to a demand that is expected to grow to 3.5 million tons by 2020. By introducing a heterologous fatty acyl-CoA reductase (FAR1) gene into the yeast Saccharomyces cerevisiae, it is possible to induce fatty alcohol production in a fastgrowing organism at a low cost to the environment. One of the fatty alcohol products, monounsaturated palmitoleyl alcohol, is a high value but low abundance oleochemical. Although the yeast produces large amounts of monounsaturated acyl-CoA most of the fatty alcohols synthesized are saturated. Far1 displays no preference for saturated acyl-CoA in vivo and so we hypothesize that once acyl-CoA is desaturated by Ole1 it becomes sequestered into native lipid synthesis pathways and is not accessible to Far1. This was investigated by testing for proteinprotein interactions among proteins required for synthesis of lysophosphatidate, phosphatidate and triacylglycerol. Preliminary data from membrane yeast 2-hybrid studies supports the contention that protein-protein interactions within the cells channel acyl-CoA towards fates not related to fatty alcohol production. Interactions were found between Ole1 and Dga1, the terminal enzyme in the triglyceride synthesis pathway, as well as Ole1 and Slc1, a protein that acylates lysophosphatidic acid. Further research aims to determine the proteins in complex with Ole1 through a co-immunoprecipitation and direct interactors through rabbit reticulocyte assays. This project aims to decipher the regulation of lipid biosynthesis in yeast and determine how best to disrupt these interactions to increase the flux of unsaturated acyl-CoA toward fatty alcohol synthesis.

M36 Genetic engineering of *Aspergillus pseudoterreus* for 3-hydroxypropionic acid production within the Agile BioFoundry

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The organic acid, 3-hydroxyprionic acid (3HP) is a platform chemical that can be converted into various additional high value chemicals, such as, acrylonitrile. Biological fermentation is the main route for 3HP production and has been actively investigated for its productivity improvement in the past decade. Naturally, there exist several 3HP production processes, such as, glycerol fermentation, acrylic acid degradation, CO2 assimilation, and uracil catabolism. However, the yield and efficiency of 3HP production in native microorganisms are low. Therefore, genetically engineered industrial microorganisms with novel 3HP production pathways have been examined for improvement of 3HP production. The industrial filamentous fungus *Aspergillus pseudoterreus* is well known as a platform strain for itaconic acid production and is genetically tractable. In this study, the genes of aspartate decarboxylase, beta-alanine-pyruvate aminotransferase, and 3-hydroxypropionate dehydrogenase under the control of constitutive promoters were introduced into the *cis*-aconitate decarboxylase gene locus in *A. pseudoterreus*. The selected transgenic *A. pseudoterreus* strains can accumulate about 1.8 g/l of 3HP in shake-flask cultures. The culture conditions for 3HP production in *A. pseudoterreus* were also optimized. Over-expression of pyruvate carboxylase and aspartate aminotransferase under the control of constitutive promoter were further examined for their effects on 3HP. The results indicate that *A. pseudoterreus* can serve as an industrial filamentous fungus host for the 3HP production.

M38 Effects of Biomass Comminution on Hot Water and Dilute Acid Pretreatment

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Comminution of lignocellulosic biomass, such as milling and cutting, results in different biomass shapes and sizes, thus significantly affects sugar yields of pretreatment and sequential enzymatic hydrolysis as well as the overall cost competitiveness. Kinetics of hot water-only and dilute acid pretreatments followed by enzymatic hydrolysis of hardwood (e.g. poplar wood) and softwood (e.g. Douglas fir) substrates with different particle sizes resulted from two different cutting approaches (e.g. Shards, Cubic prismatic) were investigated in this study. Results showed enzymatic hydrolysis of pretreated biomass, the rotary shear cutting showed 5-15% higher sugar yields. It was found that the total sugar yields of biomass with different particle sizes prepared by two cutting methods at similar particle sizes were comparable. On the contrary, the energy consumption of crumbling was three times less than traditional hammer milling. This study provides new insights in developing novel biomass preprocessing methods.

M39 Hot Water-Only and Alkali Flowthrough Pretreatment of Softwood

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Flowthrough pretreatment provides valuable insights in the fundamentals of deconstruction of plant biomass. In this study, the potential softwood degradation pathways under water-only and alkali conditions were determined by elucidating the

deconstructed biomass-derived products at 0-270 °C for 2-10 min at a flow rate of 25 mL/min, at initial pH of 6.4 (water-only), 8, 9, 11, and 12, respectively. Results suggested that the initial pH provided a convenient indicator along with the severity parameter for the potential biomass degradation pathways by flowthrough pretreatment, including non-oxidative (pH<9) and oxidative (pH≥9) pathways. Up to 100% hemisugars, 90% cellulose, and 70% lignin yielded under the non-oxidative conditions (pH<9) with the severity parameter $LogR_0$ around 5.5, respectively. On the contrary, at pH 12, the oxidative degradation resulted in pretreated hydrolysate rich in monomeric and oligomeric phenolic products as well as glycolic acid, acetic acid, and formic acid from carbohydrates. The 2D ^{1}H-^{13}C NMR revealed that unlike non-oxidative degradation (pH<9)leading to mainly cleavage of β-O-4 linkages, all of β-β, β-5, and α-O-4 linkages were cleaved while substantial aromatic rings with limited β-O-4 structures were remained at pH12.

M40 Oxidation of Lignin-Rich Residue from Deacetylation, Mechanical Refining, and Enzymatic Hydrolysis of Lignocellulose

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Production of aromatic aldehydes, such as vanillin, from lignin, has been an intriguing process for decades, but widespread implementation has been inhibited by the high hydroxide:lignin ratios required for significant aldehyde yields, as well as the degraded chemical structure of industrial lignins. In this work, we explore oxidation of the lignin-rich residue isolated from corn stover after deacetylation, mechanical refining, and enzymatic hydrolysis (DMR lignin), including a survey of reaction conditions and detailed product characterization that accounts for greater than 60% of substrate carbon as quantified products (aromatic monomers, aliphatic acids, and carbonate) and the remainder as an oligomeric oil. We also discuss strategies for maximizing selectivity to desired products and separating them from the alkaline solution.

M41 Production of nutraceutical value fatty acids (eicosapentaenoic acid and docosapentaenoic acid) from algae by using forest biomass

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The long-chain omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are considered as essential fatty acids as they cannot be synthesized by humans but have to be provided through the diet. As their production from fish oil is environmentally unsustainable, there is demand for new sources of omega-3 fatty acids. The aim of the present work was to establish a microalgae platform for the production of nutraceutical-value omega-3 fatty acids from forest biomass. To this end, we chose the use of two significant tree species, namely Norway spruce and silver birch that were pretreated with a hybrid organosolv-steam explosion method and hydrolyzed with commercial cellulolytic enzymes prior to algae cultivation. Initially we tested the growth of *Phaeodactylum tricornutum* under autotrophic mode cultivation and further compared with glucose synthetic media as well as birch and spruce hydrolysates under mixotrophic condition. The total lipids produced were similar among the different substrates for mixotrophic growth (1.21-1.29 g/L), which was higher than the results obtained in autotrophic cultivations (0.57 g/L). The highest EPA production (256 mg/L) and productivity (19.69 mg/L/d) were observed on spruce hydrolysates with the corresponding values for DHA to be 63.08 mg/L and 4.85 mg/L/d, respectively. These values were considerably higher than those obtained during autotrophic growth for EPA (79.80 mg/L and 6.14 mg/L/d, respectively) and DHA (9.75 mg/L and 0.75 mg/L/d, respectively). To the best of our knowledge, this is the first report where forest biomass is used for microalgae growth and production of nutraceutical lipids.

M42 Comparative Proteomics Studies of Non-Model Yeasts to Understand Low pH Tolerance Mechanisms in Hydrolysate Cultivations

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Industrial production of platform chemicals and fuel precursors from lignocellulose represent a more desirable alternative to those processes based on petrochemical sources. However, the use of model microorganisms in these applications could be limited for the adaptability of the microbial chassis to the specific culture conditions. Stressors, including pH, substrate inhibition, temperature, osmolarity, or optimal downstream conditions, could hinder the efficiency of the complete process. Although non-model organisms can usually provide solutions to overcome all these challenges, it is often essential to study and characterize their specific capabilities to develop efficient procedures. In this work we evaluated the low-pH adaptation cellular machineries of the three different acid tolerant yeasts *Pichia kudriavzevii*, *Zygosaccharomyces parabailii*, and *Dekkera bruxellensis* during fermentation of corn stover hydrolysate under anaerobic conditions. Comparative proteomics studies were performed for the three yeast strains at pH 5.5 and 3.0, and analyzing the data collected, sets of 194, 220, and 430 differentially and uniquely expressed proteins were detected within *Z. parabailii*, *P. kudriavzevii*, and *D. bruxellensis*, respectively. Among these proteins, we were able to identify individual and common cellular mechanisms that could be involved in the low-pH tolerance. The targets identified from the three selected strains were further explored to determine whether modulation of their expression was sufficient to affect acid tolerance in *Saccharomyces cerevisiae*.

M43 Homoethanol production from glycerol and cellobionate using recombinant *Klebsiella oxytoca* strains

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Cellobionic acid, an oxidized cellulose degradation product, could be produced from cellulosic biomass. Glycerol is a cheap and renewable resource for fuels and chemicals production and is available as a byproduct of biodiesel production. Cellobionate is a more oxidized substrate than cellobiose, whereas glycerol is a more reduced substrate than glucose. While the production of homoethanol from cellobiose can be achieved, the conversion of cellobionate to ethanol is accompanied by the production of oxidized byproduct such as acetate, and reduced byproducts such as 1,3-propanediol are produced along ethanol when glycerol is used as the carbon source. When cellobioante and glycerol were used as the sole carbon source by *Klebsiella oxytoca* BW 21, ethanol yield was about 62%-67%. Co-utilization of both cellobionate and glycerol in batch fermentation increased the yield of ethanol to about 77% and decreased by-product accumulation (such as acetate and 1,3-propanediol) substantially. However, the rate of cellobionate utilization was substantially slower than glycerol. The strain WT26 was constructed by deleting the *pta, frd, ldh, pflA,* and *pduC* genes in strain BW21. Strain BW26 can barely use glycerol fermentatively as the sole carbon source. However, the strain can use glycerol and cellobionate simultaneously and the ethanol yield from the glycerol and cellobionate mixture was about 95%.

M44 Innovative Pretreatment and Fractionation to Transform Biorefinery Design for Lignin-based Products

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The utilization of the lignin-containing biorefinery waste as feedstock for renewable products offers a unique opportunity to improve the sustainability and economic viability of lignocellulosic biorefinery. Nevertheless, the fundamental understanding of relationship between lignin chemistry and processibility indicated that traditional pretreatment and fractionation strategies need to be tailored to derive more reactive lignin for bioconversion and biomaterials. In particular, we have shown combinatorial pretreatment at low holding temperature can derive lignin with less inhibitor, diverse molecular weight, fermentable monomers and oligomers. In addition, we have also established low concentration of sugar can synergize with lignin to improve biorefinery performance. Based on the discovery, we have advanced innovative fractionation strategies to break down the LCC structure and release carbohydrate in biorefinery waste, which enabled record bioconversion yields for various products from lignin-containing biorefinery waste. In addition, innovative pretreatment and fractionation strategies were developed to tailor lignin chemistry for stable and uniform nanoparticles. Overall, these innovative pretreatment and fractionation strategies can transform the biorefinery design, where lignin reactivity for bioconversion and biomaterial can be improved to enable value-added biproducts to maximize economic return and sustainability.

M45 Nature inspired reductive cleavage of lignin β-O-4 bonds using organic thiols

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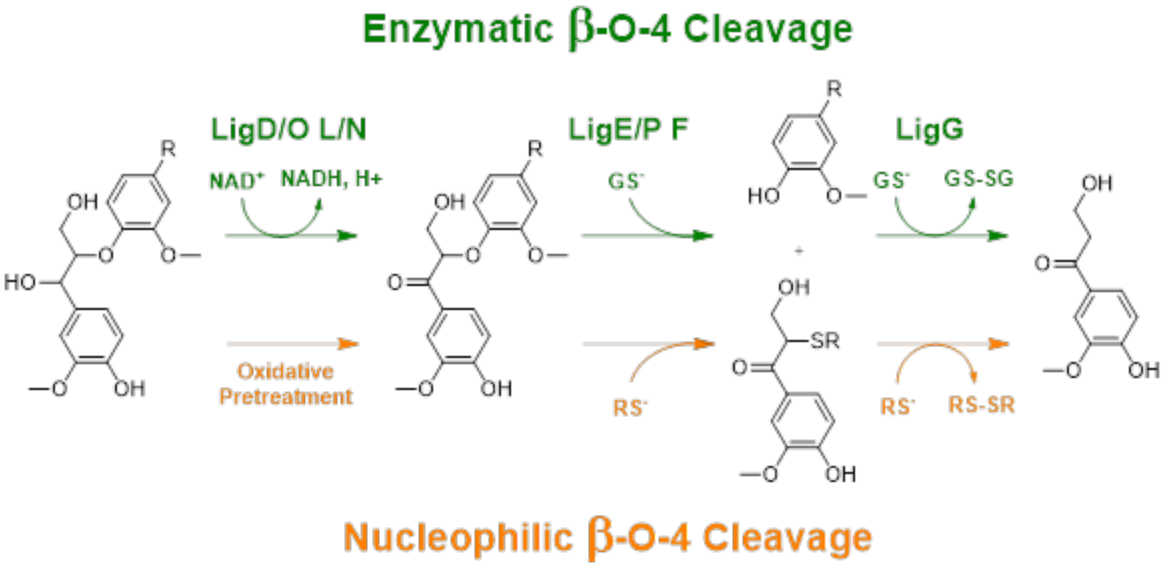
The replacement of petrochemicals with lignin is challenging due, in part, to the expensive inputs and/or caustic depolymerization techniques typically required. Nature provides examples of lignin deconstruction that can be modeled for a simple and green cleavage method. In the β-aryl ether cleavage pathway of wood degrading bacteria, the cleavage of β-O-4 bonds occurs in a three-step process: 1) oxidation of the α-carbon hydroxyl group, 2) glutathione nucleophilic attack on the β-carbon, displacing the phenoxide, and 3) reduction of the above-formed glutathione ether's S-C bond with a second glutathione, releasing the second lignin fragment and the glutathione disulfide. This work focuses on replicating the nucleophilic and reductive chemistry of the protein pathway using only small organic thiols without the aid of proteins or metals. A range of ether bonds were tested to determine necessary functionality needed for cleavage. Among these findings, the most important functional group needed was the oxidized alpha hydroxy group, mimicking the requirements of the protein being modeled. In addition, adding functionality to the aromatic ring (e.g., authentic S-S and G-G dimers) did not prevent the β-aryl ether cleavage. Yields of 30-100% were obtained from the oxidized lignin dimer models treated with thiol. Importantly, applied to real lignin, the process achieves approximately 68% molecular weight reduction. This work exemplifies a reductive biomimetic approach to lignin depolymerization by mimicking the nucleophilic thiol-mediated ether cleavage found in the enzymatic β-aryl ether cleavage pathway.



M46 Scale-up from lab to pilot scale of a simple and mild acid pretreatment process of hard-wood to lignocellulosic sugars

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Cellulose and hemicellulose in biomass, are potential sources of sugar to produce biochemicals, biofuels and biomaterials in industrial fermentative processes. Mild-acid pretreatment, followed by an enzymatic hydrolysis step belongs to the group of most economical and feasible technologies (Harmsen et al. 2013).

Within the BIOFOREVER EU project, optimal process conditions for mild acid pretreatment were selected on lab scale for four types of wood (see abstract of Smits et al, entitled “Fast optimization of acid-pretreatment conditions in the conversion of wood to lignocellulosic sugars using the Estimated Residual Glucan level”).

The results of the screening were used in scaling up the pretreatment and enzymatic hydrolysis processes to pilot-scale of about 20 kg wood/h. The basic set-up of the pretreatment at BPF consists of a two-stage pretreatment process. However the results of the screening and considerations for simplicity and operability drove the selection for a one stage process.

During one-stage pilot trials we confirmed the optimal process conditions determined on the lab resulting in a feasible continuous process on pilot scale, including the projected enzymatic hydrolysis results. The pretreated slurry was easily processed and, after enzymatic hydrolysis, the solid/liquid separation was straightforward and effective. The resulting sugar syrup was demonstrated to be well fermentable. Details of the scale-up process and results will be discussed in the presentation.

We want to thank **Bio-Based Industries Joint Undertaking who supported us within the Bioforever project and our partner in this project DSM.**

Harmsen P., Lips S., Bakker R., 2013. Pretreatment of lignocellulose for biotechnological production of lactic acid. Research review Wageningen UR Food & Biobased Research, Wageningen, The Netherlands

M47 Fast optimization of acid-pretreatment conditions in the conversion of wood to lignocellulosic sugars, using the Estimated Residual Glucan level.

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Lignocellulosic biomass can be a potential source of sugar to produce biochemicals and biofuels in industrial processes. This can be an alternative for sugar currently made from starch and sucrose; herewith reducing the demand for food-grade sugar in non-food applications.

Mild-acid pretreatment, followed by enzymatic hydrolysis, is one of the most economically feasible technologies to release sugars from biomass. The variation in biomass with according variation in composition; and the natural variation within each type of biomass makes it challenging to predict optimal pretreatment process conditions to obtain maximal sugar output.

We developed a quantitative method for screening optimal pretreatment settings and applied it to four types of wood: soft wood, hard wood and mixtures thereof. The method judges sugar release after enzymatic hydrolysis in a set of more than 30 differently pretreated samples and can be concluded in a 5-day work week per type of wood.

The method developed uses the Estimated Residual Glucan (ERG) level after pretreatment as variable to evaluate pretreatment settings by sugar release after pretreatment and enzymatic hydrolysis. The results show the potential of ERG to define optimal pretreatment conditions, enzyme activities and process settings. Moreover, the method can be used to control the effect of

batch-to-batch variation on sugar production.
The work was executed in conjunction with Bioprocess Pilot Facilities (BPF) within the BioForEver project (BIO-based products from FORestry via Economically Viable European Routes), and received funding from the BioBased Industries Joint Undertaking under the European Union's Horizon 2020 research and innovation program.

M48 Developing novel continuous enzymatic hydrolysis processes for lignocellulosic biomass deconstruction

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Continuous enzymatic hydrolysis processes have been suggested as a method to improve overall process performance in industrial biorefineries. In this work, we present work towards demonstrating a novel configuration for continuous enzymatic hydrolysis. This configuration allows for 'high solids'-like final product concentrations while minimizing the solids liquefaction step, thus removing the need for specialized solids hydrolysis reactors. In this work we show how the process effects total hydrolysis yields, enzyme utilization, and conversion rates for acid pretreated spruce produced in a pilot scale pretreatment reactor, as compared to a standard high solids batch hydrolysis. We present our most recent findings, and discuss the possible merits and difficulties with such an approach. We as well present work discussing the nature of the liquefaction stage with regards to biomass water interactions, and constraint of water by pretreated lignocellulosic biomass.

M49 Potential of Ensiled Sorghum Biomass as Feedstock for Bioethanol Production

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The development of a cost-efficient lignocellulosic biomass deconstruction process with high sugar conversion yields is key to the success of bio-based industries. Pretreatment is required to reduce biomass recalcitrance, which typically involves severe physical or chemical approaches coupled to subsequent enzymatic hydrolysis. Even though these pretreatment methods are effective, they are also costly and energy-intensive. On the other hand, ensiling is a well-known method for preserving green biomass through the anaerobic production of organic acids, a process that could reduce the required pretreatment severity and improve enzymatic conversion of cellulose. Specifically, dry ensiled sorghum biomass was identified as a promising candidate because it contains ~15% of organic acids (mainly lactic and acetic acids). Therefore, this study explored the impact of pretreating ensiled sorghum using biocompatible cholinium-based ionic liquids (IL) in a 'one-pot' configuration, as a strategy to minimize IL and enzyme loadings. Enzymatic saccharification of the pretreated sorghum silage released 87% and 85% of the maximum theoretical yield of glucose at 10% and 5% IL loading rates, respectively. Additionally, the ensiled biomass generated higher glucose concentrations than the dry biomass, and it was determined that the glucose yield observed with the low (5%) IL concentration could reduce the minimum selling price of ethanol by 19%, relative to the higher (10%) IL load. These results indicate that the ensiled biomass could be a potential solution to decrease pretreatment severity, while increasing sugar yields and reducing the minimum selling price of a desired biofuel.

M50 Evaluation of corn steep liquor (CSL) addition on isopropanol-butanol (IB) production from sugarcane-sweet sorghum juices by *Clostridium beijerinckii* DSM 6423

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Both isopropanol and butanol are important bulk chemicals with a large market which also present good properties to be used as biofuel. They can be produced by isopropanol-butanol fermentation. CSL is a byproduct of the wet milling of corn process, rich in protein, vitamins and minerals. It has been reported that it can act as a low-cost source of nitrogen and minerals. The main aim of this work was to evaluate CSL addition as nutrient on isopropanol and butanol production from an industrial mixture of sugarcane (75%) and sweet sorghum (25%) juices by *Clostridium beijerinckii* DSM 6423. Fermentations were carried out in bottles of 250 mL with 100 mL of medium. Addition of CSL as a replacement of both commercial vitamin complex (Dispert®) and yeast extract was evaluated. Different CSL concentrations were also studied (5, 10, 15, 20 g/L). A control fermentation supplemented with yeast extract (1 g/L), buffer and mineral (P2) stock solutions and a commercial vitamin complex (1% (v/v)) was performed. Fermentation conditions were 35ºC, 150 rpm, and initial pH 6.0. The results showed that CSL could replace both vitamin complex and yeast extract. No significant difference was observed for solvent concentration, yield and productivity for different CSL concentrations ($p > 0.05$). Butanol and isopropanol concentrations of 6.9 and 3.4 g/L were obtained (total solvent 13.1 g/L). Solvent yield and productivity were 0.34 g/g and 0.26 g/Lh, respectively. The supplementation of the media with 5 g/L of CSL could be a cheaper alternative for isopropanol and butanol production.

