43rd Symposium on Biomaterials, Fuels and Chemicals

Monday, April 26 – Wednesday, April 28, 2021

Monday, April 26

8:10 AM - 9:00 AM Monday Keynote

8:10 AM Discovering novel carbohydrate-active enzymes

B. Henrissat, Ph.D., D.Sc., Technical University of Denmark, Lyngby, Denmark*

Carbohydrate-active enzymes (CAZymes) assemble and breakdown glycans and glycoconjugates, and thereby find numerous applications in the paper, textile, detergent, food and feed industries as well as in the health and nutrition sectors. Some CAZymes have been discovered and utilized long ago. For instance, amylase and invertase were isolated in the second half of the 19th century while lysozyme was the first enzyme whose 3-D structure was determined. In spite of this early start, the number of families and the diversity of carbohydrate-active enzymes continue to grow steadily in the early 21st century. We will review current methods employed to discover novel CAZymes, progressively uncovering the massive diversity of glycans whose breakdown requires an equal diversity of bespoke enzymes.

8:25 AM Q&A

8:30 AM Discovery of Novel Compounds and Pathways through Identification of Bioprivileged Molecules

L. Broadbelt, Northwestern University, Evanston, IL, USA*

Bioprivileged molecules are biology-derived chemical intermediates that can be efficiently converted to a diversity of chemical products including both novel molecules and drop-in replacements. Bridging chemical and biological catalysis by bioprivileged molecules provides a useful and flexible new paradigm for producing biobased chemicals. However, the discovery of bioprivileged molecules has been demonstrated to require extensive experimental effort over a long period of time. To meet this need, we developed a computational framework for identification of candidate bioprivileged molecules from $C_6H_xO_y$ (C6), $C_4H_xO_y$ (C4), $C_5H_xO_y$ (C5), and $C_7H_xO_y$ (C7) molecule subspaces that ranks the molecules according

to a number of diverse criteria and generates products emanating from them using automated reaction network generation. All top candidates were analyzed for their key functional moieties using a random forest model, and this algorithm was applied to compare the functional group space occupied by bioprivileged molecules of various databases of molecules with a focus on evaluating how closely the molecules were aligned with those known to biology. The framework is sufficiently automated and flexible that it can be easily expanded to include other chemical formulae to screen for bioprivileged candidates. This in turn facilitates the retrosynthesis process inherent in the framework to identify those bioprivileged intermediates in other subspaces that lead to target molecules. The application to discovery of known and novel monomers for poly(hydroxyurethanes) that are derived from biobased molecules and lead to recyclable materials will be discussed.

8:45 AM Q&A

9:00 AM - 10:00 AM Session: 1: Emerging plastics research

Conveners: **Gregg Beckham**, National Renewable Energy Laboratory, USA, CO, USA and **Allison Werner**, National Renewable Energy Laboratory, USA

9:00 AM Bio-upcycling of plastic waste

T. Narancic, University College, Dublin, Ireland*

In addition to creating a serious environmental concern, the “take-make-dispose” culture associated in particular with single-use petrochemical plastics results in loss of valuable carbon. The circular economy of plastics aims to retain the value of the material through reuse, recycling, and other recovery. However, recycling of post-consumer plastic remains largely unexploited, likely due to the high price and low quality of the recyclate. The conversion of post-consumer plastic to value-added molecules i.e. upcycling should be a perspective of the circular economy of plastics. The enzymatic hydrolysis of PET has been widely demonstrated, and recently shown to be feasible on industrial scale [1]. We have built on this idea and demonstrated that PET monomers obtained by hydrolysis can efficiently be converted into biodegradable plastic, polyhydroxyalkanoate (PHA) and a novel, partly bio-based poly(amide urethane) (bio-PU) using native and engineered capacity of bacteria [2, 3]. The use of enabling technologies, such as systems and synthetic biology allows us to further expand the spectrum of non-conventional feedstocks beyond PET on one end, and the repertoire of products on the other end of bow-tie architecture of microbial metabolism.

1. Tournier, V., et al., *An engineered PET depolymerase to break down and recycle plastic bottles.* Nature, 2020. **580**(7802): p. 216-+.
2. Narancic, T., et al., *Genome analysis of the metabolically versatile Pseudomonas umsongensis GO16: the genetic basis for PET monomer upcycling into polyhydroxyalkanoates.* Microbial Biotechnology, 2021.
3. Tiso, T., et al., *Bio-upcycling of Polyethylene Terephthalate.* Metabolic Engineering, 2021.

9:15 AM Q & A

9:20 AM Developing a novel microbial host for upcycling waste polyethylene terephthalate

J. Diao, Y. Hu and T.S. Moon, Washington University in St. Louis, St. Louis, MO, USA*

Polyethylene terephthalate (PET) represents 8% (by weight) of global solid waste. PET chemical recycling has been an option to solve this global problem, but it has one main challenge: its relatively high process cost and the extremely low price of virgin PET. One solution to address this issue is to upcycle waste PET rather than recycle it to generate the same PET typically with low quality. PET upcycling can be achieved by depolymerizing PET into terephthalic acid (TPA) and ethylene glycol (EG) and biologically converting these monomers into value-added products. However, there are only a handful of reports demonstrating microbial strains capable of growing on both TPA and EG generated from PET as sole carbon sources. To overcome this critical limitation, we have performed strain screening to discover a *Rhodococcus* strain (named RPET) that can grow well on the alkaline hydrolysis products of PET as the sole carbon source without any purification step. Notably, this strain was able to tolerate and grow on a mixture of TPA and EG at extremely high concentrations (up to 0.3M each, total 0.6M) and high osmolarity resulting from alkaline hydrolysis and pH neutralization. Specifically, a simple depolymerization process led to a monomer yield up to ~97%. The resultant pH neutralized media supported RPET's growth (up to 0.4 g dry cell weight per g PET) without any purification and sterilization step except for their dilution to make up to 0.6M of monomer concentrations. In addition, many synthetic biology tools, developed for *Rhodococcus opacus* (1), were functional in RPET, facilitating its engineering. In this presentation, we will discuss our effort to develop this novel chassis for waste PET valorization with PET conversion into carotenoids and muconate as two demonstration products.

1. DeLorenzo DM, Rottinghaus AG, Henson WR, Moon TS. 2018. *ACS Synthetic Biology* 7:727-38

9:35 AM Q & A

9:40 AM Discovering terephthalate transporters by gene amplification, laboratory evolution, and biosensor screening

I. Pardo[*]

New strategies for the chemical recycling and biological upcycling of polyethylene terephthalate (PET) are sought as an alternative to current mechanical recycling methods, which mostly result in cascade recycling or down-cycling of PET to products of lesser value. In this endeavor, the microbial conversion of the PET constituent monomer terephthalic acid (TPA) to value-added bioproducts is considered key for the valorization of plastic residues. Here, we will present recent work in which we engineered the model organism *Acinetobacter baylyi* ADP1 to catabolize TPA, through a combination of gene amplification and adaptive laboratory evolution. With the aid of a fluorescent biosensor engineered *ad hoc* for the detection of intracellular TPA, we further identified a native transporter capable of importing TPA in *A. baylyi*, and isolated evolved variants of this transporter that more efficiently uptake this substrate. This work is an example of the power of evolution to enable the biological degradation of xenobiotic compounds, and also provides valuable tools for future efforts in the biological upcycling of PET.

9:55 AM Q & A

9:00 AM - 10:00 AM Session: 2: One-carbon metabolism

Conveners: **Calvin Henard**, University of North Texas, Denton, TX, USA and **Marina Kalyuzhnaya**, San Diego State University, San Diego, CA, USA

9:00 AM Establishing *Eubacterium limosum* as a model methylotrophic acetogen

B. Woolston, Northeastern University, Boston, MA, USA*

Single-carbon (C1) compounds including carbon monoxide, methanol and formic acid have emerged as promising feedstocks for biofuel and biochemical production. These substrates can be produced renewably from CO_2 through electrocatalysis or hydrogenation with renewable hydrogen, thus bypassing food security and land conversion concerns raised over traditional biofuel feedstocks. Acetogenic microbes are a particularly attractive class of organisms for the biological upgrading of C1 compounds, and use the Wood-Ljungdahl pathway (WLP) to form carbon-carbon bonds between C1 compounds at electron efficiencies greater than 80%. In particular, *Eubacterium limosum* is capable of robust growth on all three reduced C1 compounds. However, compared to microbes used in traditional bioprocessing, and other acetogens used in the context of industrial syngas-to-ethanol fermentation, a systems-level understanding of *E. limosum* is lacking, and genetic tools for establishing heterologous product pathways are underdeveloped. We are working on expanding the genetic toolbox for *E. limosum* by developing tools for high-efficiency recombineering, as well as robust promoter and ribosome binding site (RBS) libraries. We are also employing metabolic flux analysis through isotopic tracer experiments to more fully elucidate the underlying metabolic network topology under both unitrophic and mixotrophic growth conditions. This talk will elaborate on results from both of these efforts. Ultimately, this work will dramatically enhance our ability to engineer *E. limosum* for the production of biofuels and bioproducts from single-carbon substrates.

9:08 AM Q & A

9:11 AM Sustainable bioproduction via microbial electrosynthesis

A. Bose, Washington University at St. Louis, St. Louis, MO, USA*

Anthropogenic carbon dioxide (CO_2) release in the atmosphere from fossil fuel combustion has inspired scientists to study CO_2 to fuel conversion. Oxygenic phototrophs such as cyanobacteria have been used to produce biofuels using CO_2. However, oxygen generation during oxygenic photosynthesis affects biofuel production efficiency. To produce *n*-butanol (biofuel) from CO_2, here we introduced an *n*-butanol biosynthesis pathway into an anoxygenic (non-oxygen evolving) photoautotroph, *Rhodopseudomonas palustris* TIE-1 (TIE-1). Using different carbon, nitrogen, and electron sources, we achieved *n*-butanol production in wild-type TIE-1 and mutants lacking electron-consuming (nitrogen-fixing) or acetyl-CoA-consuming (polyhydroxybutyrate and glycogen synthesis) pathways. The mutant lacking the nitrogen-fixing pathway produced highest *n*-butanol. Coupled with novel hybrid bioelectrochemical platforms, this mutant produced *n*-butanol using CO_2, solar panel-generated electricity, and light, with high electrical energy conversion efficiency. Overall, this approach showcases TIE-1 as an attractive microbial chassis for carbon-neutral *n*-butanol bioproduction using sustainable, renewable, and abundant resources.

9:19 AM Q & A

9:22 AM A Synthetic Acetyl-CoA Bi-cycle Synergizes the Wood-Ljungdahl Pathway for Efficient Carbon Conversion in Syngas Fermentation - CANCELLED

W. Xiong, National Renewable Energy Laboratory, Golden, CO, USA*

The Wood-Ljungdahl pathway (WLP) is a natural carbon fixation pathway capable of converting one-carbon (C1) compounds (CO_2, CO, formate) to two-carbon (C2) metabolite acetyl-CoA or coordinating with canonical glycolysis to convert sugar feedstocks to acetyl-CoA with high carbon yield. The catalytic inefficiency and engineering difficulty in key enzymes, however, limit the biosynthetic potential of this pathway. Here we design a synthetic acetyl-CoA bi-cycle to synergize the WLP for efficient C2 metabolite synthesis. This pathway produces an acetyl-CoA by fixation of two CO_2 equivalents *via* three functional modules acting in series: carbon fixation, gluconeogenesis, and non-oxidative glycolysis. We examine the pathway through comprehensive *in silico* thermodynamic and kinetic analyses. The prototypic pathway is

implemented in a syngas-fermenting organism, *Clostridium ljungdahlii* DSM 13528, by expressing a heterologous phosphoketolase which can work jointly with other pathway enzymes that are native in the host acetogen. We demonstrated the effectiveness of this synthetic pathway in carbon conversion under various growth conditions, which complements the WLP for valorization of syngas as well as sugar feedstocks with high catalytic efficiency. This study underscores the reductive acetyl-CoA bi-cycle as a practical strategy to improve carbon conversion and redox homeostasis in the acetogenic host for industrial applications of syngas fermentation.