M51 Evaluating the impacts of alkaline pre-extraction during two-stage Cu-AHP pretreatment on enzymatic hydrolysis and lignin properties in diverse hybrid poplar cultivars

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We have recently developed a two-stage pretreatment comprising an alkaline pre-extraction followed by a Cu-catalyzed alkaline hydrogen peroxide (Cu-AHP) treatment that is effective at pretreating hardwoods at relatively mild reaction conditions. We

previously demonstrated that this process is capable of achieving high yields of sugars from enzymatic hydrolysis and high yields of aromatic monomers from the catalytic depolymerization of lignin. In this work, we focus on characterizing how biomass source and reaction conditions used during the alkaline pre-extraction impact the subsequent processing stages as well as lignin yields and properties. Specifically, five hybrid poplars were subjected to alkaline pre-extraction under various conditions including differences in time (15-300 min), temperature (90-155 ^{o}C), alkali loading (0.1-0.2 g/g biomass), and solid to liquid ratios (10-20% wt/v) and the impact on lignin recovery and purity was determined. Empirical models were developed to relate reaction severity to lignin extraction and recovery during the pre-extraction stage. For select conditions, lignin properties were assessed including β-O-4 content determined by ^{13}C NMR, molecular mass distributions as determined by gel permeation chromatography, and susceptibility to depolymerization to aromatic monomers using thioacidolysis and formic acid-catalyzed depolymerization. These lignin properties are demonstrated to be functions of the pre-extraction conditions utilized. Finally, the alkaline pre-extracted biomass from select conditions was evaluated for its response to the subsequent Cu-AHP treatment and enzymatic hydrolysis and the overall impact of processing conditions on process economics are determined through a technoeconomic analysis.

M52 Process benefits of using biomass pellets in a biorefinery

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Consistent supply of low-cost biomass with cost effective processing is necessary for commercializing cellulosic biofuels or other biobased chemicals. Biomass pelleting is one option to simplify biomass supply chain logistics and minimize downstream processing costs in a biorefinery. Work from our research group had previously shown that use of pelleted biomass with soaking in aqueous ammonia pretreatment (SAA) enables higher pretreatment solid loadings and reduced pretreatment severity, enzyme loadings, and hydrolysis time. This study compares hydrolysis results using loose and pelleted biomass across a range of low to high severity pretreatment conditions and enzyme loadings. Experiments were conducted to determine the conditions that result in 90% glucose yields within 48 hours. Use of pelleted biomass allows flexibility to reduce pretreatment severity, enzyme loadings, hydrolysis time, or combinations of these. Economic factors which will impact optimal processing conditions include capital, energy, enzyme, and chemical costs. Under high enzyme loadings, hydrolysis time can be reduced by 58% using pelleted biomass. At high pretreatment severity, enzyme loadings can be reduced by 80%, whereas, at low pretreatment severity, either 40% reduction in enzyme loadings or 48% reduction in hydrolysis time can be achieved using pelleted biomass. A comparative techno-economic analysis is also being performed based on the above results to determine economic repercussions of the processing options. The economic analysis compares the operating and capital costs for both forms of biomass. A sensitivity analysis is also being done to see the effect of variation in input parameters on the minimum selling price of ethanol.

M53 Impact of ionic liquids on biomass recalcitrance

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Lignocellulosic biomass valorization requires either pretreatment or fractionation to recover their building block components, which are then converted into valuable and sustainable chemicals and products. Exposure to select ionic liquids (ILs) has been shown to reduce recalcitrance and increase activation of the resulting biomass. In this work, hybrid poplar biomass was exposed to two carboxylate ILs, 1-allyl-3-methylimidazolium formate ([AMIM][HCOO]) and 1-ethyl-3-methylimidazolium acetate ([EMIM][CH_3COO]), and the regenerated IL-biomass was compared in terms of activation, physical and chemical changes, and enzymatic saccharification. The regenerated biomass was examined via Fourier transform infrared spectroscopy and X-ray diffraction. Results showed that activation using [AMIM][HCOO] did not deacetylate hybrid poplar as readily as with [EMIM][CH_3COO]. On the other hand, activation with [EMIM][CH_3COO] made the cellulose and hemicellulose more accessible. Almost twice the amount of cellulose and hemicellulose present in the [EMIM][CH_3COO]-regenerated biomass was enzymatic hydrolyzed when compared to the [AMIM][HCOO]-activated biomass. Overall, our results highlighted that exposure to [AMIM][HCOO] is favored for biomass dissolution and direct product formation, while the use of [EMIM][CH_3COO] results in biomass that is less recalcitrant and therefore more amendable to enzymatic saccharification and fractionation.

M54 Development of a robust, high-throughput DNA sequence validation workflow

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High-throughput sequence validation is essential to controlling the quality of ever increasing rates and scales of DNA construction. We have developed a low-cost DNA sequencing workflow along with informatic tools for its optimization. We used a greedy algorithm to select custom barcode tags (maximizing where possible color balance and pairwise edit distance) for increased multiplexing. Towards achieving optimal cluster density on the MiSeq, we applied a machine learning approach to predict cluster density as functions of library fragment size distribution and loading concentration. We are also developing an automated analysis pipeline for reference mapping-based analysis as well as de novo assembly, as we expand throughput to 1536 samples per MiSeq run. These advances reduce our reagent costs and increase useful MiSeq data output as we seek to

identify correct constructs.

M55 Design and Construction of a Microfluidic Reactor for Real-Time Imaging of Pretreatment and Enzymatic Hydrolysis

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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 a single PDMS layer (75 x 50 mm) which accommodates six reaction chambers (20 mm diameter each) on six Thermanox microscopic coverslips (25 mm diameter each), 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. 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 carried out fluorescence microscopy before and after pretreatment and enzymatic hydrolysis, with Calufluor-white stain to observe specific changes in the cell wall during the process.

M56 The response of *Pseudomonas putida* to a complex lignolysate

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There is strong interest in lignin valorization as an economical feedstock to produce valuable products. Nevertheless, the bioprocessing of lignin into fungible bioproducts is a major bottleneck to microbial lignin valorization because of its structural complexity. Here, we employed ionic liquid pretreatment to obtain a soluble aromatic-rich fraction by base-catalyzed depolymerization (BCD) of the solid fraction after saccharification. Growth of *Pseudomonas putida* KT2440 on BCD liquor coincided with disappearance of aromatic peaks, consistent with the complete utilization of *p*-coumarate, ferulate, and the other aromatic monomers present in the depolymerized substrate. The growth of *P. putida* in the BCD liquor was higher than that on individual aromatic substrates, suggesting that the BCD liquor contained additional carbon sources beyond lignin-related aromatics. Aromatic independent growth was confimed in a *P. putida* mutant strain that was unable to grow on *p*-coumarate and ferulate. A combination of proteomic and metabolomic analyses demonstrated that the BCD liquor is a complex substrate containing at least four distinct substrates for *P. putida* (aromatic monomers, amino acids, cholinium and fatty acids). Comparative proteomic analysis revealed the significant upregulation of aromatic catabolic, protocatechuate ortho-cleavage, and beta-ketoadipate pathways, indicating the complete utilization of lignin-derived monomers to central metabolism in BCD liquor. Interestingly, beta-oxidation, acetyl-CoA synthetase and isocitrate lyase were also significantly upregulated probably due to requirements of efficient energy production by alternative pathways in the absence of glucose. Glutaminase was also upregulated, which may be related to amino acid utilization. Cholinium-based ionic liquid degradation pathway was upregulated due to the residual cholinium (~150mg/L) in BCD liquor.

M57 Enhancing 3-hydroxypropionic acid production in combination with cell surface display and metabolic engineering of S. pombe

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3-hydroxypropionic acid (3HP) is the important chemical for building bio-sustainable society. Here, we describe metabolic engineering of fission yeast *Schizosaccharomyces pombe* for 3HP production *via* malonyl-CoA pathway from glucose and cellobiose. Genes encoding malonyl-CoA reductase (MCR) of *Chloroflexus aurantiacus* was dissected into two functionally distinct fragments (MCR-C and MCR-N) and the activity between MCR-C and MCR-N was balanced. To increase the cellular supply of malonyl-CoA and acetyl-CoA, we introduced genes encoding endogenous aldehyde dehydrogenase, acety-CoA synthase from *Salmonella enterica*, and endogenous pantothenate kinase. The resultant strain produced 1.0 g/L of 3HP from 50g/L consumed glucose. We also engineered sugar supply by displaying beta-glucosidase on its cell surface. When 50 g/L of cellobiose was used, the engineered strain efficiently consumed cellobiose and produced 3HP at 3.5 g/L. Finally, this strain produced up to 11.4g/L of 3HP under fed-batch condition from cellobiose with a yield of 13% (g-3HP/g-sugar).

M58 Organosolv Pretreatment of Miscanthus x giganteus Using Bench-Scale Ball Milling for Effective Enzymatic Saccharification

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The effect of organosolv pretreatment on *Miscanthus x giganteus* (MG) in a large scale ball mill are investigated for improved enzymatic saccharification. In order to study the synergetic effects of ball milling with organosolv treatment in a single reactor, a specially-designed temperature controlled bench-scale ball mill with 30 L reactor volume is constructed and used in this study. Various reaction conditions are attempted to find the optimal conditions, which minimize cellulose loss and maximize the enzymatic saccharification of (MG), with conditions varying from room temperature to 170 °C for reaction temperature, from 30 to 120 min of reaction time, from 30% to 60% ethanol concentration, and a liquid/solid ratio (L/S) of 10–20 under non-catalyst conditions. The effectiveness of ball milling together with ball-milling is evaluated and confirmed by compositional analyses, enzymatic saccharification yield and X-ray diffraction analyses of the treated solids. In this paper, the pretreatment effects on chemical compositional changes and weight loss of pretreated solids, such as delignification and hemicellulose removal, and structural changes of treated solid using X-ray diffraction test will be reported along with enzymatic saccharification test results.

M59 Recovering Valuable Bioactive Compounds from Potato Peels via Sequential Hydrothermal Extraction

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Potato peels are a major waste stream from the potato processing industry and a potential source of valuable functional and bioactive compounds. Recovering these compounds would valorize this agricultural byproduct, however, traditional extraction methods have several drawbacks: they are energy and chemical intensive and have low yields. Moreover, conventional methods require completely dry samples, necessitating pretreatment. In contrast, Sequential Hydrothermal Extraction (SeqHTE) is a unique, environmentally friendly two-stage process ideal for processing wet biomass. This tunable extraction platform employs subcritical conditions of water to enhance mass transfer and break up solid materials into smaller, more soluble components, leading to higher selectivity and higher extraction yields.

This study evaluated performance of SeqHTE treating different potato peels in terms of process versatility, extraction yields, bioactive quality, and soluble nutrient recovery. Polyphenol recovery compared favorably with previous studies, and owing to the increased presence of bound and complex polyphenolics, the extracts exhibited significant antioxidant activities. Moreover, the SeqHTE process allowed the recovery of important quantities of potato glycoalkaloids, polysaccharides, and water-soluble nutrients. The results correlated well with previous studies. The fundamental data collected lays the groundwork for further development of the custom extraction platform, and emphasizes the suitability of the process for the recovery of structurally diverse and complex bioactive compounds from potato peels. Furthermore, this work provides a preliminary understanding of the process chemistry, valuable insight on the effect of cultivar-specific attributes, determining sources of potential issues and highlighting key topics for future research efforts.

M60 Synthesis and characterization of lignin-based epoxy rigid foam using inorganic ultra-accelerator-blowing agent

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Lignin is the second most abundant biopolymer isolated from plants through pulping and biofuel industries. In this study, aminated kraft softwood was synthesized and it was used as curing agent for epoxy resin. Differential scanning calorimetry (DSC) analysis showed the ability of aminated lignin to replaced up to 50 wt% of petroleum-based curing agent (isophorone diamine). In addition, a novel inorganic accelerator/blowing agent was developed to prepare epoxy rigid foam at room temperature in very short time (around 1 minute). Three types of epoxy foam samples with different amounts of accelerator/blowing agent and lignin (either unmodified or modified) were prepared: pure epoxy foam, epoxy foam with unmodified lignin as filler and epoxy foam with aminated lignin as curing agent. Thermal properties of all samples were studied by DSC and thermogravimetry analysis (TGA) and it was found that epoxy foam sample with 15 wt% aminated lignin has excellent thermal stability. Furthermore, replacing the petroleum-based curing agent with 50 wt% aminated lignin, increased the compressive strength by 113 %. The effect of accelerator/blowing agent as well as lignin addition (both modified and unmodified lignins) on the foam cell size were studied by field emission scanning electronic microscopy (FE-SEM). Analysis of cell size by FE-SEM showed that by increasing the amount of accelerator/blowing agent up to 10 wt% the cells become larger, but after that the size of cells were reduced due to fast curing reaction of epoxy resin with 15 wt% accelerator/blowing agent. This could be explained by the fact that the function of the accelerator is more effective than the blowing agent. The results of this study proves that the aminated lignin can work as an active curing agent in the presence of accelerator/blowing agent to prepare rigid epoxy foam with excellent mechanical properties.

M61 Can lignin enhance the hydrophobicity of microfibrillated cellulose (MFC) for thermoplastic applications?

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Cellulose is the most abundant natural polysaccharide on earth and has huge potential to be used in applications such as pulp & paper, construction, food, pharmaceuticals, packaging and renewable energy. The vast majority of cellulose exists as an intricate matrix with hemicellulose and lignin, making it difficult to deconstruct. Lignin has been shown to be a major impediment to both deconstruction and in the production of high value pulps such as Kraft and dissolving pulps, with the latter uses to make high value products such as rayon. Recent work has looked at the potential of nanocrystalline (NCC) and nanofibrillated

cellulose (NFC), with the expense of removing lignin contributing to the cost of these pulps. Microfibrillated cellulose (MFC) is a relatively new product with excellent physical properties and good hydrophilicity which is favourable for wide applications including food and pharmaceuticals. However, the hydrophilicity of MFC has limited its application in sectors such as the packaging and polymer industries. As will be described in the presentation, we have assessed several ways of making MFC's hydrophobic. Most current techniques involve the usage of petroleum based organic material to modify the surface of MFCs'. However, as will be described, lignin offers considerable potential as a relatively inexpensive way to produce MFC-based-thermoplastics which could have application in the packaging and automotive sectors. The various approaches to incorporating lignin onto MFCs, to prepare highly hydrophobic material which can reinforce thermoplastics, will be described.

M62 Astaxanthin Production by *Phaffia rhodozyma* from Structural and Non-Structural Sweet Sorghum Sugars for Co-Product Generation

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Sweet sorghum possesses favorable cultivation characteristics such as drought tolerance and short growing season that make it a favorable crop for biorefinery applications. Similar to sugar cane, sweet sorghum also generates both non-structural sugars in the form of juice and structural polysaccharides from lignified stalk tissue that can be converted biochemically to high-value chemicals. One such chemical is astaxanthin, a carotenoid which has high market value as both an aquaculture feed supplement and nutraceutical for human consumption. This research involved cultivation of the yeast strain *Phaffia rhodozyma* on sugars from sweet sorghum to determine the production potential of astaxanthin. *P. rhodozyma* grew very well on supplemented sweet sorghum juice producing biomass titers around 30 g/L and astaxanthin titers of 65 mg/L at a volumetric productivity of 0.389 mg/L/hr. Hydrolysate generated from alkaline pretreated sweet sorghum bagasse could only produce about 15 g/L of *P. rhodozyma* biomass and 9.5 mg/L of astaxanthin. The detoxification of sweet sorghum bagasse hydrolysate was necessary to improve biomass titers and astaxanthin output. The presence of dissolved phenolic components originating from lignin appear to negatively impact the cultivation of *P. rhodozyma* in the bagasse hydrolysate. Improvements in biomass and product titers can be achieved with better bioprocessing strategies.

M63 Performance prediction using dynamic simulation of (Acetone)-Isopropanol-Butanol-Ethanol fermentation coupled toÂ gas stripping

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Alcohols such as n-butanol and isopropanol, obtained from fermentation processes using Clostridia, are among most promising petroleum substitutes due to their wide range of applications. In order to make the large scale production economically viable, several *in situ* butanol recovery techniques were already described in the literature to overcome end product inhibition in ABE fermentation and maximize the microorganism performance in the bioreactor.

In this work, as a first step of a larger study, gas stripping was applied to a fermentation process using the natural isopropanol producer *C. beijerinckii* DSM6423 for (A)IBE fermentation. A comparative analysis between batch and continuous fermentation without and with intermittent gas stripping was performed by means of dynamic process simulation including both biological and thermodynamic phenomena occurring in the bioreactor. The reactor was modeled by a simplified kinetic model and simulated using Excel, coupled to AspenPlus software which integrated the gas-liquid phase separation, at each time step of the calculation. This methodology allowed to consider the impact of a separation process on the two distinct fermentation phases (acidic and solventogenic), in terms of volumetric productivity, yield, conversion and final product concentration.
The volumetric productivity was found to be doubled when switching from batch to continuous process and even improved of 17 % when intermittent gas stripping was applied. Sensitivity analysis to dilution rate and vvm were also performed. Other ISPR techniques, such as liquid-liquid extraction will be implemented. A global optimization will be performed minimizing the total annual cost of products.

M64 Depolymerization of Corn Stover Lignin with Bulk Molybdenum Carbide Catalysts

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Depolymerization of lignin to aromatic compounds in high yield for production of fuels and chemicals is vital to realize an economically competitive biorefinery. In this study, undoped and nickel-doped bulk molybdenum carbides were synthesized and evaluated in reductive depolymerization of lignin model compound and corn stover lignin. The highest lignin monomer yield of 37.3 % was obtained with Ni-Mo_2C and ethanol/water solvent. Nickel doping promoted the depolymerization performance of Mo_2C, by increasing the number of metallic sites while decreasing that of acidic sites. This change in active site distribution mitigated C-C coupling reactions and coking. Ethanol addition to the water solvent also significantly suppressed C-C coupling. Physically mixing carbides with zeolites decreased monomer yields, presumably due to excessive repolymerization of reactive intermediates over the acidic zeolite. This work highlights the feasibility of developing effective lignin depolymerization strategies by fine-tuning the properties of molybdenum carbide catalysts and solvent systems.

M65 Application of industrial amylolytic yeast strains for the production of bioethanol from rice waste

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The valorisation of industrial waste streams holds promising solutions regarding the global search for inexpensive alternative fuels. With a global paddy production of 760 million tons in 2017, rice processing waste is an ideal contender for waste stream utilisation strategies and can be used as a feedstock for bioethanol production. However, CBP (Consolidated Bioprocessing) requires genetically modified yeast to simultaneously hydrolyse complex starch-rich substrates and produce ethanol in a fermentation process. The heterologous production of α-amylases and glucoamylases by industrial yeast strains would therefore reduce enzyme costs in industrial processes and could eliminate them completely.

In this study, amylolytic industrial *Saccharomyces cerevisiae* yeast strains were evaluated for their ability to hydrolyse and produce bioethanol from a rice waste substrate, namely broken rice. The recombinant strains, containing integrated fungal amylase genes, were assessed through enzyme characterisation, hydrolysis trials and fermentation studies. The heterologous enzymes were able to hydrolyse broken rice without any substrate pre-treatment. During the fermentation of 200 $g.l^{-1}$ broken rice, the amylolytic strains could ferment rice starch to ethanol in a single step and produce high ethanol titers. After 72 hours at 30°C, 85% of the theoretical ethanol yield was obtained. Furthermore, the addition of STARGEN 002™ (10% of the recommended dosage) in combination with the amylolytic yeasts did not significantly improve the maximum ethanol production or the rate at which it was produced when compared to CBP. Thus, these recombinant yeast strains are considered ideal candidates for CBP to produce bioethanol from starch-rich waste streams.

M66 Retrosynthesis of all available pathways to microbial production of precursors to target chemicals based on chemical separation characteristics

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Biological production of target chemicals is often limited by ease of separating the chemical of interest from the bioreactor. In practice, chemicals that do not separate easily create inefficiencies in production that limit the viability of biological routes to production. In many cases it may be advantageous to prefer limiting biological production of material before it reaches the target chemical, in order to more easily separate material of interest from the general bioreactor substrate. In this work we use our previously reported RetSynth tool to characterize trade-offs in separation between purely biological and hybrid bio/chemical routes to synthesis of target chemicals. We set up an optimization protocol using boiling point and solubility of chemical intermediates as a proxy for separability of chemical from a liquid substrate. The RetSynth tool provides six combined biological and chemical reaction databases, with tens of thousands of biological reactions and millions of chemical reactions. This tool allows us to characterize and optimize production using differential halting, enzyme addition and hybrid routes to production. We initially start by characterizing alcohols, an important class of chemicals for both Spark Ignition and Mixed Compression Controlled Ignition. There are over 300 alcohols that have the potential to be produced biologically. We demonstrate under what conditions strictly biological vs. hybrid routes to approaches to production are optimal and define a strategy for ranking paths to production more generally.

M67 Next Generation Logistics Systems for Delivering Optimal Biomass Feedstocks to Biorefining Industries in the Southeastern United States

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The diverse portfolio of biomass sources that is available in the Southeastern U.S., including a significant supply of pine “residue”, represents a valuable strategic position for the region. Through blends formulated based on critical properties, this project will take full advantage of the range in biomass properties afforded by the portfolio to produce a consistent, high-performance feedstock for the industry, while lowering cost. Key developments being targeted to enable this potential include whole-tree transport to a state-of-the-art merchandising depot that will further access biomass from ongoing, forest industry operations. The approach will more effectively utilize the tree and distribute cost, while minimizing in-woods contamination of the woody biomass component. To implement this vision, information on the chemical composition and changes that are induced during multiple preprocessing steps (size reduction, moisture removal, densification, etc.) is needed. New NIR sensor technology will be developed for online monitoring of important biomass properties. The data will be incorporated into a statistical process control platform to improve process efficiency and meet required specifications. Advanced process models are being developed to inform the techno-economic and life-cycle assessment of the program’s impact. The new system will ultimately reduce operational risks from supply chain disruptions, and allow operation of larger-scale biorefineries.

M68 Bioprocess development for muconic acid production by engineered *Pseudomonas putida*

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Process scale and bioreactor conditions can lead to important variations on the performance and metabolism of microbes, which may become an issue during commercialization efforts. This work aims to validate the performance of engineered *P. putida* KT2440, in the Agile Biofoundry framework, for the production of muconic acid at near industrial scale and with various

bioreactors conditions to understand its robustness and optimal production parameters. Sensitivity to process variation (i.e. pH, oxygen level, temperature) and process feeding techniques was studied in 0.5 L bioreactors and optimized conditions were used to scale-up the muconic acid producing-strain in a 500 L bioreactor in National Renewable Energy Laboratory's pilot plant.