9:30 AM Q & A

9:33 AM Blending industrial blast furnace gas with H_2 enables *Acetobacterium woodii* to efficiently co-utilize CO, CO_2 and H_2

K. Novak, C.S. Neuendorf and S. Pflügl, Technische Universität Wien, Vienna, Austria; I. Kofler, K1-MET GmbH, Linz, Austria; N. Kieberger, voestalpine Stahl GmbH, Linz, Austria; S. Klamt, Max Planck Institute for Dynamics of Complex Technical Systems, Magdeburg, Germany*

Acetogens comprise interesting organisms for fixing industrial carbon emissions into fuels and chemicals. The model organism *Acetobacterium woodii* efficiently utilizes H_2/CO_2 for growth and acetate production. In this study, we aimed to determine the impact of gas composition (i.e. CO, CO_2 and H_2 partial pressures) on CO_2 utilization, growth, and acetate production by *A. woodii*. Based on the gas composition of an industrial blast furnace gas (BFG), H_2 blending was used to study the impact of H_2 availability on CO_2 fixation alone and together with CO using idealized gas streams. The key hypothesis was that gas-limited batch and continuous cultures would allow to overcome CO inhibition of the *A. woodii* H_2-dependent CO_2 reductase. Moreover, it was hypothesized that H_2 blending could also be a useful tool to enable co-utilization of CO, CO_2 and H_2. Indeed, with H_2 available as an additional energy source, net CO_2 fixation and CO, CO_2 and H_2 co-utilization was achieved in gas-limited fermentations. Using industrial BFG+high H_2, *A. woodii* efficiently produced up to 15.1 g L^{-1} acetate in continuous fermentations. Furthermore, metabolic modeling showed that intracellular carbon flux distributions and total ATP production were dependent on the availability of H_2 and CO. Collectively, H_2 blending was shown to be a suitable control strategy for gas fermentations and demonstrated that *A. woodii* is an interesting host for CO_2 fixation from industrial gas streams. Finally, successful upgrading of acetate produced from industrial BFG to 2,3-butanediol and isopropanol by *E. coli* will be briefly presented.

9:41 AM Q & A

9:44 AM Methane to biopolymers: production of sustainable plastic-replacements using gas fermentation

A. Pieja, Mango Materials*

Mango Materials is a Bay Area-based start-up that uses methane gas to produce biodegradable biopolymers that are economically and functionally competitive with conventional, oil-based plastics. Mango Materials produces powder or pellets of poly-hydroxyalkanoate (PHA), a valuable product that can be converted into a variety of high-margin or high-volume, environmentally friendly goods such as textiles, injection-molded packaging or other products, or films.

Methane is a potent greenhouse gas often produced as a byproduct at sites such as wastewater treatment plants, landfills, and agricultural facilities. Mango Materials' process incentivizes facilities to capture their methane and transform it into a useful product.

Mango Materials is currently scaling up its process and operates its Launch facility at a local wastewater treatment plant (Redwood City, CA). This talk will discuss the company's scale-up journey from

theoretical lab studies to a demonstration-scale facility and will identify the challenges and next steps as we look forward to commercialization.

9:52 AM Q & A

10:00 AM - 11:00 AM Session: 3: Advantaged Performance Bioproducts and Separations

Conveners: **Brent Shanks**, Iowa State University, Ames, IA, USA and **Jean-Philippe Tessonnier**, Iowa State University, IA, USA

10:00 AM Methods and Applications of Machine Learning Tools to Accelerate Discovery of Performance Bioproducts

O. Dollar, N. Joshi, W. Tatum, C. Ashraf, J. Kong, J. Pfaendtner and D. T.M. Beck, University of Washington, seattle, WA, USA*

Production of high value specialty and fine chemicals through biological routes holds tremendous promise due to the immense diversity of chemical functionalities and species that can hypothetically be produced from microbial sources. However, our ability to identify lead target molecules for R&D continues to challenge the overall pace of progress in discovering and developing production routes for high performance molecules. This presentation will share a few examples of how our team is using statistical and machine learning tools to speed the pace of new lead target discovery. We will highlight our work using natural language processing (NLP) to improve autonomous extraction of molecules and their associated properties from a large corpus of chemical and materials research literature. In addition, we will highlight new ways that we are using databases of chemical information in conjunction with variational autoencoders to improve both the fidelity and diversity of generative molecular data science models.

10:08 AM Q &A

10:11 AM Machine Learning for Advantaged Biobased Polymer Discovery

N. Wilson, P. St John, M. Nimlos and M. Crowley, National Renewable Energy Laboratory, Golden, CO, USA*

Developing sustainable polymers that can be sourced from biomass or waste streams is key for establishing a circular carbon economy. The design space accessible through biocatalysis is immense and cannot be reasonably probed using an Edisionian approach. To aid experimentalists in the down selection of material targets a high-throughput machine learning tool capable of predicting polymer properties from monomer structures was developed. The approach used message passing neural networks (MPNN) to predict 8 material properties: glass transition temperature, melt temperature, density, modulus, and the gas permeability of O_2, N_2, CO_2, and H_2O. To establish this tool, database development, automated 2D polymer structure generation, MPNN architecture training and optimization, and domain of validity testing were performed. The prediction pipeline was applied to monomers sourced from the KEGG database which generated >1,500 polyester target candidates. These targets were further down selected based on theoretical yield, biosynthetic pathway, material performance, and material synthesis scheme to identify advantaged biobased polymers for the replacement of polyethylene terephthalate (PET). The development of a machine learning polymer prediction tools will increase the rate at which new, sustainable materials can be discovered.