M69 Structural and chemical characterization of lignin obtained from protic ionic liquid pretreatment

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Lignin is an abundant biopolymer which can be obtained as a by-product in the production of second generation ethanol. Recent studies have shown that some protic ionic liquids (PIL) are capable to solubilize nearly 80 wt% of lignin during the pretreatment process. On the other hand, its rather complex and diverse chemical architecture does represent a major problem to its widespread characterization and valorization. In this way, the goal of this study was the extraction and lignin recovery from sugarcane bagasse applying two PILs: ethylamonium formate - [Eti][For] - and ethylamonium acetate - [Eti][Ac] -, both not yet reported by literature; and to analyze them by 2D-NMR, GPC and SAXs, searching for new high value added applications. The structural analysis revealed that recovered lignins had a relatively low amount of syringyl, guaiacyl, p-hydroxyphenyl units, and other common aromatic fractions such as p-coumarate, which presented a high concentration for the [Eti][Ac] lignin. It was observed a low molecular weight of lignin recovered from [Eti][For] (1,921 g/mol), which shows a great potential for applications in pharmaceutical or even as antioxidants.

M70 Open culture fermentation for biofuels and biochemicals production – caproic acid production

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Open culture fermentation main advantage is that it does not require sterile process conditions, therefore the cost of sterilization of substrate can be omitted. During that process, variety of chemical compounds originating from waste can be converted into a mixture of short chain carboxylic acids and alcohols. Subsequently, the intermediates can be elongated into medium chain carboxylic acids – e.g. caproic acid which could be applied in chemical industry or further upgraded to bio-jet fuel. There is still a knowledge gap on how to optimize and stabilize the process and to make it environmentally and economically efficient. Required external addition of ethanol as electron donor was indicated as the main factor influencing the carbon footprint during caproic acid production, in-situ production of it could significantly decrease it. Moreover, exchanging ethanol for lactic acid could lead to use of broader spectrum of substrates originating from waste. With rising interest in applying open microbial consortia, finding new and efficient ways for managing reactor microbiomes became equally important. Here, we investigate different approach to optimize the process either by spiking it with additional electron donor or by bioaugmentation. Traditional approach on bioaugmentation with microbes responsible for final products generation were conducted with minor success. New approach has been considered, i.e. identification of hub (keystone) microorganisms for further process optimization. We developed caproic acid production process from acid whey with high productivity of 3.2 g/L/d and high specificity of 83%. Initial techno-economic analysis indicates that it could be produced under current market price.

M71 Experimental, techno-economic and environmental evaluation of enzymatic cellulose hydrolysis of switchgrass at high solids content in an energy-driven biorefinery

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High solids content could lead to high ethanol concentrations and reduce energy consumption but can also inhibit hydrolysis and fermentation. Experimental assays were performed using liquid hot water pretreated switchgrass solids following a Box-Behnken experimental design to analyze the effect of solids content (15, 20 and 25%), enzyme dosage (10, 40 and 70 $mg_{protein}/g_{glucan}$) and enzyme synergy (0, 10 and 20% cellulases substituted by xylanase) on the enzymatic hydrolysis of cellulose. Enzyme dosage had a more significant impact than solids content on hydrolysis efficiency. Inhibition of the hydrolysis reaction related to the increase of solids content was noticeable for low enzyme dosages, but it seemed to be avoided when enzyme was used in high dosages. High hydrolysis efficiencies were found for high solids content (>90% for 25 %) and high enzyme dosages (40 and 70 $mg_{protein}/g_{glucan}$). Experimental results were used in models developed for life cycle assessment and techno-economic analysis to obtain the greenhouse gases (GHG) emissions and the minimum ethanol selling price (MESP) respectively, in a biorefinery producing ethanol, electricity, furfural, acetic and formic acid. A multi objective optimization was used to find an economical-environmental optimal based on the quadratic models found to represent variations of GHG and MESP as a function of solids content and enzyme dosage. An enzyme dosage of 36.8 $mg_{protein}/g_{glucan}$ and solids content of 20.7 % were the optimal conditions, producing bioethanol with an associated "Well to Tank" GHG value of -68 ± 5 g $CO_{2eq}/MJ_{ethanol}$ and a MESP of 0.838 $/L.

M73 Optimizing operating conditions for production of 2,3-butanediol using *Zymomonas mobilis*

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USA

2,3-Butanediol (BDO) has many advantages over ethanol that include lower toxicity to organisms, a higher heat capacity for fuels and a precursor to a large number of valuable applications. We have genetically modified *Zymomonas mobilis* to produce BDO for fuel upgrading and successfully removed ethanol production. A plasmid bearing strain was used to determine operating conditions for increased titers and productivity. Because of the engineered BDO pathway and loss of ethanol production, redox balancing using micro-aeration was crucial to reducing byproduct formation. *Z. mobilis* is a facultative anaerobe that requires a very narrow dissolved oxygen range for adequate BDO production. When aeration levels are too high, acetoin, the precursor to BDO, is produced as a byproduct. If aeration levels are too low, glycerol forms as an undesired byproduct. Due to the requirements of less than 1% dissolved oxygen, multiple micro-aeration conditions were determined through experimental design that boost titers, reduce byproducts, and increase productivity. BDO titers as high as 45 g/L with a productivity of 2 g/L/h were achieved on glucose. Future research will include fed-batch fermentations for higher titers, using pretreated hydrolysate sugars as feedstock and showing scalability in a pilot plant setting.

M72 Enhancing titers of 2, 3 Butanediol through fed-batch fermentation using an engineered strain of Zymomonas *mobilis*

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Recent advances in *Zymomonas mobilis* strain development has produced an engineered strain that can metabolize glucose and xylose to 2,3 butanediol (BDO) and no ethanol. End-product inhibition from BDO is expected to be much less than ethanol paving the way for developing a fed-batch fermentation process to achieve high BDO titers. A higher titer is desirable to reduce water removal during downstream separations and catalytic upgrading and reduce capital costs of the process. There are challenges to a fed-batch process using the BDO producing *Z. mobilis*, particularly when biomass sugars are the carbohydrate source, that need addressed to achieve titers above 100 g/L. *Z. mobilis* can utilize both glucose and xylose, but they are consumed at different rates. Additionally, the biomass sugars are not produced at high enough concentration to achieve 100 g/L of BDO and even if the sugars are concentrated, *Z. mobilis* has a threshold level before there is inhibition from osmotic pressure due to sugar concentrations above 160 g/L. The strain also has a redox imbalance as a result of the BDO engineered pathway and the removal of ethanol production. The redox imbalance results in by-product formation which reduces titer. We present fed-batch fermentation strategies that address sugar feeding and redox to produce BDO at up to 100 g/L while minimizing by-products. The strategy is demonstrated at bench scale using pure sugar and biomass sugar.

M75 Green biomass as a viable feedstock for fractionation to biofuels and value-added products

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Fresh green biomass is high in protein and cellulose while low in lignin. A mechanical pretreatment involving maceration and screw pressing fractionates fresh biomass (*Medicago sativa*, lucerne) to a juice and pulp fraction. The juice fraction, which is high in protein, is heated to 85°C to precipitate the proteins out of solution and further decanted to produce a concentrated leaf protein paste of high value for animal feed. The remaining pulp fraction is rich in cellulose, an ideal feedstock for 2nd generation bioethanol production and other sugar platform fermentation products. After a short extraction process to remove chlorophylls and other ethanol-soluble extractives, hydrothermal pretreatment of the pulp fraction revealed higher cellulose conversion to glucose yields when treated with Novozymes Cellic CTec3. Supplementation of the cellulases with pectinases also increased the cellulose and hemicellulose hydrolysis yield by 25%. Some of the pulp produced was ensiled and stored for one month to test the effect as pretreatment, which would be relevant when large biorefineries need ways to store excess feedstock. The results showed that while some of the sugars have been converted to lactic and acetic acid during the ensiling process, the enzymatic hydrolysis yields are comparable to those using fresh pulp.

M76 Lipids and carotenoids production from wheat straw hydrolysates using oleaginous yeasts

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Oleaginous yeasts have been identified as a promising microorganism for lignocellulosice biomass valorization, since they can use different carbon sources to produce lipids and value-added natural pigments. In this study, the performance of *Rhosdosporidium toruloides* NRRL Y-1091 and *Lipomyces starkeyi* NRRL Y-1389 to growth and produce lipids and carotenoids from hemicellulosic and cellulosic hydrolysates obtained by hydrothermal pretreatment and enzymatic hydrolysis of wheat straw was evaluated. The results showed that phenolic compounds, acetic acid and high concentration of sugars can inhibit yeast growth, and yeasts grow better in detoxified hydrolysates. The highest concentration of lipids (3.99±0.35 g/L with 17.32±0.12 g/L of cell mass) was obtained by cultivating *L. starkeyi* in cellulosic hydrolysate containing 46.40±0.18 g/L glucose, 11.36±0.02 g/L xylose and 0.63±0.03 g/L phenolic compounds. The highest production of carotenoids (24.58±1.88 mg/L) was found in cellulosic hydrolysate containing 43.60±0.01 g/L glucose, 12.34±0.03 g/L xylose, 1.14±0.03 g/L acetic acid and 0.19±0.02 g/L phenolic compounds. In this hydrolysate, cell mass, lipids and carotenoids production by *R. toruloides* reached 17.95±0.42 g/L, 3.36±0.29 g/L and 24.58±1.88 mg/L, respectively. In brief, this study demonstrated that oleaginous yeasts can use both cellulosic and hemicellulosic hydrolysates to produce lipids and carotenoids simultaneously. However, the poor tolerance of these yeasts to inhibitors derived from lignocellulosic biomass pretreatment and high concentrations of sugars are still important challenges for process scale-up.

M77 The dynamics of the lignin glass transition

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At room temperature, lignin is mechanically rigid, which impedes industrial processing. High temperature is employed to soften it. At the molecular scale, this is achieved by enhancing the underlying lignin atomic dynamics. We combined molecular dynamics simulations with quasi-elastic neutron scattering and dielectric spectroscopy experiments to probe the dependence of lignin dynamics on hydration and thermal history. We found a dynamical hysteresis: at a given temperature, the dynamics of lignin molecules are faster when the lignin is cooled than when heated. The simulations revealed syringyl units to be more dynamic than guaiacyl and the three-carbon chains to be more dynamic than the phenol rings. Heating biomass above the lignin glass transition temperature T_g is expensive. Our results show that T_g is lower when lignin is cooled than when heated, and therefore extending the cooling phase of processing and shortening the heating phase may offer ways to lower processing costs. We further characterized the atomic motions giving rise to the technologically important lignin glass transition and how they differ above and below T_g. Below T_g, lignin exhibits mainly internal and localized motions. Above T_g, the mobility of segments, consisting of 3–5 lignin monomeric units is enhanced. The temperature dependence of the lignin relaxation time was found to switch from Arrhenius to non-Arrhenius as the temperature increased above T_g. Despite the heterogeneous and complex structure of lignin, its glass transition dynamics can be described by concepts developed for chemically homogeneous polymers.

M78 Production of a biofuel intermediate from biomass sugars by *Clostridium tyrobutyricum*

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Butyric acid is a valuable chemical that can be produced fermentatively from glucose in *Clostridium tyrobutyricum* and catalytically upgraded to drop in biofuels. Despite considerable study of glucose fermentation in *C. tyrobutyricum*, information about glucose and xylose co-fermentation to produce butyric acid in this organism is limited. In this work, fed-batch fermentations of corn stover hydrolysate at pH 5.0 demonstrated the co-fermentation of glucose and xylose. However, the fermentation stalled at approximately 48 hours after reaching inhibitory levels of butyric acid (18 g/L). When the fermentation pH was increased to 6.0, higher butyric acid titers (50 g/L) were acheived but the fermentation again stalled after 98 hours due to product inhibition. To demonstrate the potential of a continuous co-fermentation process, *in situ* product recovery via membrane-based liquid-liquid extraction (pertraction) was applied to remove butyric acid. The extracted acids were then recovered from the organic solvent via back extraction into an alkaline solution, producing a high purity product. Results from pertractive fermentations of synthetic and real corn stover hydrolysate will also be shown.

M79 *Komagataella phaffii* (*Pichia pastoris*) response to hydrolysate-derived inhibitors and construction of recombinant strains for production of xylonic acid

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Lignocellulosic biomass, which is rich in pentose and hexose sugars, is an inexpensive and abundant substrate that can be used for production of fuels and chemicals. Yeasts easts can be interesting alternatives to produce polyols, organic acids and bioethanol. In this study, the yeast *Komagataella phaffii* (*Pichia pastoris*) response towards hydrolysate-derived inhibitors was evaluated through transcriptomic analysis and thereafter; recombinant strains capable of producing xylonic acid were constructed. Initially, tolerance of *K. phaffii* to acetic acid, furaldehydes (HMF and furfural) and sugarcane biomass hydrolysate was evaluated by fermentative assays. Than, RNAseq analyses were performed to evaluate the yeast response towards the same inhibitory compounds. Genetic response analysis revealed the enrichment of different GO categories, which varied according to the compound evaluated. In addition, a data set containing genes differentially expressed in each condition or in all of them could be identified. In parallel, the yeast *K. phaffii*, which is able to grow at very high cell densities, was engineered to produce xylonic acid. For this, new putative xylose dehydrogenase (XDH) genes from bacteria and fungi were identified by phylogenetic analysis and ten of those were chosen for expression in *K. phaffii*. Recombinant strains expressing each gene were evaluated by the ability to produce xylonic acid and the best candidate genes were chosen for further analysis. The effects of co-substrates on xylonic acid production were evaluated in different fermentation setups. Strains were able to produce xylonic acid with yield up to 0.95 g/g, under the best-evaluated conditions. Results will be presented and discussed.

M80 Production of bacterial cellulose by static fermentation of *Gluconacetobacter xylinus* (ATCC®23768™) using cacao mucilage exudate as a substrate

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At present, cellulose can be synthesized by bacteria of the genera *Enterobacter*, *Rhizobium*, *Agrobacterium*, *Azotobacter*, *Gluconacetobacter* among others; being this mechanism of obtaining the polysaccharide of broad biotechnological interest for the purity, resistance and crystallinity with which the material is obtained. However, industrial production of bacterial cellulose (BC) has limitations due to low production rate and high costs; which has fostered the search for new sources of carbon that

allow greater performance and productivity at low cost. Given that Colombia is the tenth producer of cacao worldwide and in this agro-industry, per ton of dry cacao beans is possible obtain: cacao shells and cacao mucilage; the latter is drained as an exudate rich in sugars such as glucose, fructose and sucrose, making it possible to postulate the evaluation of cacao mucilage exudate (CME) as an alternative for the production of cellulose by the static fermentation of *Gluconacetobacter xylinus* (ATCC®23768™). The production of BC was carried out using mucilage as a culture medium in different proportions and supplemented with nitrogen sources. The bioreactors were inoculated with 10% (v/v) pre-inoculum of the strain. The production was evaluated during 15 days, monitoring the pH and consumption of monosaccharides and disaccharides by HPLC. Finally, it was possible to demonstrate BC production between 0.43 ± 0.16 g/L up to 14.06 ± 0.69 g/L using CME in different proportions, being similar or superior to that observed with other carbon sources evaluated worldwide.

M81 Design and synthesis of biobased-flame retardant from novel phosphorylated downstream corn oil for engineering plastics

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Flame retardants (FRs) are an important class of additives used in combination with many engineering plastics to support electronic and automotive applications. FRs help to fulfill the industry fire standard requirement. Halogen free and biobased-FRs are gaining substantial attention in recent years in order to reduce the toxicities of conventionally used FRs. In this work, a phosphorylated biobased-FR was synthesized from downstream corn oil. Unlike conventional FRs, the incorporation of only 7.5 wt% of this biobased-FR was found to be effective in enhancing flame retardancy within the tested engineering plastics, poly(butylene terephthalate) (PBT) and poly(trimethylene terephthalate) (PTT). The UL-94 fire class of neat PBT improved from no rating to a V-2 rating after blending with biobased-FR. The utilization of a co-product from ethanol industry to develop a value added and eco-friendly product in this project supports the circular economy model and makes for a better future.

M82 Development of a novel and biobased modifier from bioethanol coproduct for improving the toughness of poly(lactic acid), PLA

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Poly(lactic acid) (PLA), a bioplastic, has outstanding mechanical strength, biocompatibility and biodegradability. However, it suffers from the low elongation at break and toughness which significantly restrict its applicability as the alternative to certain traditional commodity plastics. To address this shortcoming, development of toughness modifier is highly required. Here, a novel and biobased toughness modifier (BioTM) was synthesized from downstream corn oil of bioethanol industry to improve the properties of PLA. Different loading percentage of the BioTM (5-15 weight %) was incorporated into PLA matrix to process blends through extrusion and injection moulding processing. The fabricated biobased PLA blends showed a remarkably enhanced elongation at break, tensile toughness, and notched Izod impact compared to the pristine PLA. The synthesized BioTM will pave a way to other biopolymers for expanding their performances and applicability.

M83 Using Anaerobic Digestion to Break Down Microalgae Cell Walls and Enhance Lipid Extraction

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Breaking the cell wall is a key step for extracting intracellular components of algae such as proteins and lipids. Microalgae cell wall degradation using anaerobic biological hydrolysis was investigated in this study as an alternative to energy intensive options. The release of undigested microalgae lipid fractions as long chain fatty acids through pH controlled alkaline conditions was also examined. The hydrolysis rate of microalgae was significantly improved with pH elevation. The soluble chemical oxygen demand (SCOD) results showed that hydrolysis rates were highest in an alkaline environment, especially at pH 11.0 (12,273 g/L SCOD). Under alkaline pH conditions, the activities of acetogens and methanogens were low or severely limited, resulting in the release and accumulation of more long-chain fatty acid (LCFAs) than at acidic or neutral conditions. The maximum LCFAs concentration of 1.15 g/L was observed on the fourth day of anaerobic digestion at pH 11.0. The results provide insight for developing low-cost options using known and proven technologies for harvesting lipids from algal biomass.

M84 Impact of Moisture, Ash, and Bale Degredation of Preprossessing Performance and Feedstock Quality

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The Idaho National Laboratory, Department of Bio Energy, operates a research scale biomass preprocessing system, the Biomass National Feedstock User Facility (BFNUF). This system is composed of multiple grinders, conveyors, screeners, a rotary biomass drier, and densification equipment. In 2018, the BFNUFconducted research on corn stover and pine at varying moisture and ash concentrations to determine the effect that those parameters have on resulting quality and system efficiency. Resulting product quality (moisture, ash, ash and particle size) were measured after each unit operation to determine how input parameters impact unit operation and performance. Data loggers were connected to every electrical motor in the system, measuring energy performance as the varying biomass properties and operational parameters changed. Experimentation showed a strong dependence of grinding energy on moisture. Also particle size was impacted by initial bale quality. Initial bale moisture

also impacted the uniformity of material flow during the grinding process. High moisture bales caused uneven flow while low moisture bales resulted in more uniform material flow through the processing system composed of a two stage grinding process and associated conveyors. This report will present the findings from that research.

M85 Comparative Evaluation of 11 Industrial Hemp Varieties as Potential Energy Crops

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Industrial hemp (IH) has been a commodity crop grown in United States for various applications and is getting increasing interest due to recent legal amendments and new incentives towards hemp related research. Despite existing applications in hemp derived fiber and oil products, IH can be a potential energy crop for biofuels and bioproducts. However, technical uncertainty and economic feasibility remains a challenge for hemp cultivation. This study aims to combine agronomy, laboratory, and economic analysis approaches to evaluate and compare the potential of eleven IH varieties grown in Kentucky. Based on the agronomic data, the per hectare stem biomass yield for fiber only varieties ranged from 2933 kg to 8340 kg, while for the dual variety it ranged from 3582 kg to 7665 kg. The biomass yield of IH stem alone was at a similar level to the other energy crops such as switchgrass and sorghum. The eleven IH varieties underwent dilute acid pretreatment followed by enzymatic hydrolysis and fermentation to evaluate the ethanol production. The highest ethanol yield of 91.1 gallons /dry ton hemp stems was observed from Futura 75, while the lowest ethanol yield of 70.6 gallons /dry ton hemp stems was obtained in Codimone. In addition to the hemp stems, field trial for the dual variety also showed a grain yield of 1082 kg/ha for Bialobrzeskie, whereas Santhica 27 obtained a grain yield of 554 kg/ha. Lastly, cost analyses were conducted to compare the economics of IH based biofuels production, combined with sensitivity analysis on scenarios to coproduce fiber, grain, and biofuels. These collective evaluations illustrate that IH has significant potential to become a promising regional commodity crop for boosting bio-economy.

M86 Native basidiomycete fungi screening for future production of enzymatic cocktails to be used in simultaneous pretreatment and saccharification of cocoa shells lignocellulosic biomass

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Cocoa shells are compound by lignocellulose mainly, and to represent around 60% of content in cocoa cobs, showing the lignocellulose biomass from cocoa shells as a promising source for second-generation bioethanol. otherwise, the second-generation ethanol production demands a pretreatment and saccharification steps before sugars fermentation. One alternative to reduce the costs in second-generation ethanol production is simultaneous pretreatment and saccharification. Basidiomycete fungi are organisms that release cellulolytic and ligninolytic enzymes for lignocellulose degradation. Our goal was to select native Colombian basidiomycetes to produce enzymatic cocktails for the simultaneous pretreatment and saccharification of lignocellulosic biomass from cocoa shells. A qualitative screening of the native basidiomycetes *Hyphodontia sp.*, *Byssomerulius sp.*, *Dictyopanus sp.* and *Trametes sp.* to evaluate ligninolytic and hydrolytic activities in solid culture media were done. Ligninolytic activity was measured by oxidation of ABTS and guaiacol, and cellulolytic activity was measured by hydrolysis of carboxymethylcellulose (CMC) using red congo to show cellulose breakdown. *Dictyopanus sp.* and *Trametes sp.* were the isolations that shown the major ligninolytic and cellulolytic activity in the culture media with guaiacol and CMC at 15 days of growth. *Dictyopanus sp.* and *Trametes sp.* showed a guaiacol oxidation halo of 35,96 mm y 22,76 mm and CMC breakdown halo of 37,60 mm and 27,60 mm, respectively. These results confirm ligninolytic and cellulolytic activities for the native basidiomycete isolations *Dictyopanus sp.* and *Trametes sp.* in this work, profiling those two isolations as a source to produce enzymatic cocktails for the future simultaneous pretreatment and saccharification of cocoa shell biomass.

M87 Furfural from pulp mill prehydrolysate

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The interest on enhancing the biomass utilization at pulp mills is growing as it allows them to diversify their product portfolio and increase their revenues. It is estimated that a significant amount of hemicelluloses (mainly C5 sugars in the case of hardwood operations) and lignin are present in the prehydrolysate of kraft dissolving pulp mills or other mills in which a wood prehydrolysis step is employed. Presently, this sugar-rich stream is sent to the recovery cycle where it is mixed with weak black liquor and burned in the recovery boiler after being concentrated in the evaporators to produce energy. There is a potential of producing high value products from the C5 sugar-rich stream after pretreatment to remove inhibitors before its conversion (biologically or chemically). This work deals with the production of furfural from a prehydrolysate solution rich in pentoses. Furfural production was investigated under various conditions. The optimum operating conditions for maximum furfural production from sugar solutions were determined. Furfural yields as high as 77% using sulfuric acid as a catalyst were achieved by introducing the catalysts at the desired temperature and removing furfural from the reaction vessel as it is formed. A significant amount of acetic acid is available for recovery. The possibility of the integration of a furfural plant at a kraft dissolving pulp mill was evaluated.