10:19 AM Q & A

10:22 AM Performance Advantaged Polymers and Additives - Benefits in Performance and Manufacturing

N. Rorrer, W. Henson, C. Hoyt, A. Singh and S. Nicholson, National Renewable Energy Laboratory, Golden, CO, USA; G. Beckham, National Renewable Energy Laboratory, USA, Golden, CO, USA*

The unique functionality afforded to biomass possess the opportunity to re-design todays chemicals and materials to have enhanced performance. In general, this enhanced performance can manifest as advantages in manufacturing (e.g. use of less toxic intermediates, faster reaction times), life time performance (e.g. better thermomechanical performance), or at the end-of-life. In the current work, we demonstrate that the extended functionality of β-ketoadipic acid and methyl muconates, namely ketones, methyl groups, and conjugate double bonds, can lead to enhanced performance for polymers and polymer additives. Advantages in manufacturing are evaluated by the generation of techno-economic and supply chain models which reveal that the feedstock type influences both the final selling price of a monomer as well as its supply chain energy and greenhouse gas emissions. Results from this work demonstrate the promise of leveraging performance advantaged bioproducts for multiple applications beyond just material performance. Future work will continue to explore how heteroatoms from biomass enable the manufacturing benefits discussed here.

10:30 AM Q & A

10:33 AM Developing performance-advantaged organic corrosion inhibitors utilizing bioprivileged molecules

J. Huo, University of Colorado Boulder, Boulder, CO, USA; W. Bradley, K. Podolak, B. Ryan, L. Roling, G. Kraus and B. Shanks, Iowa State University, Ames, IA, USA*

Biomass-derived chemicals and products eventually need to compete with the mature and efficient petrochemical industry. How to identify and develop biomass-derived chemicals and products with novel and/or superior performance compared with petroleum-based chemicals and products remains a challenge. Bioprivileged molecules, utilizing the integration of biological and chemical catalysis, create a platform for the development of performance-advantaged chemicals and products. In this research, two sets of organic corrosion inhibitors based on bioprivileged molecules triacetic acid lactone and 4-hydroxycoumarin were synthesized and tested on mild steel corrosion inhibition. A number of corrosion inhibitors showed promising inhibition performance in both sulfuric acid and hydrochloric acid as measured by electrochemical impedance spectroscopy and polarization techniques. The interaction of the corrosion inhibitors and mild steel surface was further confirmed and analyzed by SEM and XPS studies. Shown here is the power of bioprivileged molecules for further synthesis to novel chemical products with enhanced performance, which can be applied to other chemical products.

10:41 AM Q & A

10:44 AM Esterase catalysed biosynthesis of short-chain esters from engineered *E. coli*

A.P. Sarnaik, A. Jansen and A.M. Varman, Arizona State University, Tempe, AZ, USA; S. Shinde, A.K. Jha and R. Davis, Sandia National Laboratories, Livermore, CA, USA*

With the advent of environmental safety policies and development of sustainable industrialization practices, global research has been geared towards the development of biodegradable solvents, especially esters from renewable feedstocks. While majority of the biological esterification studies involve the use of acyltransferase enzymes, exploring esterases for esterification would be advantageous in two ways; their precursors can be synthesized in relatively high titers and is one step short as compared to the acyltransferase pathway.

To verify our approach, *E. coli* strains were constructed for the heterologous expression of four enzymes (diacylglycerol transferase, ethanol-o-acyltransferase, acetylxylan esterase, carbohydrate esterase) from *Brettanomyces bruxellensis* AWRI1499, yeast well known for its role in wine fermentation. However, the gene sequences for the selected protein candidates were not completely curated in the GenBank. Bioinformatics analysis was performed to curate distinct gene/peptide sequences to replace inappropriate nucleotides based on polynucleotide and/or polypeptide sequence homologies, followed by their codon optimization. Esterase A (EstA) from *Pseudomonas aeruginosa* was selected as a bacterial esterase. All the five sequences were cloned in *E. coli* for ethyl lactate and ethyl acetate analysis.

Based on preliminary high-cell density fermentation (lab-scale) of these five strains, two were selected for bioreactor optimizations, SSL74 (possessing CE, Carbohydrate esterase) and SSL76 (possessing EstA). Fed-batch fermentation at pH 7.0 exhibited 10 mg/L ethyl lactate and 81 mg/L ethyl acetate by both SSL76 and SSL74 strains, denoting comparable functionality of both the esterases. Fed-batch fermentation at pH 6.0 with SSL76 showed promising increasing in titers, 18.2 mg/L ethyl lactate and 225 mg/L ethyl acetate. These are the highest reported ethyl lactate titers from *E. coli*.

Importantly, the study reveals commercial potential of these underexplored esterases for industrial esterification reactions. They can be foreseen as efficient candidates for *in vivo* as well as *in vitro* enzyme catalysis at commercial scales.