M88 Controlled production of chitin nanofibers from diatom algae

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Chitin is a long-chain crystalline polymer of N-acetylglucosamine. It belongs to the family of structural polysaccharides, and is the second most abundant polymer on Earth after cellulose. It is commonly found in crustaceans, fungal cell walls, and in insect and anthropod cuticles; and is primarily used to strengthen their cell walls or skeletons. More recently, studies have reported the presence of chitin in various algae, including select species of diatom. Diatoms are single-celled photosynthetic microalgae that make cell walls of nanopatterned biogenic silica through metabolic uptake of dissolved silicon. The centric marine diatom Cyclotella sp. produces such nanofibers of the N-acetylglucosamine biopolymer beta-chitin, which are extruded from select pores in the biosilica cell wall. Unlike in crustaceans, fungi, or insects; diatom chitin is of particularly high purity, and thus extremely crystalline. In addition, diatom chitin also have unique structural properties. When produced, diatom chitin extrude as rigid nanofibrils of ~ 50 nm width out of the pores which radiate the edge of the silica valve. Immense potential exists to harness the unique biosynthetic capacities of microscopic marine organisms. Chitin fibril formation in algae is just one of many such capacities that offer numerous possibilities for new and emerging nanotechnologies, ranging from biomedical to advanced materials applications.

M89 Advanced Bioinformatics Tools to Support Metabolic Engineering

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Pathway Tools and BioCyc provide software tools and databases, respectively, to accelerate metabolic engineering. Pathway Tools can compute a qualitative metabolic reconstruction of an organism from its annotated genome. That reconstruction is stored in an integrated Pathway/Genome Database that can be queried and visualized such as to produce information pages for metabolites, reactions, and pathways, and a zoomable organism-specific metabolic map diagram. BioCyc.org contains 14,500 such databases.

The metabolic database for an organism can be converted into a quantitative metabolic flux model using flux-balance analysis to enable exploration of engineered alterations to the organism. The RouteSearch tool enables computational design of novel metabolic pathways that define optimal routes from a feedstock compound to a target compound. These metabolic routes can be constructed from reactions found in the organism database as well as from reactions from our highly curated MetaCyc database, which contains 15,000 metabolic reactions from all domains of life. Computed routes are optimal in containing as few reactions as possible, maximizing the number of atoms from the feedstock that are present in the target compound, and in adding as few reactions from MetaCyc as possible.

Several tools speed analysis of omics data. The Omics Dashboard enables interactive exploration and analysis of gene-expression and metabolomics datasets. The Dashboard is organized as a hierarchy of cellular systems and presents the user with a visual read-out of the expression status of all cellular systems. At its highest level the Dashboard contains panels for cellular systems such as biosynthesis, energy metabolism, regulation, and central dogma. Each of those panels contains a series of X-Y plots depicting the expression levels of genes within subsystems of that panel, e.g., subsystems within the central dogma panel include transcription, translation, and protein maturation and folding. The user can drill down through a series of panels to graph the expression levels of finer subcategories of genes or metabolites. Transcriptomics and metabolomics data can also be painted onto individual pathway diagrams, onto diagrams containing multiple pathways, and onto the full metabolic map diagram. Pathway enrichment analysis is provided.

M90 The effect of size distributions in microfibrillated celluloseÂ (MFC)Â on mechanical properties of MFC composite.

Y.H. Jung, Kyungpook National University, Daegu, Korea, Republic of (South)*

Utilization of fibers in lignocelluosic biomass by deconstruction to produce biosugars as well as by extraction and modification to obtain polymers can become a good strategy for the success of biorefinery. Recently, nanocellulose including micro/nanofibrillated cellulose (M/NFC) has received considerable attention because of its functionality and applicability as a biodegradable material. MFC exhibits asymmetrical properties resulting from the broad distribution of its molecular weight and various shapes of its elementary fibrils, e.g., bundled fibers, long-intact fibers, broken fibers, fragments of the fiber walls, fines, and microfibrils, which might cause heterogeneity of fibers. Thus, in this study, MFC prepared at different refining energies was fractionated and various characteristics of the fractionated fiber including size distributions were investigated. This heterogeneity in fiber sizes in MFC significantly interfered with the homogeneous interaction between the MFC and polyethylene oxide, causing strength reduction of the composite. This study emphasized the heterogeneity of MFC and its importance when using MFC as a bio-based material.

M91 Functionalized lignins as precursors for block copolymers

Z.G. Li, Oregon State University, Corvallis, OR, USA*

Lignins consist of structurally heterogeneous poly-phenylpropanoids that in their native form are low in reactivity and thus not suitable as precursors for synthesizing polymeric materials. Depolymerization of lignins yield low molecular weight products (i.e. with one or two benzene rings) that are more reactive than unmodified lignins, while pathways for synthesizing polymers from these depolymerization products are yet to be established. Fractionation of the complex mixture of lignin fragments also impedes profitable utilization of lignins in polymer synthesis.

We hereby present the use of oxidative depolymerization as an approach to obtaining oligomeric fragments (Mw~2000 Da) from various types of lignins. These fragments have aldehyde and carboxyl groups that are active in a number of cross-linking chemistries that yield block copolymers. Depending on the method of polymer synthesis, the block copolymers show diverse rheological properties that are distinct from unmodified lignins. Moreover, block copolymers with imine and oxime structures demonstrated improved adsorption affinity to metal ions, and are promising candidate materials for water treatment.

M92 Improvement of cell-surface adhered cellulases activities in recombinant strains of *Saccharomyces cerevisiae* engineered for consolidated bioprocessing.

B. Chetty and R. Den Haan, University of the Western Cape, Bellville, South Africa; T. Hasunuma, Kobe University, Kobe, South Africa; W.H. van Zyl, Stellenbosch University, Stellenbosch, South Africa*

Consolidated bioprocessing (CBP) remains an attractive option for the conversion of pretreated lignocellulosic biomass to commodity products if a fit for purpose organism can be engineered. The yeast *Saccharomyces cerevisiae* requires engineered cellulolytic activity to enable its use in CBP production of 2nd generation bioethanol. Current recombinant yeast strains engineered for this purpose must overcome the drawback of generally low secretion titres. A promising strategy for directly converting lignocellulose to ethanol is by displaying heterologous cellulolytic enzymes on the cell surface by means of the glycosylphosphatidylinositol (GPI) anchoring system. Recently, a strain producing cell adhered enzymes in a ratio-optimised manner was created that showed significant crystalline cellulose hydrolysis. However, cellulase-displaying yeast strains still secrete levels of enzyme that are insufficient for cellulose hydrolysis. Soluble *N*-ethylmaleimide-sensitive factor attachment receptor proteins (SNAREs) are crucial components of trafficking yeast proteins and are required at most membrane fusion events in the cell. SNAREs facilitate fusion between the protein transport vesicles, numerous membrane-enclosed organelles and the plasma membrane. Previous studies found that heterologous protein secretion levels were increased when overexpressing certain SNARE proteins. This study aimed to improve the amount of cell-adhered cellulase activities of recombinant *S. cerevisiae* strains through over-expression of genes identified in previous strain engineering studies. The results showed significant increases in cellulolytic activity for all three cell adhered cellulase enzyme types. This yielded improved conversion of crystalline cellulose to ethanol.

M93 Engineering a microbial consortium for advanced biofuels and bioproducts production from multisubstrate biomass streams

F. Liu, J. Jaryenneh, E. Monroe and R.W. Davis, Sandia National Laboratories, Livermore, CA, USA*

Biomass feedstocks such as distillers' grains with solubles (DGS) from first generation bioethanol production and wastewater cultivated algae provide significant supplies of feedstocks that are rich in both sugars and proteins which can be used as substrates for bio-based commodity chemicals and fuels. Here we engineered a microbial consortium for the "one-pot" conversion of the protein and carbohydrate fractions of DGS and algal hydrolysates into C4 and C5 fusel alcohols while upgrading the unutilized proteins to a high-value feed amino acid mixture. The microbial consortium incorporated two engineered *Escherichia coli* bioconversion strains. One *E. coli* strain was modified for efficient conversion of hexose and pentose sugar to isobutanol; the second *E. coli* strain was modified for the efficient utilization of the proteins in the hydrolysates to produce mixed C4 and C5 alcohols. The inoculation ratio between the two *E. coli* strains in the co-culture system was optimized to obtain up to 10.3 g/L and 5.9 g/L titers of total fusel alcohols from DGS and algal hydrolysates, respectively. 30-40% of the total proteins in the hydrolysates were converted by the co-culture. We further demonstrated the enrichment of valine in the unutilized proteins, one of the top five limiting essential amino acids in commercial mixed feeds, from 5.5% in the raw starting algae biomass to 17.3% in the fermentation broth. Therefore, the fermentation broth enriched in valine are also useful as high-value amino acids for animal feed. Furthermore, a quantitative PCR-based cell quantification method was developed to enumerate the dynamics of each individual bacterial population in the co-culture. ^{13}C-based metabolism analysis was performed to understand the biosynthesis route of each fusel alcohol product from different substrates by the *E. coli* co-culture. The *E. coli* co-culture engineered here eliminates the need for fractionation of hydrolysates and multi-step fermentation for the conversion of multi-substrate biomass streams and therefore significantly reduces the overall cost and fermentation time.

M94 Biorefinery approach for producing furfural and valorizable co-products from biomass using aqueous ChCl/MIBK biphasic solvent system

C. Wan and C. Zhu, University of Missouri, Columbia, MO, USA; X. Bai and L. A, Iowa State University, Ames, IA, USA*

This study investigates aqueous choline chloride/methyl isobutyl ketone (ChCl/MIBK) biphasic solvent system for simultaneous biomass fractionation and co-production of furfural and depolymerized technical lignin with uncondensed moieties. It aimed to address the major challenges with current cellulosic biofuel and furfural production processes as well as lignin valorization. Specially, highly digestible pulp and furfural as well as high-quality reactive lignin were produced through the proposed one-pot reaction using ChCl/MIBK biphasic solvent. The proposed biphasic solvent system can solubilize as high as 96% xylan in raw switchgrass, which was simultaneously converted to furfural with a high yield of 84.0%. Moreover, the one-pot reaction system can extract lignin with a high purity (93.1%), uncondensed moieties (i.e., Hibbert's ketone), and decreased molecular weight and polydispersity index. It is also striking to notice that biomass fractionation by the one-pot system was highly selective, persevering more than 90% cellulose in the pulp. The resultant pulp was thus enriched with cellulose (73.3%), which can be

completely hydrolyzed into glucose in 48 h via enzymatic hydrolysis. The aqueous ChCl can be successfully recycled and reused for at least four cycles with similar performance in switchgrass fractionation. This study demonstrated that aqueous ChCl/MIBK biphasic system was an effective solvent system for one-pot lignocellulose process, which resulted in direct production of furfural, high quality lignin with uncondensed moieties, cellulose-rich and highly digestible pulp.

M95 Tailoring lignin properties using a platform deep eutectic solvent

C. Wan[*] and C. Zhu, University of Missouri, Columbia, MO, USA; X. Bai and L. A, Iowa State University, Ames, IA, USA

Biomass fractionation allowing tailored lignin properties (especially abundance of β-O-4 linkages) and improved cellulose digestibility remains challenging for sustainable biorefinery. This study aimed to address this challenge using a platform DES comprising choline chloride and ethylene glycol (ChCl:EG). Lignin properties, including lignin purity, molecular weight distribution, volatility, and abundance of β-O-4 linkages can be tailored via tuning DES composition. Appealing lignin properties, such as high purity, well-preserved β-O-4 linkages and, high volatility similar to that of native lignin, are obtainable in this designer solvent system.

M96 Rational-designed nanocomposites based on biomass chitin-derived loofa sponge-like nitrogen-doped carbon nanonetworks for energy-related applications

X. Wu[*] and S. Li, Beihang University, Beijing, China; B. Yang, Washington State University, Richland, WA, USA; C. Wang, Pacific Northwest National Laboratory, Richland, WA, USA

Biomass conversion has been considered as an efficient approach to synthesize high-performance materials for energy-related applications because **biomass precursors** are **abundant, renewable**, and **low cost**. In this work, three-dimensional loofa sponge-like nitrogen-doped carbon nanonetworks derived from biomass chitin (denoted as C-Chitin) were successfully prepared by dissolution and coagulation of chitin in NaOH/urea aqueous solution using a repeated freezing-thawing process and a solution pre-gelation method followed by high-temperature carbonization under an Ar atmosphere. When composited with graphitic C_3N_4 and graphene, respectively, during the freezing-thawing process, the resulting C-Chitin/g-C_3N_4 and C-Chitin/graphene nanocomposite films both exhibited homogeneous interconnected network architectures with uniform porous features. In addition, the g-C_3N_4 or graphene nanosheets were homogeneously dispersed and immobilized in the C-Chitin matrix. Due to the well-designed architectures of high surface area and the rational composition combination, the C-Chitin/g-C_3N_4 and C-Chitin/graphene nanocomposites hold great application potentials in energy-related field. This study opened up a new avenue to large-scale fabrication of light-weight, highly effective, and low-cost N-doped carbon-based energy materials, broadening the applications of chitin.

M97 Improving process stability and biogas production via a two-stage anaerobic digestion of food waste combining solid-state hydrolysis and leachate methanogenesis/recirculation

L. Ding[*] and B. Hu, University of Minnesota, St. Paul, MN, USA

Compared with liquid anaerobic digestion (AD; total solids below 15%), solid-state AD (SSAD; total solids over 15%) of food waste (FW) holds the superiorities such as smaller reactor volumes, lower energy requirement for heating and stirring, easier disposal of digestate with lower moisture, etc. However, the higher organic contents of FW in SSAD could easily lead to accumulation of inhibitors such as volatile fatty acids (VFAs), thus resulting in subsequent acidification and even failure of the entire digester. Conventional solutions typically include extra water addition and co-digestion with bulk agents (e.g., wood chips and yard wastes), which in turn reduce the capacity of FW treatment.

To narrow this knowledge gap, a two-stage process that integrates solid-state hydrolysis of FW and subsequent leachate methanogenesis/recirculation was proposed to improve the SSAD stability and further boost the biogas production. In the first solid-state digester, FW was hydrolyzed into large amounts of VFAs which accumulated into the percolated leachate. In the second digester, the collected leachate was inoculated with previously degassed anaerobic sludge for biogas production, while the same amount of anaerobic digestate was recirculated to the first digester. The transfer of the leachate enriched with VFAs and the introduction of liquid digestate maintained the pH level of the first digester and facilitated the FW hydrolysis, whilst the VFAs were efficiently converted to biogas in the second digester. As compared to the one-stage SSAD, better decomposition of FW, higher energy recovery, and shorter retention time were simultaneously achieved in this two-stage process.

M99 Engineering an improved strain of *Pseudomonas putida* KT2440 for muconic acid production from glucose

G. Bentley, C. Johnson, D. Salvachúa and G.T. Beckham, National Renewable Energy Laboratory, Golden, CO, USA; R. Jha, N. Narayanan and T. Dale[*], Los Alamos National Laboratory, Los Alamos, NM, USA; J.R. Elmore and A.M. Guss, Oak Ridge National Laboratory, Oak Ridge, TN, USA

Pseudomonas putida KT2440 has received increasing focus as an important chassis organism for a variety of products including muconic acid, various terpenoids, polyketides, polyhydroxyalkanoates, and amino acid-derived products from various carbon

sources, demonstrating the robustness and versatility of the host. Accordingly, we sought to improve the capacity for *P. putida* KT2440 to produce a target molecule of commercial importance from an industrially-relevant carbon source, namely muconic acid production from glucose. Although *P. putida* can readily catabolize many heterogeneous substrates directly, *P. putida* contains a periplasmic route for the oxidation of glucose to gluconate and 2-ketogluconate. In a continuously-fed bioreactor, 2-ketogluconate was secreted and accumulation exceeded 80 g/L. Beyond reducing muconate yields, 2-ketogluconate acidifies the bioreactor, necessitating pH neutralization. This work shows that 2-ketogluconate accumulation can be avoided by deletion of the periplasmic gluconate pathway, but at the cost of introducing a substantial growth delay, presumably due to the absence of 2-ketogluconate as an allosteric regulator. We found that the deletion of a single transcriptional regulator can fully abrogate this growth defect. Beyond improving strain growth, the deletion of this regulator increased the muconic acid titer by 23% to 16.6 g/L at a 35% mol/mol yield from glucose. In addition to targeted engineering, adaptive evolution was used to improve the performance of engineered *P. putida*on glucose, illuminating a variety of novel targets that further improved growth and muconic acid production from glucose. The results of this work may be generalizable to improve the production of other products in *P. putida* from glucose, particularly when accumulation of 2-ketogluconate is undesirable.

M98 EVALUATION OF CENTRIFUGAL ANNULAR CONTACTOR POTENTIAL FOR OIL AND WATER SEPARATION

N. Toscano Miranda, J. Aguilar Ferreira Bernardes, J. Otavia Bahú, C.B. Batistella, R. Maciel Filho and M.R. Wolf Maciel, University of Campinas - UNICAMP, Campinas, Brazil*

The necessity of treatment of oily waters in industries or the removal of water from oils in the process of degumming and refining commercial oils have emerged several technologies over the years to optimize this separation. Decantation, solvent extraction, simple and fractional distillation, and centrifugation are some of the techniques for separating liquids. Among them, the centrifugation process deserves a highlight because it is a simple technique, without external component requirements (avoiding contamination and additional steps), performs rapid separation, has wide operating range of input flow rates, and enables different phase ratios without adjustment. In this operation unit, liquids with different densities can be quickly and easily separated due to the intensity of gravitational field (Stoke's law). For this reason, we analyzed the influence of different operational parameters on the separation of soybean oil (O) and water (W) to identify optimum operating range conditions using a centrifugal annular contactor. Thus, we conducted a 2^3 experimental design to evaluate O/W ratios (0.25 to 0.75), centrifuge feed rates (2.32 to 10.99 mL/s), and rotational speeds (1000 to 6000 RPM). The water content, density, and viscosity were measured before and after the centrifugation process to evaluate the separation efficiency. The results showed that low centrifuge feed rate (2.32 mL/s ± 0.03) and high rotational speed (6000 RPM) promote low water content (< 1.00 %) in the light phase output with high separation efficiency for different O/W ratios, being an attractive alternative for several industries that treat oil and water.

8:00 PM - 10:00 PM Hospitality Suite Sponsored by Katzen International

Regency A, 7th floor

Tuesday, April 30

7:00 AM - 8:00 AM Speaker Breakfast-Speakers and Conveners on date of your session

Duwamish - Room 306, Third Level

7:00 AM - 5:00 PM Registration

Columbia Ballroom Foyer, Third level

8:00 AM - 11:25 AM Session: 5: Enzymatic and Catalytic Deconstruction

Conveners: Anna Stenbæk, Novozymes, Baagsvaerd, Denmark and **Prof. Igor Polikarpov**, Institute of Physics, University of Sao Paulo, Sao Carlos, Brazil

Columbia C, Third level

8:00 AM 5-1: The Sabatier principle: a unifying approach to cellulase function

P. Westh, DTU BIOENGINEERING, 2800 Kgs. Lyngby, Denmark*

We have characterized a large number of fungal cellulases from different Glycoside Hydrolase (GH) families with respect to kinetic- and adsorption parameters. The substrate was pure cellulose (Avicel). The enzymes included both wild types and variants, and represented a wide range of hydrolytic cellulases (*e.g.* endo- or exolytic, processive or non-processive, inverting or retaining, with or without binding module, meso- or thermophilic). In spite of this variability among the investigated enzymes, we found distinct interdependences between functional parameters. For example, the turnover number (k_{cat}) scaled inversely with substrate binding strength regardless of GH family and catalytic mechanism. Furthermore, the density of attack sites that a given enzyme could recognize on the substrate surface increased commensurate with the substrate binding strength throughout the dataset. These relationships of binding strength functional parameters are discussed along the lines of the century-old Sabatier principle for interfacial catalysis. We propose that the observed scaling relationships reflect limited functional plasticity of cellulases, and discuss consequences of this for the design of enzymes for technical applications.

8:25 AM 5-2: Development of synthetic multifunctional cellulases in fungi

R. Brunecky, V. Subramanian, T. Vinzant, B. Donohoe, M. Himmel and S.R. Decker, National Renewable Energy Laboratory, Golden, CO, USA; J.M. Yarbrough, National Renewable Energy Laboratory, Biosciences Center, Golden, CO, USA*

Recently, a paradigm-disrupting multifunctional enzyme from the hyperthermophilic bacterium *Caldicellulosiruptor bescii,* CelA, has been promoted as a highly active alternative to multi-component fungal enzyme systems. The CelA enzyme is unique in the sense that it is a multifunctional, multimodular enzyme with multiple catalytic, linker, and binding domains contained within a single gene. Architecturally, CelA is constructed as GH9(endo)-CBM3-CBM3-CBM3-GH48(exo). CelA exhibits a novel non-surface ablative deconstruction mechanism with demonstrated superior performance over fungal enzyme systems on model substrates such as Avicel. However, the standing paradigm for enzymatic cellulose deconstruction has been the traditional fungal free enzyme model exemplified by *Trichoderma reesei*, especially its glycosyl hydrolase family 7 exo-cellulase, Cel7A (CBH1) due to the ability of fungi to secrete large amounts of cellulolytic enzymes. Both the large size and repeating domains of CelA make it difficult to express in fungal systems and its thermophilic operating optimum (85^{o}C) is incompatible with existing fungal cellulases (50^{o}C). Recently we have demonstrated expression of novel fungal-based multifunctional enzymes similar to CelA in fungal systems such as *T. reesei.* These synthetic multifunctional cellulases combine thermophilic and mesophilic catalytic domains and binding modules from both fungi and bacteria and demonstrate synergy over molar combinations of the same free enzymes on both model and real world pretreated substrates. Moreover, one of these new enzymes exhibits a novel cellulase deconstruction mechanism which is distinct from both traditional fungal cellulases and the pit formation mechanism found in CelA.