10:52 AM Q &A

10:00 AM - 11:00 AM Session: 4: Bioenergy crops and plant genetics

Conveners: **Jenny Mortimer**, Lawrence Berkeley National Laboratory, CA, USA, USA and **Wellington Muchero**, Oak Ridge National Laboratory, TN, USA

10:00 AM Production of platform chemicals in bioenergy crops: Stacking low-recalcitrance traits with co-products

A. Eudes, Lawrence Berkeley National Laboratory, Berkeley, CA, USA*

Muconic acid (MA) is used for the production of important chemicals such as adipic acid, terephthalic acid, and caprolactam. 2-Pyrone-4,6-dicarboxylic acid (PDC) is a promising building block chemical used to make diverse biodegradable polyesters with novel functionalities. There is no chemical synthesis method currently available for manufacturing PDC, whereas synthesis of MA utilizes petroleum-derived chemicals. Therefore, the development of alternative strategies for bio-based production of MA and PDC has garnered significant interest. Plants represent advantageous hosts for engineered metabolic pathways towards the production of chemicals. We demonstrate that plants can be used for the bio-manufacturing of MA and PDC by re-routing intermediates of the shikimate pathway within chloroplasts. In particular, expression of bacterial 3-dehydroshikimate dehydratase (QsuB) in plastids results in concomitant reductions of lignin and accumulation of protocatechuate (PCA) in biomass. Additional engineering strategies are currently designed to enhance PCA titers and enable its conversion into MA and PDC in-planta. Specifically, bacterial feedback-insensitive 3-deoxy-D-arabino-heptulosonate-7-phosphate synthase was overexpressed to increase carbon flux through the shikimate pathway, co-expression of PCA decarboxylase with catechol 1,2-dioxygenase allowed MA production, and co-expression of PCA 4,5-dioxygenase with 4-carboxy-2-hydroxymuconate-6-semialdehyde dehydrogenase enabled PDC synthesis. The implementation in bioenergy crops (switchgrass, poplar, and sorghum) of MA and PDC biosynthetic routes that divert phenylpropanoid pathway intermediates away from lignin biosynthesis will be presented. These engineering approaches combine in plant biomass the production of value-added chemicals with low-recalcitrance traits towards sustainable development of biorefineries.

10:08 AM Q & A

10:11 AM Genome engineering in poplar for improved biofuel feedstocks

H. Coleman, Syracuse University, Syracuse, NY, USA*

Understanding the genetic and environmental controls of secondary cell wall formation is key to harnessing the potential of plants for biofuel production. Hand in hand with this, is the modification of the cell wall to produce biomass that is more amenable to break down. To address this challenge, we have focused on better understanding both how the secondary cell wall is formed and how it can be altered to improve biomass quality. Recently we have focused on expression of cellulases and other hydrolytic enzymes in poplar for altering cell wall structure, exploring both overexpression and potentially utilizing post-harvest heat activation to improve saccharification efficiency. This work has identified a number of improved poplar lines and informed future efforts in the lab. Here I will focus on our most recent results in the area of hydrolytic enzyme expression *in planta* as well as current work focused on understanding the impact of environmental conditions, particularly nutrient availability, on growth and cell wall formation.

10:19 AM Q & A

10:22 AM Computationally guided engineering of catalytically modified plant O-acetyltransferases to alter polysaccharide structure.

H.T. Wang, D. G. Chapla, K. Moremen and B. Urbanowicz, University of Georgia, Athens, GA, USA; V. Bharadwaj and Y. Bomble, National Renewable Energy Laboratory, Golden, CO, USA*

Hemicellulosic polysaccharides account for up to 20-30% of the dry weight of plant biomass. The dominant substituents of the glucuronoxylan present in dicots are O-acetyl moieties, decorating more than half of the Xyl residues. Xylan O-Acetyltransferase 1 (XOAT1) has been shown to specifically catalyze the 2-O-acetylation of xylan. Our recent crystallographic study of XOAT1 showed it is a member of the larger SGNH hydrolase superfamily, contains a catalytic triad formed by Ser216-His465-Asp462 localized at the bottom of a heart shaped catalytic cleft formed by two unequal lobes, termed major and minor. We expanded our analyses to identify key residues involved in enzyme-acceptor substrate interactions through a combination of computational modeling followed by site-directed mutagenesis (SDM) and kinetic analysis. Our results show that the major lobe of XOAT1 interacts more with the xylan acceptors, while mutation of residues in the binding loop of the minor lobe result in modulation of catalytic activity. Taken together, we discuss how these results are applied to develop strategies for genetic engineering plants with altered xylan substitution amounts and patterning.

10:30 AM Q & A

10:33 AM Expression of a Rice Ferulate Monolignol Transferase in *Arabidopsis* Improves Cell Wall Suitability for Biorefining

C. Zhang, PhD, M.F. LaPorte and M. Lesani, University of Oklahoma, Norman, OK, USA; R. Smith, PhD and J. Ralph, PhD, University of Wisconsin, Madison, WI, USA; Y.L. Tsai, PhD and H. Scheller, PhD, Joint BioEnergy Institute, Emeryville, CA, USA; M. Peck, PhD and L. Bartley, PhD, Washington State University, Pullman, WA, USA; N. Santoro, PhD, Michigan State University, East Lansing, MI, USA*

Engineering plant lignin promises to improve efficiency of bioconversion of plant biomass to biofuels and usability of lignin for synthesizing biomaterials and other bioproducts. Previously we identified a rice “BAHD” acyltransferase, OsAT5, whose overexpression increased the abundance of monolignol ferulate esters (ML-FAs) in rice lignin (Karlen et al. *Sci Adv* 2:e1600393). Here we report confirmation of the function of OsAT5 in producing ML-FAs in *Saccharomyces cerevisiae* and Arabidopsis, both of which do not naturally form these esters. When incorporated into lignin polymers, ML-FA conjugates might improve lignin susceptibility to mild alkaline pretreatment due to the incorporation of base-cleavable ester bonds into the core of the lignin polymer. However, *Ubipro-OsAT5* rice straw did not exhibit reduced cell wall

recalcitrance, though Arabidopsis *C4Hpro-OsAT5* did. Consistent with this, gel-permeation chromatography and alkaline solubility suggest that wild-type rice lignin has shorter chains and is more alkali-labile than wild-type Arabidopsis lignin. Although introduction of *OsAT5*decreases the average molecular weight (Mw) of alkali-treated lignin in both species, the effect is more pronounced in *C4Hpro-OsAT5* Arabidopsis than in rice over-expressing *OsAT5*. This study shows the potential use of OsAT5 in structurally modifying plant cell walls and producing less recalcitrant plant biomass for biorefining and the potential of plant/lignin structure-specific engineering.

10:41 AM Q &A

11:00 AM - 12:00 PM Rapid Fire

Detecting Physiological Status of Microbial Cultures, In-situ.

H. Teel, K. Likit-anurak, P. Satjaritanun and S. Shimpalee, University of South Carolina, Columbia, SC, USA; C. Turick, Savannah River National Laboratory, Aiken, SC, USA*

Detecting Physiological Status of Microbial Cultures, In-situ.

Kris Likit-Anurak[1], Hunter R. Teel[1], Pongsarun Satjaritanun[1], Sirivatch Shimpalee[1], Charles E. Turick[2,3]

[1]University of South Carolina, Columbia, SC, USA, [2]Savannah River National Laboratory, Aiken, SC, USA, [3]ElectroBioDyne, Aiken, SC, USA

For bioconversion of organic feedstocks to fuels and chemicals to be cost-effective, bioprocesses need to operate at near optimum conditions with sufficient chemical, biochemical and microbial monitoring. To avoid conventional time and labor-intensive monitoring, a new paradigm is required for in-situ, real time analysis. Since bioconversion of organic is accomplished by microorganisms through the oxidation of feedstocks linked to the reduction of electron acceptors, microorganisms can be viewed as electrochemical catalysts. In this regard, following electron flow through well-established electrochemical techniques offers a novel and inexpensive approach to real time monitoring with the advantage of abundant data.

Here we demonstrate the use of electrochemical techniques of cyclic voltammetry (CV) and electrochemical impedance spectrometry (EIS) for monitoring microbial metabolic activity in real time, in-situ. CV provides precise information regarding extracellular electron transfer throughout growth and EIS offers a data rich platform for evaluation of microbial physiological status in real time. In addition, the problem of electrode fouling is managed with voltammetric stripping, an established electrochemical technique used to clean and condition electrodes in-situ.

The effect of organic electron donors as a function of concentration to the physiological status of *Shewanella oneidensis* was determined. In this study, the Gram-negative, pyomelanin overproducer (*S. oneidensis ΔhmgA*) and the pyomelanin deficient mutant (*S. oneidensis ΔmelA*) were chosen due to different surface electrochemical characteristics along with relative degrees of oxygen utilization efficiency. Electrochemical properties changed with growth status and correlated with electron flow from organic carbon sources and terminal electron acceptor availability. These results are compared with those of the Gram-positive *Clostridium phytofermentans* from previous studies.

Genetic manipulation of transcription factors involved in the secretion of recombinant enzymes in *Aspergillus nidulans*

E. Paschoal Antoniel, J. Aline Gerhardt, M. Alexandrino de Assis and N. Sayuri Wassano, University of Campinas (UNICAMP), Campinas, Brazil; A. Ricardo de Lima Damasio, Institute of Biology, University of Campinas (UNICAMP), Campinas, Brazil*

Filamentous fungi are capable of secreting a significant amount of proteins and are used as a platform for the production of industrially important enzymes, such as hydrolytic enzymes used in the production of bioproducts such as biofuels. However, there is still potential for progress in terms of recombinant protein production. In this regard, many techniques have been used to boost fungal cell factories production capacity; however, as consumer demand grows and productivity remains poor, identifying new genetic targets to promote increased enzyme secretion becomes increasingly important. Therefore, our aim was to evaluate the production/secretion of enzymes in strains of *Aspergillus nidulans* in which target transcription factors have been genetically manipulated. To identify these transcription factors, we used RNA-seq data from *A. nidulans* strains overexpressing three heterologous enzymes and we performed differential expression and GO enrichment analysis. Next, we used CRISPR-Cas9 to knock out these transcription factors in *A. nidulans* and analyzed their phenotype to see whether they have any effect on enzyme secretion. Based on RNA-seq data, we identified five transcription factors candidates – AN8772, AN9373, AN7331, AN7913, and AN7592 – with predict function in golgi vesicle transport, protein refolding, small molecule catabolic process and proteolysis. We found that the strain ΔAN7592 had decreased sporulation and slight resistance to osmotic stress, while the strains ΔAN9373, ΔAN7331, and ΔAN7913 had increased protease secretion. Taken together, our results suggest that these transcription factors may be involved in a variety of biological processes associated with the protein secretion pathway. We concluded that transcriptomic data could be used to identify transcription factors with possible involvement in the protein secretion pathway of *A. nidulans*, and we plan to continue our research by performing additional experiments to determine the role of these transcription factors in the secretion of biotechnologically relevant recombinant enzymes.

Characterizing fungal inhibitors from drought-stressed switchgrass

S. Chipkar, K. Smith, M. Gallmeyer and R. Ong, Michigan Technological University, Houghton, MI, USA; A.D. Jones, Michigan State University, East Lansing, MI, USA*

Development of economically viable and greener pathways to synthesize renewable energy has been an important research theme in recent years. Lignocellulosic biomass is a major resource that can be used for biofuel production. Recent research has shown that biomass characteristics are altered by environmental growth conditions, and directly influence the extent of biomass conversion to fuels. Previously it was reported that drought experienced during the growth of switchgrass led to complete inhibition of yeast growth during fermentation. In this project, we characterized specific compounds that led to this inhibition. Switchgrass harvested in drought and non-drought years were pretreated using Ammonia Fiber expansion (AFEX). Untreated and AFEX processed samples were separately extracted using solvents (i.e., water, ethanol, and ethyl acetate) to selectively remove potential inhibitory compounds and determine whether pretreatment affects the inhibition. A key goal of the project was to determine whether the microbial-inhibitors are plant-generated compounds, by-products of the

pretreatment process, or a combination of both. High solids loading enzymatic hydrolysis was performed on all samples followed by fermentation using genetically modified, xylose consuming yeast strain *Saccharomyces cerevisiae* Y330. Cell growth (OD_{600}), sugar consumption, and ethanol production were used to evaluate fermentation performance. Extracts were analyzed using liquid chromatography-mass spectrometry (LC-MS) to identify potential inhibitory compounds. We identified numerous saponins, a class of plant-generated triterpene glycosides, which were significantly more abundant in the drought-year (inhibitory) switchgrass water extracts and potential microbial inhibitors. Tandem MS analysis on the unknown characterized inhibitors was conducted to annotate their identities. Add-back fermentation experiments in non-inhibitory hydrolysates for the identified inhibitors will be conducted to replicate inhibition.