8:50 AM 5-3: Impact of biomass pretreatment on the expression of CAZymes by a lignocellulolytic microbial consortium: A metaproteomic assessment

E. Flajollet, A. Lazuka and G. Hernandez-Raquet, LISBP-INRA, UMR792, Ingénierie des Systèmes Biologiques et des Procédés, CNRS, UMR5504; Université de Toulouse; INSA,UPS, INP, Toulouse, France; N. Jehmlich, Helmholtz Centre for Environmental Research, Leipzig, Germany; S. Dejean, Institut de Mathématique de Toulouse, Université de Toulouse, CNRS, UPS, Toulouse, France; B. Henrissat, CNRS UMR 7257, Aix-Marseille University, Marseille, France*

In order to optimize the carboxylate production from lignocellulosic biomass, substrate pretreatment is frequently used to improve bioconversion rates. While the impact of pretreatment on substrate features (structure, composition) and enzymatic hydrolysis has been widely studied, its impact on the functional diversity of microbial communities has been poorly described. Currently, meta-omics technologies and particularly metaproteomics enable studying complex microbial ecosystems providing useful knowledge on the taxonomic and functional diversity associated with lignocellulose bioconversion. Here, we applied metaproteomics to assess the dynamic of the expression of Carbohydrate-Active enZymes (CAZymes) by a lignocellulolytic microbial consortium growing on raw and dry-chemically pretreated wheat straw as substrate for carboxylate production. We identified more than 10 thousand proteins, being mainly related to “translation, ribosomal structure and biogenesis”, “carbohydrate transport and metabolism” and “energy production and conversion” functions. Proteins-based taxonomy was similar between raw and pretreated substrates, being dominated by members of Bacteroidetes and Firmicutes phyla. CAZymes were mainly expressed by members of the Bacteroidetes phylum but specific CAZymes were exclusively expressed by Firmicutes. A focus on glycosyl hydrolases (GHs) enabled to identify GH16, 17, 3, 48, 5 and 9 as well as GH10, 11, 26, 28, 43, 51, 67 and 8 as the main enzyme families associated to cellulose and hemicellulose degradation, respectively. The profile of CAZymes expression revealed a different temporal dynamics for the different pretreatments. A faster carboxylate production rate and an increase of xylanase activity for chemo-mechanical pretreatments was correlated to the abundance increase of Bacteroidetes related proteins belonging to GH43 family at the early steps of bioconversion. Furthermore, metaproteomics suggest a functional interplay between Bacteroidetes and Firmicutes phyla, which produced enzymes belonging to different CAZymes families.

9:15 AM Break

9:45 AM 5-4: From enzyme discovery to applications - highlights and challenges of LPMO research

J. Rahikainen, K. Marjamaa, A. Borisova, N. Aro, H. Nygren, S. Grönqvist, N. Maiorova, M. Karjalainen, V. Pihlajaniemi, S.*

Castillo, K. Kruus and A. Koivula, VTT Technical Research Centre of Finland Ltd, Espoo, Finland
The lytic polysaccharide monooxygenases (LPMOs), capable of oxidative cleavage of polymeric carbohydrates, are an interesting group of enzymes for enhancing the total hydrolysis of lignocellulosic biomass and for introducing novel properties to carbohydrate-based biomaterials. In this work we describe screening and applications of fungal family AA9 LPMOs for lignocellulose hydrolysis and modifications of cellulosic fibres. We describe the LPMO discovery from RNAseq libraries that are prepared from various fungal cultures and development of protein production systems for the recombinant LPMOs. We also show the analytical techniques to identify and measure the soluble and insoluble reaction products and application of these methods for detection and comparison of oxidative activities between different enzymes. We also show the most interesting results regarding applicability of LPMOs for lignocellulose saccharification and fibre processing.

10:10 AM 5-5: New observed intermediate in the catalytic cycle of a copper containing lytic polysaccharide monooxygenase

R.K. Singh and M.J. Bjerrum, University of Copenhagen, Copenhagen, Denmark; B. van Oort and R. Croce, Vrije Universiteit Amsterdam, Amsterdam, Netherlands; B.M. Blossom, D.A. Russo and P.E. Jensen, University of Copenhagen, Frederiksberg, Denmark; C. Felby, U Copenhagen, Rolighedsvej 23 1958 Frederiksberg C, Denmark*
The ability of lytic polysaccharide monooxygenases (LPMO) to oxidize cellulose in crystalline form has large practical applications in the process of refining biomass. The highly conserved copper centre in LPMO catalyses the oxidation of a variety of polysaccharides. Electron paramagnetic resonance spectroscopy (EPR) and crystal structures have shown the presence of copper (II) at the characteristic flat, solvent exposed, active-site of the AA9 family of LPMO's [1].
The function of LPMO has been the subject of great controversy. In the established model, this class of enzymes was considered to be monooxygenases. However, this view is now challenged by new data indicating that both O_2 and H_2O_2 may function as co-substrates [2].
We present experimental data for the existence of a long lived intermediate in the active site of *Thermoascus aurantiacus* LPMO (TaLPMO). The active species can be formed in an aerobic environment and can exist for minutes under the right conditions without destroying the active centre. The spectroscopic properties of the intermediate as well as the kinetics of its formation and decay will be presented and discussed.
The proposed structure of the intermediate has been used to suggest a catalytic mechanism for this class of LPMOs. For the first time the full catalytic cycle of LPMOs can be explained through an experimentally observed intermediate.

1. Frandsen, K. E. H. *et al. Nat Chem Biol*. 2016 12(4): 298–303.
2. Hangasky J. A., *et al, PNAS* 2018 115(19) 4915-4920

10:35 AM 5-6: Characterization of arabinoxylanases from a newly isolated thermophilic bacterium and heterologous expression in *Thermoanaerobacterium thermosaccharolyticum* to improve fermentation of corn fiber.

D. Beri, L.R. Lynd and C.D. Herring, Dartmouth College, Hanover, NH, USA; W. York and M. Pena, University of Georgia, Athens, GA, USA*
Conversion of the fibrous portion of corn kernels could increase ethanol yields by about 10 %. We present here that *Clostridium thermocellum* is able to solubilize over 90% of carbohydrate in corn fiber without thermochemical pretreatment save autoclaving, whereas carbohydrate solubilization yields for controls using fungal cellulase are about 50%. The solubilization products, however, are rich in complex arabinoxylan (AX) that is not readily fermented by *C. thermocellum* and also not by *Thermoanaerobacterium saccharolyticum* and several other described hemicellulose-fermenting thermophilic bacteria. To find an organism capable of breaking down AX and growing in coculture with *C. thermocellum*, inoculum from a thermophilic anaerobic digester was enriched on AX and component microbes isolated. The best performing isolate, designated LL 1360, consumed 85-90% of the AX, its genome sequence was obtained. Structural analysis of the AX led to the identification of recalcitrant glycosidic linkages and informed the screening of various carbohydrate active enzymes from LL 1360. Six enzymes were found to be active on the recalcitrant corn arabinoxylan including an a-xylosidase, two a-L-galactosidases, a b-xylosidase and two a-arabinofuranosidases. The a-xylosidase and a-L-galactosidases have especially rare activities. Supplementation with these enzymes allowed *T. thermosaccharolyticum* to consume 80% of AX whereas unsupplemented controls consumed about 50%, and also helped increase the ethanol yield of a *C. thermocellum* and *T. thermosaccharolyticum* coculture on corn fiber by 25%. The enzymes were successfully expressed in *T. thermosaccharolyticum* to obtain strain AX1 which exhibited a 35% improvement in AX fermentation compared to the parent strain.

11:00 AM 5-7: Photo-activation systems for LPMO-driven cellulose oxidation

B.M. Blossom, D.A. Russo, A. Perzon, T.I. Simonsen, P.E. Jensen and C. Felby, University of Copenhagen, Frederiksberg, Denmark; B. van Oort and R. Croce, Vrije Universiteit Amsterdam, Amsterdam, Netherlands; R.K. Singh and M.J. Bjerrum, University of Copenhagen, Copenhagen, Denmark*
Lytic polysaccharide monooxygenases (LPMOs) rely on extracellular electrons donors, such as low-molecular weight lignin, which supply reducing power at different stages during the catalytic oxidation of cellulose. The specific LPMO activity varies with

the electron donating system. It has been demonstrated, that photo-activated LPMO reactions have a great catalytic potential. However, in order to increase the efficiency of such a photo-biocatalytic system, and to reduce the electromagnetic energy input necessary for substrate oxidation, we investigated the impact of sequential light cycles. In this presentation, we show how light exposure as short as 1 s/min promoted LPMO activity to the same level as observed with constant light exposure. Sequential light cycles almost doubled the level of product formation (gluconic acid) after three hours of incubation with intact cellulose fibers. For microcrystalline cellulose (avicel), it was found that constant light exposure requires a high substrate concentration to be effective, whereas sequential light-cycling can be effective at lower to medium substrate concentrations.

Additionally, we will present a novel approach to activate cellulose in a light-dependent manner by generating reactive oxygen species, i.e. OH-radicals, on the substrate surface. This photo-assisted processing approach increases the efficiency of LPMOs and LPMO-containing cellulolytic cocktails in subsequent incubations.

8:00 AM - 11:25 AM Session: 6: Industry & Start up Showcase

Conveners: Jay Fitzgerald, Department of Energy - Energy Efficiency & Renewable Energy, Washington, DC, USA and **Dr. Malgorzata Slupska**, POET, Sioux Falls, SD, USA

Columbia D, Third level

8:00 AM 6-1: Enchi Corporation

L.R. Lynd, C. Herring and B. Brady, Enchi Corporation, Hanover, NH, USA*

Enchi Corporation was founded in 2014 as a spin-off of Mascoma Corp., after Mascoma's yeast-related assets were purchased by Lallemand LLC. Enchi's mission is to develop technology for conversion of cellulosic biomass into fuels and chemicals at low cost and without added enzymes or thermochemical pretreatment. Leveraged by strategic partnerships, Enchi is pursuing disruptive reductions in the cost of cellulosic biofuel production, starting with ethanol, based on consolidated bioprocessing using thermophilic bacteria and mechanical cotreatment in lieu of thermochemical pretreatment. This presentation will outline Enchi's technical rationale and business strategy, and also offer perspectives on advancing the industry.

8:25 AM 6-2: Characterization of bacterial microbiota in ethanol biorefineries

F. Firmino, University of Wisconsin-Madison, Madison, WI, USA, J. Broadbent, Lallemand, LLC and J. Steele, Lallemand Biofuels and Distilled Spirits, Lebanon, NH, USA*

A source of inefficiency within corn-based biofuel industry is the loss of yield to bacterial contaminations, mainly lactic acid bacteria (LAB). The first step to controlling an infection is to determine which microorganism(s) are responsible, therefore we utilized both V3-V4 16S rRNA sequencing and shotgun metagenomics to characterize the bacterial microbiota of four corn-based ethanol biorefineries. These microbiotas were determined to be relatively simple, with thirteen OTUs accounting for 90% of the bacterial population. The population was dominated by *Firmicutes* (89%), with the *Lactobacillus* genus comprising 80% of the OTUs from this phylum. The alpha-diversity of the microbial community was impacted by biorefinery and processing step, and four OTUs*, Lb. helveticus, Lactococcus sp., Lb. pontis* and *Lb. delbrueckii* were responsible for 79% of the inter-biorefinery variations. Bacterial diversity was highest in the cooled mash, decreased in the fermentation samples and then decreased further in the beer well. The microbiota of one ethanol facility was determined stable over a two years period. The V3-V4 16S rRNA sequencing results revealed that bacterial microbiotas in ethanol plants were relatively simple, plant specific, typically stable, with diversity decreasing throughout the process and dominated by LAB, primarily *Lactobacillus*. Shotgun metagenomics sequencing allowed for the characterization of bacterial succession at the species level and identified *Lb. helveticus* as the dominant species in these fermentations. This greater understanding of LAB contaminants in bioethanol facilities will contribute to the development of effective approaches to control bacterial infections during ethanol fermentations.

8:50 AM 6-3: Harnessing the capabilities of Clostridia strains for industrial biotechnology and nutrition

S. Jones, White Dog Labs, New Castle, DE, USA*

White Dog Labs, Inc. (WDL) was established on the foundation of microbiology, synthetic biology, and bioprocess development to address global challenges including food sustainability and climate change. Its technology solutions are based upon harnessing the unique abilities of bacteria within the Clostridia class. These microbes can be found in diverse environments from soils to gastrointestinal tracts to extreme environments and have developed exceptional capabilities. One of WDL's first technologies is a fermentation platform called MixoFerm™. By utilizing both soluble and gaseous feedstocks, MixoFerm can improve the efficiency of fermentation systems and thus reduce production costs. We are applying this technology first for a Single Cell Protein (SCP) product called ProTyton and are looking at additional biochemical applications. WDL is also developing proprietary selection methods to isolate Clostridia strains of interest for probiotic applications, and our first probiotic product, called BioTyton, is currently being tested in vivo on poultry. We have only begun to understand the full potential of these organisms and will continue applying them to help address food sustainability, animal and human health, and climate change.

9:15 AM Break

9:45 AM 6-4: Integrated biorefinery pilot and demo-scale facilities at Aarhus University for green biorefining

M. Ambye-Jensen[*] and C.W. Hsieh, Aarhus University, Aarhus, Denmark

The Centre for Biorefinery Technologies at Aarhus University has been inaugurated in Spring 2017 with the aim of integrating several biorefining processes for the production of value-added products (feed, chemicals, and fuels) from different biomass sources. The Centre has a wide range of pilot scale facilities to process at the kg/ton per hour scale. The Green Protein pilot plant was designed for the optimization, development, process upscaling, and production of leaf protein concentrate for animal feed and food, fibre for biofuels, and chemicals. The plant has a capacity of 1-2 ton (wet weight) input biomass per hour yielding around 20-50 kg DM leaf protein concentrate per hour. The protein/residual juice separation efficiency is highly dependent on the protein precipitation method (fermentation, heat, or combination of both) and separation processes. The final protein content in the protein paste can be as high as 54%. By-products of this process include fibre pulp for cattle feed, biogas or bioethanol production, and a residual juice which is up-concentrated by microfiltration to produce a concentrate high in soluble carbohydrates and nutrients for anaerobic digestion, fermentation and fertilizer. The facility's objectives are well in line with those of the circular bioeconomy concept of creating value out of all the waste streams in the biorefinery. Additionally, a 10x upscaled demonstration facility, with improved design and control, based on production data from the pilot plant, are being constructed and due to be operational starting summer 2019.

10:10 AM 6-5: Development of an acid tolerant yeast platform for efficient organic acid biosynthesis

J. Dietrich[*], Lygos, Berkeley, CA, USA

With a full-stack engineering team, including metabolic, fermentation, and chemical engineers, Lygos specializes in developing integrated manufacturing routes from sugar through purified chemicals. A key aspect of Lygos' technology has been the development of a multi-stress tolerant yeast, *Pichia kudriavzevii*, as a platform microbe well suited for organic acid production.

Lygos has invested in critical technologies to accelerate and increase the efficiency of the *P. kudriavzevii* Design-Build-Test-Learn engineering cycle. Multiomics approaches (genome-, transcript-, protein-, and metabolite-level) are being used to assay the state of the cell's metabolic network under different conditions. Additionally, artificial intelligence and machine learning techniques are being used to interrogate these datasets, helping to accelerate cycle times and identify difficult to find genetic modifications important to delivering commercially relevant production strains.

Lygos first engineered *P. kudriavzevii* to produce malonic acid, a high-value diacid currently manufactured petrochemically using an expensive, hazardous process based on chloroacetic acid and sodium cyanide as raw materials. The malonic acid producing strain is characterized by an ability to separate growth from production, a fast sugar consumption rate, and efficient conversion of glucose to important metabolic intermediates. This platform strain is now being retooled to produce of a range of chemicals derived from glycolytic intermediates, acetyl-/malonyl-CoA, and both TCA cycle and aromatic metabolites. By leveraging our success with malonic acid we are producing new target molecules on accelerated timelines.

10:35 AM 6-6: Metabolism, reloaded:computational design of novel enzymes and synthetic pathways for the synthesis of bio-chemicals

A. Zanghellini[*], Arzeda

Nature's metabolic routes are the product of 2 billion years of evolution and extraordinary chemical versatilility. They, however, only scratch the surface of the gigantic diversity of molecules that could be produced by network of enzymes catalysts. This contrasts with the requirements of modern technology: 75% of fine and specialty chemicals on the market today are not naturally produced. Moreover, countless new molecular compounds with application from improved advanced materials to new drugs and antibiotics have not been sampled in evolution and are unreachable by synthetic chemistry.

Technological advances have poised us to provide a solution by rewiring metabolism to create synthetic strains capable of fermenting to these molecules. We (i.e. Arzeda, a Seattle, WA synthetic biology company) accomplished this by leveraging recent rapid advances in computation and machine learning. We break the problem into two parts: the first deals with the design of enzymes with non-natural activities. To this end, we have developed a high-throughput computational protein design methodology (ARCHYTAS) to rapidly craft new enzymes. The second part is to design synthetic metabolic routes by finding optimal ways to arrange natural and designed enzymes to biosynthesize any molecular target of interest. Inspired by retrosynthetic methods in organic chemistry, Arzeda's software SCYLAX draws on natural and designed enzyme reaction databases to automatically and exhaustively enumerate feasible biosynthetic routes, ranking all solutions based on their thermodynamic and mass-balance efficiencies. Very recently, we used SCYLAX and ARCHYTAS in combination to design synthetic metabolic pathways producing industrial chemicals never reported to by synthetized by fermentation, as well as new molecular backbones never synthetized before. Some of these unpublished experimental results will be presented.

11:00 AM 6-7: De novo carbon conserving biosynthetic pathways

H. Chokhawala[*], Zymochem

Acetyl-CoA is a fundamental precursor used by many native and non-native biosynthetic pathways for the production of a wide

variety of chemicals. However, production of acetyl-CoA via decarboxylation of pyruvate results in a loss of ~33% of the feedstock carbon as CO_2, limiting theoretical yields from these pathways to <67%. If microbes could conserve most/all of the feedstock carbon and direct it to the targeted end product, the resulting increase in theoretical yields (up to 50% higher) could improve production efficiencies and significantly reduce costs. At ZymoChem, our mission is to develop carbon conservation (C^2) technologies based on non-natural biosynthetic pathways within microbes, which we design to enable the fermentation-based production of industrial chemicals from various renewable feedstocks, and importantly, with little or no loss of bio-based feedstock carbon as CO_2.

9:00 AM - 3:00 PM Exhibits Open

Columbia Ballroom Foyer, Third level

11:25 AM - 1:00 PM Lunch-on your own

1:00 PM - 4:25 PM Session: 7: Synthetic and Systems Biology

Conveners: Pirkko Suominen, Cargill, Plymouth, MN, USA and **Yi Wang**, Auburn University, Auburn, AL, USA

Columbia C, Third level

1:00 PM 7-1: Systematic genome engineering of solventogenic Clostridia for biofuel and biochemical production

J. Zhang, J. Feng, P. Wang and Y. Wang, Auburn University, Auburn, AL, USA*

Clostridium is a genus of Gram-positive, rod-shaped, anaerobic bacteria. Many *Clostridium* species have great potentials for industrial biofuel and biochemical production. However, the genome engineering of *Clostridium* is generally difficult due to the lack of efficient transformation protocols and amenable genetic engineering tools. In our lab, we have developed customized CRISPR-Cas9-based genome engineering tools and applied to various solventogenic clostridial strains. Based on systematic genome engineering, we improved the n-butanol production and selectivity significantly in our clostridial hosts. Further, we also engineered our strain for isopropanol-butanol-ethanol (IBE) production by chromosomally integrating the ¡®acetone-to-isopropanol¡¯ pathway using CRISPR-Cas9, and achieved 34.5 g/L total IBE in a batch fermentation, representing the highest IBE production that has ever been reported in solventogenic clostridia. In another strain in which the CRISPR-Cas9 system was not implementable, we successfully explored the native Type I-B CRISPR-Cas system for genome engineering, and achieved multiplex genome editing (editing multiple genes) through a single transformation with high efficiency. We enabled the mutant for butanol production to historically record high of 26.2 g/L in a batch fermentation. Besides, we successfully engineered our strain for fatty acid ester production to the highest level that has ever been reported in a microbial host. The production of fatty acid ester is advantageous because the ester has much higher value than the alcohol and fatty acid precursors, and it is much easier to separate from fermentation broth. Our results demonstrated that solventogenic clostridia are an excellent platform for biofuel and biochemical production through rational genome engineering.

1:25 PM 7-2: System analysis on lignin degradation mechanisms and metabolic pathways in marine protist, *Thraustochytrium striatum*

X. Li and Y. Zheng, Kansas state university, Manhattan, KS, USA; M. Li, University of Tennessee, Knoxville, TN, USA; Y. Pu and A. Ragauskas, Oak Ridge National Laboratory, Oak Ridge, TN, USA; M. Blenner, Clemson University, Clemson, SC, USA; J.S. Yuan, Synthetic and Systems Biology Innovation Hub, Texas A&M university, College Station, TX, USA*

Lignin has been regarded as a promising feedstock for biofuel and bioproduct due to its high energy density and diverse structure. Lignin conversion through biological platforms can upgrade lignin into high value-added products. Based on lignin metabolism, strategies such as genetic modification, protein metabolic engineering can improve lignin conversion efficiency or establish novel metabolic pathways. The marine protist, *Thraustochytrium striatum* has been proven to accumulate fatty acids and carotenoids with lignin as a sole carbon source, while the metabolic mechanisms and pathways still remain unknown. This study aims at investigation on lignin degradation and construction of metabolic pathways. The black liquor from alkaline pretreatment of corn stover was used as carbon source as it contains multiple aromatic compounds and short lignin fragments, which makes it a good candidate for study of breakdown of lignin linkages, aromatic compound degradation, and interactions between different processes. An integration of omics analysis at different levels were applied during black liquor fermentation. Genomics analysis was conducted to identify genes in *T. striatum* responsible for lignin degradation. Transcriptomics and proteomics analysis were used to identify critical enzymes involved in this process. Metabolites profile is determined by metabolomics analysis. With all the results, lignin degradation pathways in *T. striatum* will be constructed. This study is the first to study lignin degradation by *T. striatum* from the fundamental aspect. The results will bridge the knowledge gap on lignin degradation mechanisms in and provide theoretical basis for development of *T. striatum* as a novel platform for lignin

bioconversion.

1:50 PM 7-3: Metabolic modeling of methanotroph biocatalysts for natural gas-to-liquids bioconversion

J. Orth[*]*, Intrexon, South San Francisco, CA, USA*

Natural gas is an extremely inexpensive source of carbon and energy for microbial fermentation, and Intrexon has developed the first natural gas-to-liquids bioconversion platform utilizing methanotrophic bacteria. Intrexon's synthetic biology tools are used to construct novel strains and engineer the metabolism of this methanotroph to produce high value chemical and biofuel products including the butadiene precursor 2,3-butanediol and the biofuel isobutanol.

One technology that has become increasingly valuable to microbial metabolic engineering is constraint-based genome-scale metabolic modeling. These models are organism-specific and allow for the rapid simulation of metabolic phenotypes and identification of novel strain engineering strategies. A methanotroph metabolic model was built at Intrexon based on the strain's annotated genome sequence using custom automated reconstruction tools and extensive manual curation. Several different types of experimental data were used to improve the model, set key quantitative parameters, and validate the model. Constraint-based analysis techniques, including flux balance analysis, are used to simulate the metabolism of different strains or different fermentation conditions.

Metabolic modeling combined with other data science approaches have been applied to improve our understanding of methanotroph biology, analyze and interpret omics data, and predict novel strain engineering strategies to reroute metabolism and improve production. These types of analysis have been incorporated into Intrexon's Design-Build-Test-Learn cycle for rapid strain engineering, resulting in significant increases in yields and titers of several products. 2,3-butanediol and isobutanol production strains have been transferred from lab scale to pilot scale, and other commercially valuable products are in earlier stages of development.

2:15 PM Break

2:45 PM 7-4: Novel synthetic pathway for production of mid-chain length fatty acids

K. Watts[*]*, Cargill, Plymouth, MN, USA*

The manufacture of fatty acids from sugar substrates has long been a tantalizing goal, but one that presents interesting economic and technical challenges. When considering longer chain fatty acids, the producer is challenged by relatively low theoretical yields and abundant supply from existing plant based sources. By contrast, if one looks to the mid chain lengths, there is substantially more constrained supply as well as some improvement in theoretical yield. The constrained supply of mid-chain fatty acids results in an unfilled market opportunity. However, these molecules are inhibitory to host microorganisms and the biological pathways to mid chain fatty acids are scarce. To meet this challenge Cargill set out to create a synthetic pathway. In order to achieve commercially relevant performance, Cargill needed to identify solutions to one of the classic challenges of metabolic engineering - simultaneously achieving high specificity and high productivity with the same synthetic pathway.

3:10 PM 7-5: Automated workflows dramatically accelerate microbial strain engineering

K. George[*] *and K. Benjamin, Amyris, 5885 Hollis Street, CA, USA*

Amyris is a pioneer in the development of fermentation-derived, sustainably-sourced products for a wide range of applications and industries. In the last 5 years, Amyris has produced 8 distinct molecules at industrial scale and reached more than 250 million consumers through products ranging from laundry detergent to skincare. Optimizing microbes to produce these molecules at high yields and productivities may require hundreds or thousands of iterations of the design-build-test-analyze (DBTA) engineering cycle. At Amyris, each "turn" of the DBTA cycle is accelerated by a persistent focus on the automation of labor-intensive metabolic engineering workflows. Simultaneously, advances in bioinformatics and data analytics continue to reduce the number of iterations needed to reach strain performance targets. Through a decade's worth of development and optimization, nearly every aspect of strain engineering has been automated, from metabolic route-finding and DNA assembly, to strain verification and high-throughput screening. This presentation will cover Amyris' systematic approach to automating metabolic engineering workflows. Current challenges, future aspirations, and key lessons from a decade's worth of development will be discussed.

3:35 PM 7-6: Evolving a terephthalic acid catabolic pathway

I. Pardo[*]*, C. Johnson and G. Beckham, National Renewable Energy Laboratory, Golden, CO, USA; E. Neidle, University of Georgia, Athens, GA, USA*

Terephthalic acid (TPA) is a commodity chemical mostly employed as a precursor to the widely-used polymer polyethylene terephthalate (PET). While several bacterial strains capable of catabolizing TPA have been isolated, the degradation of this xenobiotic compound remains inefficient. In this work, we describe the introduction and Evolution by Amplification and Synthetic biology (EASy) of a TPA catabolic pathway in *Acinetobacter baylyi* ADP1, a strain that is natively unable to utilize

TPA. The EASy method involves increasing the copy number of a chromosomal segment encompassing the foreign genes, a process that facilitates the selection of beneficial mutations during growth on TPA in serial culture. This way, we have obtained several populations capable of consuming TPA at >100 mg/L/h. In the future, the engineered TPA-degrading strain will in turn be used as a host for the EASy-based optimization of TPA biosynthetic pathways. Currently, TPA is produced by the catalytic oxidation of *p*-xylene, and only a few examples of enzymatic or whole cell biosynthesis have been described. By using EASy, we will be able to evolve biosynthetic pathways for the efficient production of this commodity chemical using alternative substrates such as biomass.

4:00 PM 7-7: A multiobjective strain design platform for modular cell engineering

C.T. Trinh, S. Garcia, J. Lee and H. Seo, University of Tennessee, Knoxville, TN, USA*

Metabolic engineering has enabled the use of microbial cell factories for industrial production of biochemicals. However, developing an optimal strain for synthesis of one product with the conventional strategy is laborious and expensive. To accelerate and reduce the cost of strain engineering, we formulate the modular cell (MODCELL) design principles by exploiting the modular organization of metabolic networks and combinatorial possibilities of metabolic modules that enable the synthesis of a large space of biochemicals. Using the multiobjective optimization methods, we develop novel algorithms to implement the MODCELL design for genome-scale metabolic networks and the associated software ModCell2.0. We demonstrate ModCell2.0 for design, construction, and validation of an *E. coli* modular cell for combinatorial synthesis of biochemicals, e.g., alcohols and bioesters from fermentable sugars and organic wastes. We envision MODCELL will provide a useful platform for modular cell engineering.

1:00 PM - 4:25 PM Session: 8: Everything Lignin

Conveners: Roberto Rinaldi, Imperial College and **Arthur Ragauskas**, Oak Ridge National Laboratory, Oak Ridge, TN, USA

Columbia D, Third level

1:00 PM 8-1: Multi-stream Integrated BioRefinery (MIBR) for sustainable and cost-effective biofuels and bioproducts

J.S. Yuan, Synthetic and Systems Biology Innovation Hub, Texas A&M university, College Station, TX, 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 byproducts 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.

1:25 PM 8-2: Outer membranes vesicles: a proposed mechanism for lignin depolymerization and catabolism by Pseudomonas putida

D. Salvachúa, A. Werner, I. Pardo, S. Notonier, B.S. Donohoe and G.T. Beckham, National Renewable Energy Laboratory, Golden, CO, USA; P.E. Abraham, R.J. Giannone and R.L. Hettich, Oak Ridge National Laboratory, Oak Ridge, TN, USA; M. Michalska and P.D. Laible, Argonne National Laboratory, Lemont, IL, USA*

Pseudomonas putida KT2440 is a Gram-negative soil bacterium reported to catabolize aromatic compounds and utilize high molecular weight lignin. The intracellular mechanisms implicated in the catabolism of aromatic compounds have been extensively studied, but the enzymes involved in the breakdown of oligomeric lignin and/or their spatial location remains unknown. To identify the enzymes involved in lignin breakdown, we performed a differential proteomic study in the intracellular and extracellular loci of *P. putida* when grown in lignin and minimal media. The number of proteins found exclusively in lignin cultures was considerably higher in the extracellular fraction than in the intracellular one and the former fraction contained a high number of enzymes that had been previously described to be intracellular. To discern between cell lysis and secretion of enzymes to the extracellular milieu, we conducted cytometry, GFP-labeling in selected enzymes, and scanning and transmission electron microscopy. This work uncovered the presence of numerous outer membrane vesicles (OMVs) in the

bacterial cultures. To understand the function of these vesicles, we isolated them from *P. putida* cultures and analyzed their cargo through proteomic analysis. The results showed that some enzymes, previously reported to be involved in the catabolism of aromatic compounds or correlated with lignin breakdown, were enriched in the OMV fraction compared to the supernatant. This discovery opens new directions for investigation, from fundamental research to applications, to understand how bacteria interacts with lignin or aromatic compounds in the extracellular locus and to engineer improved microbes for the lignin conversion into renewable chemicals.

1:50 PM 8-3: Creating a new field of lignocellulose valorization by the novel sustainable process: Simultaneous Enzymatic Saccharification and Comminution

Y. Otsuka, R. Navarro, T. Araki and M. Nakamura, Forestry and Forest Products Research Institute, Tsukuba, Ibaraki, Japan; K. Shikinaka, National Institute of Advanced Industrial Science and Technology, Sendai, Japan; E. Masai, Nagaoka University of Technology, Nagaoka, Japan; Y. Katayama, Nihon University, Fujisawa, Kanagawa, Japan*

Lignin is a major component of all plants, and is the most abundant aromatic biomass on earth. Lignin is a very delicate substance that is easily denatured by strong acid, alkali and high temperature conditions. This is one of the major obstacles towards the creation of a valuable utilization technology for lignin. Recently, we have developed the simultaneous enzymatic saccharification and comminution (SESC) as a mild lignocellulose pretreatment system that is not dependent on extreme conditions of pH and temperature. By SESC method, lignocellulosic biomass can be separated into its sugar and lignin (SESC-lignin) components. Since the generated SESC-lignin is not denatured, it can be directly used as a raw material for functional materials or decomposed efficiently by chemical treatment into aromatic monomers. The aromatic mixture can then be transformed into various platform chemicals by microbial fermentation. In this regard, our research group has developed a process to convert lignin aromatics into 2pyrone4,6dicarboxylic acid (PDC) by metabolic engineered microorganism. We have also demonstrated that various polymer products can be synthesized by using PDC as a base component. In this presentation, we would like to discuss how the combination of SESC process and microbial fermentation could make a new field of lignocellulose valorization.

2:15 PM Break

2:45 PM 8-4: Identification, reconstruction, and optimization of enzymatic pathways for lignin valorization

J. Michener, G.N. Presley, J. Cecil, D. Garcia and R.J. Giannone, Oak Ridge National Laboratory, Oak Ridge, TN, USA*

Cellulosic biofuel production yields a substantial lignin byproduct stream that currently has few applications. Biological conversion of lignin compounds into chemicals and fuels has the potential to improve the economics of cellulosic biofuels. Microbial strains have been isolated with the ability to degrade challenging aromatic compounds, and the associated pathways could substantially improve the conversion of thermochemically-depolymerized lignin into valuable bioproducts. However, substantial work will be required to enable cost-effective lignin valorization, both to broaden the range of compounds that production strains can assimilate and to improve conversion into the desired product. We have used transposon insertion sequencing to rapidly identify entire catabolic pathways in genetically-intractable microbes such as the lignin-degrading strain *Novosphingobium aromaticivorans*, leading to the discovery of multiple new enzymes for transformation of lignin-derived aromatic compounds. Simultaneously, we are reconstructing pathways for lignin catabolism in *Escherichia coli* to enable pathway characterization in a tractable and well-defined host. Evolutionary optimization of these heterologous pathways identified factors limiting pathway activity and will allow the design of characterized, optimized synthetic operons for aromatic degradation. Finally, we have developed a new method for selecting non-model microbes with improved production of target bioproducts, such as synthesis of muconic acid from aromatic compounds. In combination, these efforts improve our ability to engineer microbes for lignin valorization.

3:10 PM 8-5: Enzymatic modification of lignin

T. Gronroos, K. Birikh, V. Hamalainen, A. Suonpaa, J. Antunes, M. Heikkilä and P. Ihalainen, METGEN Oy, Kaarina, Finland*

Controlled fragmentation of lignin could lead to its use in higher value products such as binders, coatings, fillers and other. Oxidative enzymes, (laccases and peroxidases) have long been proposed as a potentially promising tool in lignin depolymerization. However, their application was limited to ambient pH, where lignin is poorly soluble in water. MetGen Oy, a Finnish biotechnology company that designs and supplies industrial enzymes, has developed and brought to market several lignin oxidizing enzymes, including an extremely alkaline lignin oxidase MetZyme® PURECO™, a genetically engineered laccase of bacterial origin. This enzyme can function at pH values as high as ten to eleven and at elevated temperatures, addressing lignin at its soluble state. Lignin modification by MetZyme® PURECO™ was characterized by size exclusion chromatography, UV-spectroscopy, NMR and dynamic light scattering for monitoring particle size of solubilized lignin. The results showed that the balance between polymerization and depolymerization is pH and aeration dependent. Under highly alkaline conditions and strong aeration, depolymerization prevailed over polymerization. Thereby, laccase treatment not only decreased the lignin molecular weight but also increased its solubility in water, altering its dispersion properties. NMR results showed over 75 % increase in phenolic- and aliphatic-OH groups. Therefore, the enzyme also partially demethylates lignin, increasing the number of hydroxyl groups, and alters its surface properties. These observations have been found to apply to a wide range of different type of lignins. These enzyme-based solutions open new opportunities for biorefinery lignin valorization,

thus paving the way for economically viable biorefinery business.

3:35 PM 8-6: Impact of dilute acid pretreatment conditions on corn stover lignin properties and their suitability as a phenol replacement in phenol formaldehyde resins

B. Saulnier, A. Savoy, S.K. Singh and D. Hodge, Montana State University, Bozeman, MT, USA; M. Nejad, Michigan State University, East Lansing, MI, USA*

Lignin derived from a biorefinery utilizing dilute acid pretreatment of corn stover was recently demonstrated to be suitable as a 100% replacement for phenol in phenol formaldehyde (PF) resins used as an adhesive in engineered wood products applications. In this work, we will build on these findings and investigate the how processing during pretreatment and recovery/purification of lignin from the hydrolysis residue ("lignin cake") impact the suitability of these lignins as a phenol replacement. As the lignin cakes may be comprised of <60% lignin, for the first part of this work we investigate the purification of lignin from lignin cake by extraction with organic solvents or alkali and assess the impact of solvent and extraction temperature on the recovered lignin yields, purities, and solubilities in select organic solvents. In the second part of this work, we investigate how pretreatment conditions and natural lignin diversity impact the properties of lignin following pretreatment. Importantly we hypothesize that the preservation of *p*-coumaric acid (*p*CA) in corn stover lignins during dilute acid pretreatment results in its improved incorporation into PF resins. To address this, 6 different maize genotypes representing a diverse range of *p*CA contents are subjected to dilute acid pretreatment at a range of pretreatment severities. The resulting pretreated biomass samples are evaluated for lignin content, response to enzymatic hydrolysis, *p*CA and ferulic acid content, and response to lignin recovery/recovery. Additionally, select recovered lignins from this sample set are evaluated for their potential as a phenol replacement in PF resins used as a wood adhesive.

4:00 PM 8-7: The fate of lignin after biomass conversion and pulping processes: What is the value proposition?

R.P. Chandra, Innotech Alberta, University of British Columbia Bioenergy Group, Edmonton, AB, Canada; T.D. Ngo and B. Ahvazi, Innotech Alberta, Edmonton, AB, Canada; J.N. Saddler, University of British Columbia, Vancouver, BC, Canada*

Biomass conversion and pulping processes both aim to recover cellulose and hemicellulose while minimizing the impact of lignin. In the case of biomass conversion, lignin adversely affects enzymatic hydrolysis, while during pulping processes, depending on the product, the presence of lignin contributes to inferior fibre properties, yellowing etc. Therefore, in both cases, lignin modification and/or removal is critical towards obtaining the final product. Consequently, there are approximately 70 million tons of lignin available from kraft pulping processes, while another 200-300 million tons of lignin are forecast to become available from current and future biorefineries. This lignin represents the largest reservoir of aromatics on the planet and thus has tremendous potential for application development. Therefore, the critical need to valorize lignin through the development of chemicals and materials is widely acknowledged as the one of the main hurdles to be overcome to realize biomass-based biorefineries. However, depending on the type of biomass, the chemistry of the isolation process (acid vs alkaline vs mechanical), and the recovery process, the resulting lignin exhibits diverse characteristics that influence its downstream utilization. Therefore, this presentation will discuss the characteristics of the lignin that result from various biomass pretreatment and pulping processes. Several examples will be detailed including the development of lignin nanoparticles, novel materials from various lignin sources, and the isolation, characterization and application of lignin obtained from emerging lignin processing systems such as deep eutectic solvents and hydrotropes.

6:00 PM - 7:00 PM Exhibits Open

Columbia Ballroom Foyer, Third level

6:00 PM - 9:00 PM Banquet and Award Presentations: Banquet Speaker, Michael Lakeman

Conveners: Michael Lakeman, Boeing

Columbia C-D, Third level

Wednesday, May 1

7:00 AM - 8:00 AM Speaker Breakfast-Speakers and Conveners on date of your session

Duwamish - Room 306, Third Level

7:00 AM - 3:00 PM Registration

Columbia Ballroom Foyer, Third level

8:00 AM - 11:25 AM Session: 12: Technoeconomic, Geopolitical, and Life Cycle Analyses

Conveners: Mary Biddy, National Renewable Energy Laboratory, Golden, CO, USA and **Tom L. Richard**, The Pennsylvania State University, University Park, PA, USA

Columbia D, Third level

8:00 AM 12-1: How can cultivation of perennial species in riparian zones meet renewable energy goals and improve ecosystem health?

C. Costello and S. Jose, University of Missouri, Columbia, MO, USA; N. Ayoub, Helwan University, Cairo, Egypt*

Given the pressures on land use to produce food crops, many have looked at cultivation of perennial plant species on marginal lands, particularly in riparian zones, as a potential feedstock for bioenergy. Cultivation of perennial species in riparian zones that exhibit physical deficiencies, such as erosion, could lead to improvements in ecological function, such as soil stabilization, carbon sequestration, and improved habitat opportunities. Further, utilization of waterborne transportation can reduce life cycle energy and greenhouse gas emissions associated with these cellulosic biofuels. In this work, we have estimated the quantity of marginal land available within the Missouri/Mississippi River Corridor given a variety of physical and policy-driven definitions of marginal land. Physical reasons for classifying land as marginal include high erodibility and prone to flooding as classified by NRCS-SSURGO data. Three cellulosic, perennial species (poplar, willow, and switchgrass) were considered for planting in these zones. The range of estimates vary widely, primarily due to the uncertainty about the true conditions of forested areas and lands enrolled in the Conservation Reserve Program, which does not allow cultivation for bioenergy. Conservative scenarios that do not include forested or CRP lands result in estimates of 27.6 – 30.4 million hectares of land potentially available or up to 360 million tons of biomass. Preliminary estimates of the life cycle energy and greenhouse gas emissions for the most plausible biomass planting scenarios will also be shared. Timing of harvest to maximize habitat benefits for wildlife and logistics are key considerations.

8:25 AM 12-2: Economic and environmental benefits of increased nitrogen use efficiency on crops

L.M. Eaton, B. Davison, L. Baskaran, C. Brandt, E. Webb, M. Davis and M. Langholtz, Oak Ridge National Laboratory, Oak Ridge, TN, USA*

Nitrogen is a key input to food production. Its use in agriculture increases crop productivity, but at the same time is responsible for environmental issues. A number of concepts are being pursued to increase nitrogen use efficiency (NUE), which would result in lower-cost food production, better income for farmers, and decreased environmental impacts. While many published reports discuss the benefits of increased NUE, we quantify them using an economic simulation model of US agriculture. In this analysis we estimate the economic and environmental impacts of increased NUE (i.e. price impacts, net farmer incomes, land allocation, and water quality changes). Results suggest that a 20% increase in nitrogen use efficiency in row crops would result in reduction of 1.58 million tons of applied N, an annual 1.6% increase in farmer net returns ($743 million), and a cumulative economic benefit of $5.5 billion over a 10-year period with full adoption. Overall consumption increases and expenditures for commodity crops decrease. Land effects are inconclusive. Using a watershed-scale hydrological model of the Arkansas-White-Red river basin (AWR) we estimate the water quality impacts of increased NUE. Results suggest that a 20% reduction in N use corresponds to a 5.8% reduction in nitrate loadings and 2.6% decrease in overall nitrogen concentration in drainage waterbodies. The report concludes with a discussion of the application of these results to the social value of nitrogen and nitrogen reduction strategies.

8:50 AM 12-3: A biomass supply analysis for a bioconversion facility located in Southwest Washington

A. Chowyuk, R. Bura, H. El-Husseini and R. Gustafson, University of Washington, Seattle, WA, USA; N. Parker, Arizona State University, Tempe, AZ, USA*

Cellulosic biofuels have been considered as replacement fuels for petroleum-derived fuels to mitigate climate change, reduce greenhouse gas emissions, contribute towards energy independence and security, and promote sustainable economic development. While biofuels offer many environmental and socioeconomic benefits, commercial scale biorefineries have not been developed. High feedstock costs are one of the most significant barriers to the advancement of commercial scale biofuel production. This research investigated obtaining biomass from various sources – including purpose grown poplar, residuals, poplar grown for water treatment, and switchgrass grown to clean roadside stormwater – to reduce biorefinery feedstock cost.

A moderate sized sugar platform based biorefinery located in Southwest Washington was assumed to consume 250,000 tonnes

of biomass per year. It was found that plenty of purpose grown poplar could be cultivated on local pastureland for the factory, thus avoiding food vs. fuel conflicts, but that the plant-gate cost was $83/tonne. To reduce the cost of biomass we investigated obtaining lower cost biomass from the following sources:

- Poplar grown at waste treatment plants for water management. A practice already in place in several Washington waste treatment facilities as they cannot discharge in receiving rivers in summer months.
- Residuals from sawmills, agriculture, and forest harvesting
- Switchgrass grown on highway vegetative filter strips to clean stormwater runoff.

For the poplar grown at the waste treatment facilities and along highways it was assumed that the biorefinery would pay for harvesting and shipping of the biomass. Discussions with waste treatment managers using poplar for water reclamation showed that they were already concerned about the cost of harvesting and shipping the trees once they had reached a maximum allowable age.

The research showed that about 55% of the biomass necessary to run the biorefinery could be obtained from the lower cost sources and that biorefinery gate biomass cost could be reduced by over $20 per tonne of biomass. This talk will present the specifics of how the costs of each biomass source was calculated, including appropriate supply curves, and the impact of the lower cost biomass on the viability of bioconversion facility based in Southwest Washington.