Lactate overloading phenomenon in carboxylate platform: stable caproate and hydrogen co-production

F. Brodowski, M. Lezyk, N. Gutowska and P. Oleskowicz-Popiel, Poznan University of Technology, Poznan, Poland*

The emerging carboxylate platform, producing medium chain carboxylates (MCC) during the chain elongation process in an open culture fermentation, is an alternative to popular biotechnology technologies producing biogas and ethanol. The most popular MCC is caproate produced in ethanol-based or lactate-based carboxylate platform. The conversion of lactate to caproate is becoming more and more popular, however there are many limitations affecting production stability, e.g. lactate overloading. Lactate overloading not only affects caproate production, but can also activate propionate producers that can outcompete chain elongators. The main objective of the study was to investigate the effect of external acetate addition (electron acceptor) on lactate overloading in the continuous caproate production process. The process was carried out in two 1L continuous stirred-tank reactors. In the first one denoted as B1, lactate conversion to caproate was carried out without the external acetate, and in the second one denoted as B2, with the addition of acetate. The concentration of lactate in the feedstock (inflow) was increased until lactate overloading occurred. In B1, fluctuations in caproate production were observed as a result of the appearance of residual lactate, however no propionate production was observed. The addition of external acetate to lactate-overloaded B1 recovered stable caproate production and induced hydrogen co-production. In B2, the external acetate influenced the shift of the lactate overloading limit (the lactate overloading occurred at a higher concentration of lactate in the feedstock than in B1) and despite the presence of residual lactate, a stable caproate production was achieved. Additionally, hydrogen production in lactate-overloaded B2 was observed. Thus, the addition of external acetate was proposed as a viable strategy to stabilize caproate production in lactate-based carboxylate platform and recover production stability in the case of lactate overloading.

Glucose Transport Systems for Upgrading Farm Residue-Derived Hydrolysates to Fine Chemicals via Shikimate Pathway Biosynthesis

K. Draths, Y. Jadidi, C. Saffron, H. Frost and W. Liao, Michigan State University, East Lansing, MI, USA*

Examination of Glucose Transport Systems for Upgrading Farm Residue-Derived Hydrolysates to Fine Chemicals

Yasheen Jadidi, Henry Frost, Chris Saffron, Wei Liao and Karen Draths

The natural product shikimic acid (SA) is the starting material for synthesis of oseltamivir phosphate (Tamiflu®), an antiviral agent effective against influenza. SA may be isolated from plants; however, methods for biosynthesizing SA from glucose via fed-batch fermentation have also been developed. Our research aligns with the developing trend to utilize organic farm waste in place of corn-derived feedstocks for microbial synthesis of chemicals. Data presented will encompass an effort to add to the “agricultural residue economy”, utilizing mixed sugars derived from lignocellulosic corn stover for SA biosynthesis. *Escherichia coli* K-12 strains expressing different glucose transport systems – native phosphotransferase (PTS), heterologously expressed *Z. mobilis* GIF and GIK, and an uncharacterized evolved uptake

mechanism – were examined in a fed-batch fermenter for their abilities to co-utilize glucose and xylose for SA synthesis. Strain YJ1.144/pSC6.090B expressing GlF and GlK performed better than a strain expressing the native glucose PTS and another PTS^- strain evolved to take up glucose. YJ1.144/pSC6.090B achieved an SA titer of 94.9 g L^{-1} when grown on a 70:30 (*w*/*w*) mixture of glucose/xylose. CO_2 emission data collected from the fed-batch fermentations were used to perform a comparative life-cycle assessment (LCA) and techno-economic analysis (TEA). In the LCA/TEA, a fair techno-economic and life cycle impact (LCI) comparison was made between use of corn-derived glucose and stover-derived glucose/xylose mixtures for SA production. These results will be presented.

Microbiome Analysis of Iodoform Effects on Advanced Anaerobic Digestion Processes for Production of Fatty Acids

J.L. Rico Reyes, R. Daly, K. Wrighton, PhD, K. Reardon, PhD and S. De Long, PhD, Colorado State University, Fort Collins, CO, USA*

Advanced anaerobic digestion (AD) technologies have the potential to convert organic residues into added-value chemicals and fuel precursors. This rewired AD (RWAD) approach requires selectively directing feed carbon into fatty acids (FA) instead of methane. For such an approach, studies in RWAD have used methanogen inhibitors, like iodoform, and demonstrated success in arresting methanogenesis while producing FA. However, RWAD is a complex biological system. A single perturbation can significantly impact the microbiome structure and its metabolic pathways. To date, there is a lack of understanding of how iodoform impacts the microbiome in RWAD. This knowledge gap is one of the limitations in the field for optimizing process performance. This study investigated the effects of iodoform addition in RWAD of food waste using anaerobic wastewater sludge as a microbial inoculum. Microbiome reactors were acclimated to operating parameters for one month, followed by a five-day experimental run with five reactor replicates per treatment (with and without iodoform addition). Significant differences were observed in the production of gases and the FA product spectrum. As expected, methane was produced in controls but not in iodoform-treated reactors. Interestingly, acetic and propionic acid production was highest in controls, while butyric and hexanoic acids were highest in iodoform treated reactors. Comparative microbiome analysis via 16S rRNA gene and metagenomic sequencing revealed differences in the microbiome structure and the metagenome. These differences were driven by *Prevotella* and *Peptostreptococcacea*, which dominated iodoform reactors, and *Alcaligenes* and *Rombustia*, which dominated in controls. Metagenome assembled genomes were reconstructed and linked to specific taxa, genes, and metabolic pathways. This is the first comparative microbiome and metagenomic analysis of iodoform effects on RWAD and provides new insights into microbiomes' genetic potential to transform organic residues into FA. Further, this study demonstrates the value of integrated meta-omics studies for advancing knowledge of RWAD systems.