9:15 AM Break

9:45 AM 12-4: 2018 NREL design report: Integrated biorefinery pathways for biochemical conversion of biomass to hydrocarbon fuels and bio-products – process design and techno-economics

R. Davis, N. Grundl, L. Tao and M. Biddy, National Renewable Energy Laboratory, Golden, CO, USA*

While economics and process understanding for biochemical production of cellulosic ethanol (and to a lesser extent, hydrocarbon fuel) have been reasonably investigated and refined in recent years, similar considerations for more complex biorefineries to produce both fuels and bio-derived products have not yet been well-established publicly. Moreover, techno-economic models have demonstrated that, particularly for fungible hydrocarbon fuel production, biochemical conversion to fuels will not achieve cost goals when limited to utilization of biomass carbohydrates alone. In support of an expanded focus to highlight paths towards economic viability for a multi-fuel/product biorefinery concept, this work presents a detailed techno-economic analysis (TEA) focused on biochemical hydrocarbon production via deconstruction and upgrading of cellulosic biomass carbohydrates coupled with conversion of lignin and other residual constituents to value-added coproducts, to improve revenues and maximize biomass utilization. This work as documented in a recently-published “design report” expands from earlier analyses widely circulated for biochemical fuel processes (Wooley 1999, Aden 2002, Humbird 2011, Davis 2013) with key differences and new challenges highlighted to ultimately achieve minimum fuel selling price targets below $2.50/gallon gasoline equivalent (GGE) in the future. The TEA evaluates two sugar-to-fuel pathways via fermentation and catalytic upgrading through carboxylic acids and 2,3-butanediol (BDO), with associated upstream and downstream process integration considerations included as well as process and cost evaluation for lignin conversion to adipic acid (as a representative example bio-advantaged coproduct). Additionally, the work also presents a cost sensitivity analysis to quantify impacts of uncertainties and identify key cost drivers and future research priorities.

10:10 AM 12-5: CANCELLED - Techno-economic analysis and exergo-environmental performance of integrated first- and second-generation bioethanol production plants through biochemical and thermochemical conversion pathways

P. Silva Ortiz, University of Campinas-UNICAMP, Campinas, Brazil, A.P. Mariano, University of Campinas, Campinas, Brazil and R. Maciel Filho, State University of Campinas, Campinas, SP, Brazil*

Driven by a range of bioenergy sustainability challenges, advanced conversion technologies are required to reduce costs, environmental impacts, and increase the productivity efficiency to continue the transition of lignocellulosic biofuel production from pilot scales to industrial implementation. Thus, biorefinery technologies could play an important role to produce a comprehensive range of marketable products in a sustainable way from widely available lignocellulosic residues. This study analyses the integrated first (1G) and second-generation (2G) ethanol production plants via biochemical and thermochemical pathways to improve sustainability-related indexes of sugarcane-based biorefineries in Brazil. The integrated 1G + 2G process designs (combining biochemical and thermochemical pathways) for bioethanol production from sugarcane bagasse aiming to develop a thermodynamic-based approach for integrating large resources use efficiency with advanced conversion technologies from a technical, economic and environmental perspective. Thus, several techno-economic and environmental performance parameters are using in the assessment: *i). Energy and exergy efficiency, ii). Average unitary exergy cost (AUEC), iii). Irreversibility/Exergy products ratio, iv). Global CO2 emissions, v). CAPEX and OPEX (capital and operational expenditure)*. Results regarding the technical conversion of these systems indicated that the higher exergy efficiency (37%) was presented in the integrated 1G + 2G biochemical process and consequently a lower average unitary exergy cost (AUEC=2.7 kJ/kJ). Furthermore, the global CO2 emissions was 4.04 kgCO2equiv./kg ethanol and the CO2 equivalent index in exergetic base was 149 gCO2/MJ ethanol for the biochemical process. Lastly, this process shows a reduction of 20 % on the capital investment cost in comparison with the thermochemical pathway.

10:35 AM 12-6: Near-term opportunities for carbon dioxide removal from bioenergy

D.L. Sanchez, PhD, University of California-Berkeley, Berkeley, CA, USA*
Capture and permanent geologic sequestration of biogenic CO2 emissions may provide critical flexibility in ambitious climate change mitigation. However, most bioenergy with carbon capture and sequestration (BECCS) technologies are technically immature or commercially unavailable. Here, I evaluate low-cost, commercially ready and/or small scale BECCS technologies. These include CCS at existing biorefineries, carbon sequestration from biogas upgrading, biochar production, and other advanced systems. These systems embrace modularity, low- or no-cost CO2 capture, and satisfy regional policy conditions or niche market demands to provide cost-effective CO2 sequestration from bioenergy.

11:00 AM 12-7: Analyzing the integrated social and environmental dimensions of biofuels: A political-industrial ecology perspective

J. Baka, Penn State, University Park, PA, USA*
Biofuels have transformed energy and agricultural systems and political landscapes in both developed and developing economies. While scholars have acknowledged the cross-cutting impacts of biofuels, integrated assessment tools/frameworks remain underdeveloped, particularly those that combine social and environmental sciences. This paper addresses this gap by utilizing a political-industrial ecology (PIE) framework to evaluate biofuels. PIE is an emerging subfield of geography that brings together methods/thinking from industrial ecology and political ecology to simultaneously evaluate the biophysical and political dimensions of environmental change from a systems perspective. I illustrate the potential of PIE through a case study of Jatropha biofuel promotion in India. Jatropha was promoted as a miracle crop that could help to overcome the food versus food security challenges of first generation biofuels, particularly when grown on “marginal” lands. As the PIE analysis reveals, the concept of marginal lands is a political construction, which obscures the livelihood significance of such lands to rural communities. Locating Jatropha projects on marginal lands tended to increase energy poverty in rural communities because a traditional bioenergy system was uprooted to clear lands for Jatropha plantations. The traditional energy economy provided approximately 3-10 times more useful energy than would the proposed Jatropha system. Lastly, Jatropha markets failed to emerge, further increasing India’s reliance on fossil fuels. As such, a PIE analysis of biofuels offers broader insights into the social and environmental dimensions of biofuels/bioenergy than other impact assessment frameworks currently allow.

8:00 AM - 11:25 AM Session: 9: Waste and Gaseous Feedstocks

Conveners: Ian Rowe, Department of Energy - Energy Efficiency & Renewable Energy, Washington, DC, USA and **David Babson**, U.S. Department of Energy, Washington, DC, USA

Columbia C, Third level

8:00 AM 9-1: Hydrothermal conversion of sawdust into value added products

B. Nanayakkara, K. McGrouther and D. Gapes, Scionresearch, Rotorua, New Zealand; R. Syed, S. Zhang and P. Rose, Callaghan Innovation, Lower Hutt, New Zealand; L.P. Padhye, D. Weerakoon and S. Raj, University of Auckland, Auckland, New Zealand; N. Wijaya, Agl energy Ltd, NSW, Australia*
The project described here aimed at converting sawdust into two value added products i.e. a microbial based protein or nutrient feed and a selective adsorbent, by a two stage hydrothermal process. Pine sawdust was subjected to hydrothermal processing (170°C with 1 wt % sulphuric acid) and the resulting liquors (HTP1) which had 22-33 g/L of sugars, was utilized for growing cultures of *Pseudomonas putida* and *Saccharomyces cerevisiae*. Undiluted HTP1 liquor did not support growth; but 10-fold dilution of liquor, also amended with nutrients, supported the growth. *Saccharomyces cerevisiae* produced 2.5 g/L of protein and *Pseudomonas putida* produced 1.3 g/L of protein. Further studies are warranted to test other high protein yielding microbes, and to increase the biomass & protein yield using continuous fermentation techniques.

The hydrochar resulting from first hydrothermal processing stage (HTP1), was subjected to a second stage hydrothermal processing (HTP2, 200°C/300°C with sulphuric acid/KOH) to produce an efficient adsorbent capable of removal of ammonium in aqueous media. The hydrochar produced with sulphuric acid in HTP 2 resulted in removal of 0.18 mg ammonium adsorption/g of hydrochar after 24 hr, but removal rates more than doubled to 0.38 mg ammonium adsorption/g of hydrochar, when KOH was used in HTP2 reaction. Adsorption isotherm data for hydrochars showed that Freundlich model was the best-fit model. The ammonium removal efficiency of hydrochar was comparable to removal efficiency of activated carbon, demonstrating that adsorbent produced through waste biomass can be a potential solution for aqueous contaminant remediation.

8:25 AM 9-2: Hybrid electrochemical-biological process for biochemical production

S. Jones, White Dog Labs, Inc., New Castle, DE, USA and K. Kuhl, Opus 12, Inc., Berkeley, CA, USA*
Relative to CO_2 fixation by photosynthesis as the basis for biofeedstocks, industrial-scale electrochemical CO_2 conversion could improve the overall efficiency by more than 10X and use a fraction of the land area. By coupling electrochemical CO_2 reduction,

which is efficient but limited to low carbon molecules, with biological systems, which can more easily manipulate simple organic molecules to produce complex products, a new type of industrial chemical production can be realized. Such hybrid systems would be modular, efficient, highly selective, and offer significant environmental benefits. Opus 12 and White Dog Labs (WDL) have partnered to develop and demonstrate this hybrid electrochemical-biological system for biochemical production. First, a proton exchange membrane (PEM) electrolyzer (developed by Opus 12) produces a CO-rich syngas mixture from waste CO_2, and then this syngas is upgraded into a biochemical in a MixoFerm process (developed by WDL). MixoFerm uses microbes capable of utilizing both gaseous feedstocks and soluble feedstocks to maximize product yield. In the hybrid process, the goal is to minimize sugar addition while maintaining a high product yield. The demonstrated hybrid unit is a technology platform that can easily be modified for various biochemicals of interest.

8:50 AM 9-3: Biological conversion of methane to multi-carbon chemicals using metabolic engineered methanotrophs

E.Y. Lee, KyungHee university, Yongin-si, Korea, Republic of (South)*

Methane is an abundant and low-priced carbon feedstock for industrial biotechnology. Methanotrophs are microbes that utilize methane as a sole carbon and energy source. Biocatalytic capability of wild-type methanotrophs are rather limited for efficient biocatalysis and production of non-natural metabolic target products. With aid of recent progress in genetic tool development and system biology-based understanding of methanotrophs' microbial physiology, methanotrophs can be metabolically engineered to produce organic acids, alcohols, amines from methane. In this presentation, methane-to-succinate, 2,3-butanediol and other multi-carbon chemicals using metabolic engineered methanotrophs including *Methylomicrobium alcaliphilum* 20Z and *Methylomonas* sp. DH-1 will be discussed.

9:15 AM Break

9:45 AM 9-4: Innovative feedstock design through repartition of photosynthetic carbon to terpene production

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

Photosynthetic terpene production efficiently transforms sunlight energy for reducing inorganic carbon and represents one of the most carbon efficient route for hydrocarbon production from CO_2. Despite the advantages, enhancing photosynthetic terpene production has been highly challenging. We have systemically designed alternative pathways to repartition photosynthetic carbon into terpene biosynthesis to enhance productivity. First, we have identified the key metabolic bottleneck for limonene production in the cyanobacterium *Synechococcus elongates and* achieved >100 fold increase in limonene productivity. The engineered strains allowed us to discover sucrose accumulation at low limonene productivity stage. Further knock-down of sucrose and glycogen metabolisms have enabled the repartition of carbon from sugar metabolism to terpene metabolism to enhance limonene production to a higher level. Second, we have designed C2 redirection pathway to channel photorespiratory glycolate to pyruvate and subsequently terpene biosynthesis in tobacco using squalene as a model terpene. Metabolomics analysis revealed a significant carbon repartition, as a decrease in the intermediates of sucrose and starch biosynthesis correlated with an increase in malate and pyruvate in the C2 redirection lines. Third, we have developed a C5 redirection pathway to channel carbon directly from Calvin Benson cycle to terpene biosynthesis and by-pass the speed-limiting step in both plants and cyanobacteria. Overall, we have established repartition of photosynthetic carbon from sugar metabolism to terpene metabolism as an efficient approach for enhancing hydrocarbon yield in next generation plant and algae feedstock.

10:10 AM 9-5: Production of volatile fatty acids through arrested anaerobic digestion

V. Sànchez i Nogué, D.C. Thomas, P.O. Saboe, H. Monroe, G.T. Beckham and E.M. Karp, National Renewable Energy Laboratory, Golden, CO, USA*

Anaerobic digestion is an effective, scalable, and industrially proven technology to convert wet waste feedstocks to methane for use in multiple heat and power applications, depending on the available energy distributions systems. Microbial communities present in anaerobic digestion units hydrolyze polysaccharides (and other organic content), and convert the resulting intermediates to volatile fatty acids, which are subsequently converted to methane by methanogens. Being able to produce volatile fatty acids as primary products, instead of methane, would enable catalytic conversion of acids into valuable co-products and hydrocarbon fuels. To that end, we present recent results aimed to produce volatile fatty acids via arrested methanogenesis. Residues from food processing companies were used as feedstock, whereas activated sludge from current anaerobic digestion units were used as microbial inocula. This screening study will establish the basis for further process engineering, including semi-continuous bioconversion systems, adaptive evolution, and cost-effective*in situ* product recovery.

10:35 AM 9-6: Development of a synthetic biology platform for acetogens

R. Jensen, Lanzatech, Skokie, IL, USA*

As world demand not just for energy but also chemicals continuing to rise concerns about the economic and social impact of climate change grow and the need for strategies to minimize the use fossil carbon resources in the production of fuel, chemical and nutritional products demanded by societies globally intensifies. Gas fermentation using C1 utilizing microorganisms allows

for the sustainable production of fuels, chemicals and feed by recycling carbon from local, highly abundant, low-cost waste resources.

LanzaTech has pioneered development and scaled up a gas fermentation process using autotrophic, acetogenic microbes for sustainable production of fuels and chemicals from a diverse range of C1 feedstocks, including waste gases from industrial or syngas generated from any biomass resource.
Less than 10 years ago, acetogens were considered genetically inaccessible and poorly characterized on both genetic and metabolic level. LanzaTech and partners has developed a first genetic toolbox (including genome editing tools and large libraries of validated genetic parts), a comprehensive Systems Biology platform (including multi-omics workflows) and predictive models (including integrated metabolic and process models and machine learning algorithms) for an acetogenic organism.
A particular challenge has been the development of cost-efficient high throughput methods, given the anaerobic nature of the process and requirement to screen in context of gases, but recent advancements allow for a new biofoundry concept to address these challenges.
Using the developed platform, production of over 50 molecules have been demonstrated directly from gas and successful transition to scale has been demonstrated for first products.

11:00 AM 9-7: Biomethanation: A renewable energy storage and CO_2 utilization option

N. Dowe and K.W. Harrison, National Renewable Energy Laboratory, Golden, CO, USA*
We are faced with the reality that, as a planet, we must reduce carbon dioxide (CO_2) emissions now or face the consequences of irreversible damage to earth's climate. The move to renewable energy is happening on a global scale to mitigate this crisis. This has come with the challenge of how to store the increasing amount of renewable electricity generated from intermittent sources that can overwhelm the grid during peak production times. While batteries are very efficient and can store the excess electrons over a period of several hours, batteries cannot, yet, store these electrons over several months. Moving the renewable electrons to hydrogen (H_2) and then to methane (CH_4) will enable longer term storage of renewable electrons in the natural gas grid for months at a time. Biomethanation is a biological process where archaea microorganisms convert H_2 and CO_2 to CH_4, water, and heat. The process is simple, and the organisms are robust enough to handle various waste streams. The process has the potential to provide a mechanism for long term renewable energy storage and recycle CO_2. The National Renewable Energy Laboratory, Southern California Gas Company, and Electrochaea are developing this unique process in a first-of-its kind pressurized bioreactor located at the National Renewable Energy Laboratory. We will discuss the process, the benefits, the challenges, and some early operating data from this new area of research.

10:00 AM - 2:00 PM Exhibit Dismantle

Columbia Ballroom Foyer, Third level

10:00 AM - 2:00 PM Poster Removal

Columbia A, Third level

11:30 AM - 1:00 PM Lunch-on your own

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

Chelais - Room 305, Third Level

1:00 PM - 4:25 PM Session: 10: Enabling Analytical Technologies

Conveners: Carlos Driemeier, Brazilian Center for Research in Energy and Materials - CNPEM, Campinas, Brazil and **Hugh O'Neill**, Oak Ridge National Laboratory, Oak Ridge, TN, USA

Columbia D, Third level

1:00 PM 10-1: Tracking changes in cellulose surface structure during cellulose deconstruction with fluorescence-tagged Carbohydrate Binding Modules (CBMs), confocal laser scanning microscopy, and quantitative image analysis

V. Novy, K. Aissa, F. Nielsen and J. Saddler, University of British Columbia, Vancouver, BC, Canada; C.G. Hunt, US Department of Agriculture, Madison, WI, USA*

Overcoming biomass recalcitrance is key to the success of biorefinery processes. A more recently established factor with profound impact on cellulose hydrolysis rates and yields is the accessibility of cellulose to enzymes (CAE). CAE, or the ability of the cellulolytic enzymes to access their binding sites, has proven difficult to analyze and quantify. We present a novel method based on two different fluorescence-tagged CBMs, confocal laser scanning microscopy, and quantitative image analysis. Based on the emitted fluorescence of the respective probes, the structure of the cellulose surface and CAE can be analyzed and potential changes during enzymatic deconstruction quantified. The method was applied to northern bleached softwood kraft (NBSK), which was hydrolyzed using commercial enzyme cocktails. Fiber length analysis, SEM imaging, and NMR provided supporting information. Additionally, cultivations of *Trichoderma reesei* on NBSK were investigated to assess the impact of the cascaded fungal enzyme production on the cellulose surface structure and CAE.

NBSK shows the distinct pattern of a mainly crystalline cellulose surface, interlaced at regular intervals by hotspots of paracrystalline cellulose, confirming the debated paracrystalline structure of dislocation zones. Analyzing CAE over hydrolysis time further generated strong evidence that enzymatic hydrolysis predominates at the dislocation zones at the initial phase of the reaction, causing their fast degradation and resulting in short fiber fragments enriched in crystalline cellulose.
The presented method provides advanced information about the cellulose surface structure as well as CAE, and thus can help us unravel the complex mechanisms of cellulose deconstruction.

1:25 PM 10-2: Understanding thermal transformations in cellulose by in-situ Nonlinear Vibrational Spectroscopy

Z. Xu, L. Zhang and B. Yang, Washington State University, Richland, WA, USA; Z. Wang and P. ElKhoury, Pacific Northwest National Laboratory, Richland, WA, USA; H. Wang, Fudan University, Shanghai, China*
A combination of Total Internal Reflection Sum Frequency Generation Vibrational Spectroscopy (TIR-SFG-VS) and conventional SFG-VS together allow probing and understanding the molecular structures at the surface and in the crystalline core of cellulose, as well as the correlation between structural motifs and biomass recalcitrance. From the recorded SFG spectra in the C-H and O-H regions, we describe - for the first time - that the surface layers of Iβ cellulose feature distinct structures and structural motifs as compared to their analogues in the crystalline core. Our derived picture thus challenges the traditional understanding of cellulosic materials, which neglects the differences between its structural motifs at the surface and in the core. Furthermore, this work describes the effect of structure on cellulose reactivity and decomposition. To this end, the implementation of aqueous pretreatment of cellulose using a heated fluid test bed with dynamic TIR-SFG-VS allows probing the thermally-induced structural changes of Avicel and cellulose Iβ throughout heating and cooling cycles. Naturally, the observed recrystallization into a new crystalline structure in the process of cooling could strongly affect the recalcitrance of cellulose. This is important from a practical point of view, where by novel insights on the effectiveness of biomass pretreatment may arise from the improved understanding that is afforded by our data. If time permits, we will also go over on-going combined AFM-Raman measurements that are ultimately aimed at establishing a complementary approach to observing and understanding topography and chemistry at solid-air and potentially solid-liquid interfaces.

1:50 PM 10-3: Leveraging unlimited diffraction photoactivated localization microscopy (PALM) to probe the distribution of cellulosomes at the microbial-substrate interface

J.M. Yarbrough, N. Hengge, Q. Xu, Y. Zeng, B. Donohoe, T. Vinzant, A. Mittal and Y. Bomble, National Renewable Energy Laboratory, Golden, CO, USA; D. Stitch, University of Colorado Denver - Anschutz Medical Campus, Aurora, CO, USA*
Clostridium thermocellum is one of the most efficient microorganisms, so far isolated, for the deconstruction of biomass. To achieve this high level of cellulolytic activity, *C. thermocellum* uses large multienzyme complexes known as cellulosomes to breakdown polysaccharides found in plant cell walls. The attachment of bacterial cells to the nearby substrate via the cellulosome has been hypothesized to be the reason for this high efficiency. The region lying between the cell and the substrate has shown great variation and dynamics that are affected by the growth stage of cells and the substrate used for growth. Many aspects of plant cell wall deconstruction by cellulolytic bacteria that directly bind to substrates remain unknown and this knowledge gap is crucial for consolidated bioprocessing (CBP) applications. Therefore, it is imperative that we obtain a better fundamental understanding of the interactions that exist between the cellulosomes, bacteria, and the substrate. To address this question, we are utilizing unlimited diffraction photoactivated localization microscopy (super resolution microscopy) to probe the distribution of cellulosomes at the microbial-substrate interface.

2:15 PM Break

2:45 PM 10-4: Real-time visualization of biomass deconstruction during co-solvent reactions

S.V. Pingali, H. O'Neill, L. Petridis, U. Volker, J. Smith and B. Davison, Oak Ridge National Laboratory, Oak Ridge, TN, USA; C. Cai and C. Wyman, University of California Riverside, Riverside, CA, USA; A. Ragauskas, The University of Tennessee - Knoxville, Knoxville, TN, USA*
In-situ small-angle neutron scattering (SANS) and extensive molecular dynamics (MD) computer simulation were used in a combined approach to examine real-time breakdown of biomass and the temperature dependence of specifically cellulose and

lignin structure and dynamics. Lignin, a major polymeric component of plant cell walls, forms aggregates in vivo during pretreatment of lignocellulosic biomass for ethanol production. The aggregates are thought to reduce ethanol yields by inhibiting enzymatic hydrolysis of cellulose. Cellulose, a major carbohydrate is understood to undergo significant changes in its crystallinity and porosity. These characteristic changes also influence the efficiency of subsequent enzymatic hydrolysis. Here, we report on real-time SANS experiments during steam explosion, dilute acid pretreatment and co-solvent like THF:water of biomass using a temperature-pressure reaction cell. The temperature of the cell was ramped-up from 20 to 150-180 °C, then maintained at that temperature and finally the cell was cooled down to room temperature. In dilute acid, a clear characteristic structural feature first appeared at 120°C representative of a particle size. This structural feature with increasing temperature and residence time at 180°C, progressively increased in size. We have identified the particle that appears at 120°C as lignin aggregates. Additionally, cellulose fibril show coalescence behavior indicated by the formation of larger fibril characteristics. Consistent to this observation, simulations results of lignin and cellulose in these solvent will be presented to highlight particular interactions that stabilize solvation of lignin and carbohydrates in co-solvent reactions.

3:10 PM 10-5: Unveiling the inside of lignocellulose with synchrotron X-ray microtomography

C.E. Driemeier, CTBE/CNPEM, Campinas, Brazil*

Lignocelluloses are cellular solids made of cells encased by µm-thick cell walls. The cellular and tissue levels of the biomass structure can be probed non-invasively using synchrotron X-ray computed microtomography (µCT). In this work, we employ µCT to investigate the 3D structures of sugarcane bagasse and straw, the vast lignocellulosic feedstocks associated with the sugarcane industry. We present three case studies where we emphasize the importance of quantitative image analysis and we explore consequences for biorefinery processes. In the first case study, we detected and analyzed mineral particles trapped in the biomass, which are a main cause of harm for biorefinery equipment. In the second study, we unveiled the location of water in fresh sugarcane bagasse, solving an old mystery of sugarcane technology. Finally, in the third study we used 3D images to create pore network models of the bagasse structure, informing on the main channels of intraparticle connectivity. These results demonstrate µCT is a valuable technique to advance the understanding of biomass feedstocks and their conversion processes.

3:35 PM 10-6: Applications of NMR spectroscopy for comparative analysis of the products of lignocellulosic biomass conversion

J. Cort, PhD, Pacific Northwest National Laboratory, Richland, WA, USA*

Conversion of lignocellulosic biomass to liquid oils and fuels or higher value chemicals must be efficient if biomass is to displace fossil carbon as a feedstock for these products. Towards this end, researchers have subjected many different types of biomass to a wide range of thermochemical and biological conversion processes and conditions. Understanding the chemistry of biomass conversion requires thorough characterization of the chemical composition of starting materials and products—either or both of which may be complex mixtures. Multidimensional heteronuclear correlation NMR spectroscopy (e.g. HSQC, HMBC) can be highly informative for characterization of complex mixtures produced from biomass conversion, because the dispersion of proton and carbon or nitrogen chemical shifts in each frequency dimension resolves resonance peaks from different functional groups into characteristic regions. These spectra can be useful for comparing two or more samples to identify similarities and differences, for making assignments by comparison to a library of chemical shifts for standard compounds, or as a signature-like set of features that can be associated with properties. In combination with high-resolution/high-mass accuracy mass spectrometry, functional group information from NMR together with molecular formulas from MS can be used to make putative identifications of components that are not identifiable by other means. These instruments are available at the Environmental Molecular Sciences Laboratory (EMSL), a national scientific user facility at Pacific Northwest National Laboratory (PNNL).

4:00 PM 10-7: Modeling biomass feedstock variability to enable reliable handling, comminution, and conversion processes

P. Ciesielski, B. Pecha, N. Thornburg and J. Vermaas, NREL, Golden, CO, USA; M.F. Crowley, National Renewable Energy Laboratory, Golden, CO, USA*

The complexity and inherent variability of biomass feedstocks manifests at length scales spanning the biopolymer assemblies that compose lignocellulosic cell walls to the heterogeneous distributions of particle sizes and shapes that result from comminution methods. Recent attempts to scale-up and commercialize biomass conversion technologies have highlighted the importance of understanding and managing feedstock variability. Over the past several decades, research has been primarily dedicated to problems such as biocatalyst development and pretreatment technology, while important feedstock-centric barriers have plagued commercialization attempts have received relatively little attention. Physics-based, predictive tools that relate combinations of feedstock attributes to their behavior in handling and conversion processes will provide industry with actionable information to make informed decisions about feedstock selection and process design. In this talk I will present methods to construct procedurally generated biomass particle models directly from characterization data. The models can be used in various simulation environments to probe relationships between the attributes of a given feedstock and its response to changing conditions, such as applied heat, mechanical stress, and chemical gradients. I will describe how these models have resulted in new insight and optimization strategies for feedstock comminution, pretreatment, and enzymatic hydrolysis.

1:00 PM - 4:25 PM Session: 11: Biofuels and Biochemicals

Conveners: Dr. Solange I. Mussatto, Technical University of Denmark, Kongens Lyngby, Denmark and **Meltem Urgun-Demirtas**, Argonne National Laboratory, Lemont, IL, USA

Columbia C, Third level

1:00 PM 11-1: Biorefining impacts of novel octane hyperboosting phenomenon in prenol/gasoline blends

E. Monroe and R.W. Davis, Sandia National Laboratories, Livermore, CA, USA; A. George, Sandia National Labrotories/Joint BioEnergy Institute, emeryville, CA, USA*

This work discusses the impact of a novel discovery of “octane hyperboosting” by a potential biofuel target 3-methyl-2-buten-1-ol (prenol). Octane hyperboosting is characterized by the Research Octane Number (RON) of a mixture exceeding the RON of the individual components in that mixture. This effect has not been documented to date and the phenomenon suggests an unexplored aspect of autoignition kinetics research for fuel blends which and may provide a new mechanism for significantly increasing fuel octane number. This is necessary for increasing combustion efficiency in spark ignition engines and recent efforts from the DOE’s co-optimization of fuels and engines project have demonstrated that a 30% v/v blend of prenol in gasoline blends will lead to >10% better efficiency (and thereby lower CO2 emissions) in existing SI engines due to prenols octane hyperboosting. This suggests a paradigm shift for the biofuel industry where this effect is leveraged by biorefiners, as increasing the octane number at low blend volumes can cut costs in other parts of the refinery. Other key factors in value proposition of prenol has a high performance biofuel such as vapor pressure, energy density, infrastructure compatibility, and emissions are also discussed. Lastly, results from a survey of other biofuel molecules that may leverage this effect are highlighted

1:25 PM 11-2: Waste to bioproducts and biofuels: Challenges and opportunities in driving bioeconomy

M. Urgun-Demirtas and H. Wu, Argonne National Laboratory, Lemont, IL, USA*

This presentation includes the development and demonstration of innovative technologies to support a sustainable and circular economy by valuing and keeping negative value or low value waste resources in circulation. Our attention was focusing on the high value biproducts and biofuels production opportunities from organic waste streams including manure, food waste, and organic fraction of municipal solid waste. We have been developing sustainable and efficient approaches to resource use and management, and waste volume reduction

We successfully scaled up the patented Integrated Waste to Energy and Nutrient Production System as a sustainable and cost-competitive approach towards a circular paradigm to produce renewable methane and high fertilizer value digestate for crop cultivation. This *in situ* biogas production and upgrading process resulted in a 40-75% reduction in the CO2 volume.

In a new resource recovery concept, negative/low value cheese whey and brewery wastewater streams were converted into organic acids (lactic, acetic acid, butyric acid) through new arrested AD technologies as well as nutrient-rich edible protein source through patented fungal technology. Emergy’s versatile bio-manufacturing platform also allows to make low cost advanced porous carbon materials for energy storage and water filtration applications.

A new bioprocess has been also developed for the potential transformation of food waste streams by additive manufacturing into bioplastics. The 3D printing allowed us fine tuning of polymeric materials to produce bioplastics with desirable functionalities and forms. This new technology platform offers the possibility to produce high value biopolymers, hence develop localized, distributed low volume manufacturing within a circular economy.

1:50 PM 11-3: Developing novel anaerobic bioprocesses to recover high-value resources from urban organic waste streams

L. Raskin, University of Michigan, Ann Arbor, MH, USA; X.F. Almansa and S. Shrestha, University of Michigan, Ann Arbor, MI, USA*

Anaerobic digestion (AD) based technologies have great potential for converting the enormous amounts of organic waste generated in urban environments into valuable resources. Yet few urban organic waste streams are currently treated by anaerobic bioprocesses, suggesting that new approaches are needed. This presentation will show the development of a new approach to efficiently degrade lignocellulosic components, abundantly present in urban organic waste streams and difficult to degrade by conventional AD systems. The stomach of ruminant animals (rumen) is a natural system containing a diverse microbial community that efficiently degrades grass, a lignocellulosic substrate, under anaerobic conditions. Therefore, an anaerobic dynamic membrane bioreactor was designed to simulate the rumen and to promote the growth of rumen microorganisms to degrade lignocellulosic substrates. The rumen bioreactor was operated with food waste as the primary substrate and 60% of the lignocellulosic materials in the food waste were degraded. Approximately 40% of the volatile solids in the substrate were transformed into short chain carboxylic acids (SCCAs, C2-C5). These SCCAs can be transformed in biomethane or other valuable products, such as medium chain carboxylic acids (MCCAs, C6-C12) via chain elongation with a

reduced compound, such as ethanol. We developed a novel anaerobic dynamic membrane bioreactor (AnDMBR) and integrated it with a liquid-liquid extraction system to facilitate this chain elongation reaction by a mixed microbial community. The development of these two novel, mixed-community bioreactor systems and their integration with a liquid-liquid extraction system demonstrate the potential for recovery of high-value resources from urban organic waste stream.

2:15 PM Break

2:45 PM 11-4: Valorization of lignin and cellulose from California-relevant feedstocks into biosurfactants and nanocellulose

D. Wong, I. Sitepu, L. Lynn, P. Hernes, K.L. Boundy-Mills and T. Jeoh, University of California, Davis, Davis, CA, USA*
California is the world's leader in agricultural economy whose high crop production generates large volumes of lignocellulosic biomass waste that is a potential feedstock for conversion to bioproducts. Reducing the environmental impact and improving economic returns on these feedstocks requires valorization of both the polysaccharide and lignin fractions of the biomass. In this project, almond shells and hulls, sorghum biomass, and residues from wheat processing are pretreated and fractionated into soluble, lignin-rich, and insoluble, cellulose-rich streams. Oleaginous yeasts are screened for growth in the hydrolysates containing lignin monomers and hemicellulosic sugars, and for subsequent production of polyol esters of fatty acids (PEFA). PEFA, a high value biosurfactant, has a growing presence in the 'green' chemical industry. The efficacy of alkali pretreatment with and without metal catalysts to liberate monolignols and the extent of conversion of monolignols by the yeasts is being quantified by gas chromatography mass spectrometry (GCMS). Lignin oxidation to phenols show promising recovery compared to initial compositional analysis. The insoluble, cellulose-rich fraction is characterized and further processed into nanocellulose for incorporation into a UC Davis patented *in-situ* cross-linked alginate microcapsulation technology to improve mechanical and barrier properties of encapsulation matrices. Microcapsules achieved cross-linking of 49 % when nanocellulose composed one third of the polymer matrix. Both the PEFA and nanocellulose reinforced product streams generate revenue, improve consumer goods, and reduce the environmental impact of agricultural waste. Providing ready to scale processes to add value to lignocellulosic biomass will increase California's stronghold on environmentally conscious agriculture.

3:10 PM 11-5: Biological production of carboxylic acids from biomass sugars and further upgrading to fuels

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The production of volatile fatty acids (VFA) from biomass sugars via anaerobic fermentation has emerged as a promising approach to generate high yields of biofuel precursors. In this presentation, we will particularly focus on the production of hexanoic acid, butyric acid, and acetic acid from corn stover sugars by pure bacterial cultures and will highlight the advantages and disadvantages of different organisms (i.e. *Megasphaera elsdenii*, *Clostridium butyricum*, and *Clostridium tyrobutyricum*) and process configurations (i.e. batch, fed-batch, pertractive fermentation) to ultimately lead to high VFA titers, yields, and productivities. Lastly, VFA separations from the bioreactor broth as well as chemical catalysis of these VFAs towards the production of novel fuels will be introduced.

3:35 PM 11-6: Furan production from biomass hydrolysates: Scale-up and techno-economic feasibility study of a novel, high-yield "SIRE-BE-Dehydration" process

R. Gogar, University of Toledo, Toledo, OH, USA; S. Viamajala, United States, Toledo, OH, USA; P. Relue and S. Varanasi, The University of Toledo, Toledo, OH, USA*
Lignocellulosic biomass is a sustainable feedstock for production of furans – 5-hydroxymethy furfural (HMF) and furfural – versatile platform molecules that can be converted to drop-in fuels and bio-based polymers. Lack of efficient lignocellulosic sugars-to-furan pathways (especially HMF) constitutes a major barrier to the industrial production of furans. We have recently demonstrated high yields of furans from biomass hydrolysate using a novel Simultaneous-Isomerization-Reactive-Extraction (SIRE) followed by Back-Extraction (BE) and dehydration process (*Green Chem.*, **2017**, 19, 1782). We have developed a continuous-flow, meso-scale SIRE-BE system and this presentation will discuss furan production using concentrated AFEX (Ammonia Fiber Expansion) treated lignocellulosic biomass hydrolysate (60 g-total sugars/L). The continuous flow system consists of a packed-bed Isomerization column containing immobilized glucose/xylose isomerase to convert aldose-sugars (glucose and xylose) to more reactive keto-forms (fructose and xylulose). The isomerization column is coupled with a hollow-fiber membrane module to facilitate selective Reactive Extraction of the ketose sugars into an octanol phase using boronic acid. Thereafter, ketose sugars are Back Extracted from octanol into an immiscible acidic aqueous media. Finally, concentrated keto-sugars undergoes dehydration in acidic aqueous media in presence of acetone to yield furans at high yield of 90%. Use of acetone not only stabilizes furans to achieve high yields, but also leads to efficient product-recovery due to its low-boiling point.

We will present data from continuous-flow studies and compare with mathematical model. Techno-economic and sensitivity analysis, which reveals low cost of furans - $1.2/kg, for drop-in fuels and monomer like furandicarboxylic acid will also be presented.

4:00 PM 11-7: Carboxylate platform – lessons from the cow

M.T. Holtzapple, Texas A&M University, College Station, TX, USA*
In their 2009 paper *Lessons from the cow*, Paul Weimer et al. suggested that methods used by ruminant animals could be applied to industrial processing of biomass. In particular, ruminant animals employ mixed-acid fermentations that transform biomass into carboxylic acids (C2 to C8). As the acids are produced in the rumen, they are extracted through the rumen wall, which lowers product inhibition. Furthermore, when ruminant animals chew their cud, they subject biomass to mechanical shear stresses that tear apart the fibrous structure and improve digestibility. In many ways, the carboxylate platform mimics the rumen system because it also employs a mixed-acid fermentation. This presentation will show recent data that further mimics the cow by (1) recovering acids during the fermentation and (2) employing mechanical grinding.

6:00 PM - 8:00 PM Session: 13: Session ST-1 (IEA Bioenergy Task 39 cosponsored): Update on technical and policy aspects of drop-in biofuels

Conveners: Prof. Jack Saddler, UBC and **James D. (Jim) McMillan**, NREL, Golden, CO, USA

Columbia C, Third level

6:00 PM 13-1: Assuring performance - standards for fuel quality and sustainability in aviation fuels

M. Lakeman, Boeing*
In the development of sustainable alternative fuels for aviation, it is of utmost importance that any new fuel type is “fit-for-purpose”, i.e. it meets all the technical performance requirements for use in aircraft. Such an assurance is achieved through the development of new fuel specifications by technical standards bodies such as ASTM International. Through a robust process of testing, evaluation, stakeholder input and consensus decision-making, performance of new fuel types is assured. Likewise, validating the sustainability performance of alternative fuels is of high importance so that as new fuels are adopted, the environmental and social benefits they promise can actually be realized. A wide range of sustainability metrics and standards are in use today, each with differing relevance in differing contexts.

This presentation will discuss the development and application of these two types of standard, with special focus on how technology developers should keep them in mind as design requirements for accessing the aviation fuel market.

6:20 PM 13-2: The US federal efforts in advancing alternative jet fuels

M. Wolcott, Washington State University*

6:40 PM 13-3: The role of airports in developing a sustainable aviation fuels market

S. Meyn, Port of Seattle*
Airports are economic development engines, and are at the nexus of airlines, large fueling systems, and influential passengers and corporations. As the sustainable aviation fuel (SAF) market has evolved, Seattle-Tacoma International Airport has shifted its focus from research and development support, to direct market development. This shift began in 2016 via an agreement with Boeing and Alaska Airlines to investigate the fueling infrastructure changes needed to bring sustainable aviation fuel to the airport as economically and efficiently as possible. Since that time, the airport has developed a larger agreement among 15 airlines to develop a roadmap to greater SAF adoption. Other airports are now developing similar initiatives and working together with airlines to develop best practices for SAF-focused collaborations.

7:00 PM 13-4: Parkland fuels

D. Schick, Parkland Fuels*

6:00 PM - 8:00 PM Session: 14: Session ST-2: Global Research Consortia

Conveners: Kristiina Kruus, Aalto University, Espoo, Finland, Espoo, Finland and **Alison Goss Eng**

Columbia D, Third level

6:00 PM 14-1: Material bioeconomy flagship FinnCERES

*K. Kruus**
FinnCERES is the joint competence center for the materials bioeconomy between Aalto University and VTT Technical Research

Centre of Finland Ltd. We aim to ensure sustainable future by developing materials and applications for the future bioeconomy with a solid scientific foundation. The flagship is funded by the Academy of Finland.

The forest industry is currently going through a significant transition, needing to create new business based on novel value-added products manufactured from the wood raw material, the lignocellulose. Through its activities, FinnCERES will directly support the realization of the national bioeconomy strategy. The purpose is to develop new materials, in particular based on lignocellulose and forest biomass, for industrial scale production of packaging, textiles and separation systems, as well as semiconductors, composites and solutions for energy storage. To aid in commercial exploitation of the innovations, we are building an innovation ecosystem for the industry serving as a platform for the development of world class biomaterials.
FinnCERES addresses some of the most urgent global megatrends such as resource sufficiency and climate change. We aim to produce new biomaterials to replace or capture plastics, find alternatives to address the textile fiber gap, and develop bio-based electronics and advanced multifunctional materials. We believe that continuous demand for improved performance will guide material development and eventually the products we encounter as consumers.

6:30 PM 14-2: An overview of and recent highlights from the DOE Feedstock-Conversion Interface Consortia

B. Hoffman[*]*, Bioenergy Technologies Office, U.S. Department of Energy*
Many of the process bottlenecks and difficulties experienced in the nascent bioenergy industry are centered on feedstock handling and preprocessing, feeding feedstocks into the conversion process, conversion performance, equipment operation and process integration, which are caused by the complexity and variability in feedstock physical, chemical, biological and mechanical attributes, and recalcitrance of feedstocks to be efficiently converted into fuels and products. In FY18, the U.S. Department of Energy Bioenergy Technologies Office (BETO) initiated the Feedstock-Conversion Interface Consortium (FCIC) — a network of 8 national laboratories that leverages their core capabilities to quantify, understand, and manage variability in biomass from field through downstream conversion.

The FCIC employs a “Quality-by-Design” approach with fundamental R&D focused on:

- Quantify, understand, and manage variability in biomass to understand fundamental mechanisms underlying how biomass composition, structure, and behavior impacts unit operations through the value chain.
- Develop first principles hypotheses/mechanistic models related to flowability, physical performance, and chemical conversion in each of the steps through which the feedstock has to traverse, from field to products. Validate these models using bench scale and pilot scale data.
- Develop transfer functions (or scaling rules) which are based not only on experimental data or experience but are based on first principles conversion mechanisms that feedstock undergoes in the value chain from field to products.
- Develop unsteady TEA/LCA models which can be used to determine the value of feedstock as it undergoes conversion through the value chain and is converted to forms where it can become a commodity product.

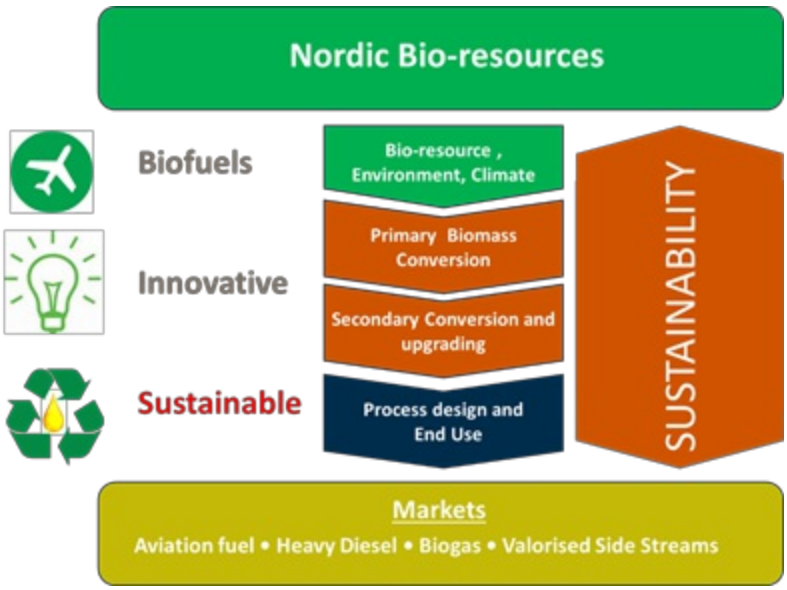
7:00 PM 14-3: An overview of and recent highlights from the DOE Agile BioFoundry

N. Hillson[*]*, LBNL, Emeryville, CA, USA*
The overarching goal of the DOE Agile BioFoundry (ABF) is to enable biorefineries to achieve 50% reductions in time to bioprocess scale-up (as compared to the current average of around 10 years) by establishing a distributed BioFoundry that productionizes synthetic biology. Toward achieving this goal, the ABF has brought together domain expertise and infrastructure that is distributed across 8 U.S. National Labs (LBNL, SNL, PNNL, NREL, ANL, ORNL, LANL, and INL). This talk will introduce the ABF, and provide some research and development highlights (including work with industry) from the first 2.5 years of its operations.

7:30 PM 14-4: Bio4Fuels – Norwegian Centre for Sustainable Bio-based Fuels and Energy

D. Akporiaye[*]*, SINTEF Industry, 0314 Oslo, Norway*

Within Norway, the dominant source of local CO_2 emissions stems from transport, accounting for over 30% of total emissions. In contrast to the significant emission reductions from the landbased industrial sector, the emissions from transport has increased significantly. Following the recommendations of a wide range of policy initiatives, the important role of advanced biofuels in achieving target reduction of emissions from the transport sector has been highlighted. As a platform for research within technologies relevant for Norwegian and Nordic production of advanced Biofuels, the Norwegian Centre for Sustainable Bio-based Fuels (Bio4Fuels) has been established.



Bio4Fuels is one of eight national centres for environmentally friendly energy research, established with combined goal of contributing to the reduction of CO_2 emissions in Norway. The Bio4Fuels centre involves seven of the main research organizations in Norway together with over 37 stakeholders, representing all aspects of the value chain and interest groups. The combined interests of the stakeholders and research partners covers four of the main value chains for converting Norwegian/Nordic sourced lignocellulosic forestry waste for the production of a range of fuel and value products. Research activities in the centre range from studies of the biomass resources availability, research on specific technologies and conversion processes, through to the techno-economics of implementation of the most relevant technologies within Norway.

References

https://www.nmbu.no/en/services/centers/bio4fuels