Engineering *Streptomyces* to Capture Value from Lignocellulosic Biofuel Conversion Residue

C. Wadler, K. Throckmorton and M. Thomas, University of Wisconsin, Madison, Madison, WI, USA*

Current methods of switchgrass hydrolysate fermentation to bioethanol leave behind about 60% of the organic material in the hydrolysate after ethanol distillation. This material is referred to as conversion residue (CR). To increase the economic viability of lignocellulosic biofuels, we are engineering *Streptomyces* species to maximize the metabolism of CR carbon into valuable bioproducts. From a library of 120 phylogenetically distinct *Streptomyces* isolates, we generated a collection of *Streptomyces* that produce lycopene from CR as a reporter for their potential to produce isoprenoids. The genetic element used in constructing this reporter is mobilizable between *Streptomyces* species and we have constructed further plasmids using a combination of traditional cloning techniques and Golden Gate assembly that allow for rapid alterations in expression levels and the generated bioproduct.
Initial screens of the engineered *Streptomyces* reporter strains showed a wide range of lycopene production levels. We were able to further increase lycopene production by introducing two different pathways that produce isoprenoid precursors: an optimized version of the native methylerythritol

phosphate (MEP) pathway or the mevalonate (MEV) pathway with a constitutive promoter. These strains also showed differences in carbon utilization of the CR, suggesting other avenues for engineering to increase bioproduct formation while we expand our repertoire of target compounds for bioproduct generation.

RuBisCO engineering and RNA-sensor based RuBisCO assay development for sustainable production of essential amino acids

A.P. Sarnaik, A. Mhatre and A.M. Varman, Arizona State University, Tempe, AZ, USA; M. Faisal, University Institute of Biochemistry and Biotechnology, Rawalpindi, Pakistan; R. Davis, Sandia National Laboratories, Livermore, CA, USA*

Undernourishment is a global issue, prevailing since decades. Hence, availability of nutritive, affordable food becomes necessary. Plant-based diet is sustainable and affordable, but there are certain essential amino acids which cannot be obtained from natural plant sources and need to be resourced from animal-based diet only.

We strategized to enrich RuBisCO, a common and abundant protein across all phototrophs, with essential amino acids, lysine and methionine. Based on Grantham's distance, the RuBisCO polypeptide sequence in *Synechocystis* sp. PCC6803 was modified. Four different RuBisCO (large subunit) variants were created, where replacements were performed distant from (rbcL1 and rbcL3) or around (rbcL2 and rbcL4) the active site, thereby increasing Lys or Met content by two-fold. *In silico* structural comparison was performed to ensure protein folding is unaffected, before analysing their *in vivo* performances.

We introduced the gene isoforms at natural *rbcL* locus in *Synechocystis* (WT) through homologous recombination. Comparative growth studies confirmed there was no effect on growth of mutants over WT, indicating neutral mutations. In parallel, we developed a sophisticated biochemical activity assay and RNA sensor-based fluorescence assay for quantitative and semi-quantitative estimation of RuBisCO activity. Results exhibited that all the isoforms of RuBisCO displayed uniform functionality, indicating successful protein engineering.

Simultaneously, genes for the RuBisCO variants were overexpressed in *E. coli* to experimentally quantify lysine/methionine levels, and to verify if the cells were able to cope up with the increased demand for lysine and methionine without further pathway engineering. GC-MS analysis of the engineered *E. coli* cell lysates established, ~75% increase in Met, and ~65% increase in Lys content in the corresponding variants relative to WT.

As RuBisCO is an integral enzyme in plant photosynthesis, prospectively these modifications can be safely translated to the food crops, seeds, legumes ultimately improving their nutritive value.

Discarded vegetal residues as low-cost carbon source for lipid production

M. Gallego-García, D. Moreno, A. González, R. Iglesias and M.J. Negro, CIEMAT, Madrid, Spain*

In South-western Europe, the horticultural intensive type systems dedicated to the production of greenhouse vegetables represents one of the main industries generating organic waste. For instance, the fruits that do not meet the required quality standards for sale are removed during harvesting and are considered as waste. These residues may, however, represent an interesting resource to use in different bioprocesses as a low-cost raw material.

Carbohydrates from discarded vegetable products can be easily extracted by collecting the corresponding juice after a crushing procedure. These carbon sources might be used, for example, in the production of single cell oils using oleaginous yeasts, to later convert these microbial oils into biofuels. This is possible because oleaginous yeasts have the ability to accumulate more than 20% lipids to their dry weight. However, with high carbon/nitrogen (C/N) ratios they can achieve a lipid accumulation of more than 70% with respect to their cellular biomass.

In this work, discarded vegetables (tomato, pepper and watermelon) were employed as raw material for microbial oil production. The liquid fraction of this residue was mainly composed by carbohydrates (including glucose, fructose and sucrose), and were used as a culture media for lipid production using the

yeast *Cryptococcus curvatus*. Depending on the studied residue, the sugar content varies from 30 g/L up to 65 g/L.

Different C/N ratios (15, 30 and 50) were tested to evaluate the percentage of lipids and the profile of fatty acids accumulated by yeast under fruit-derived media. The best result was achieved when using the discarded pepper medium with a C/N ratio of 50, obtaining a 40% accumulation of lipids and showing a fatty acid profile similar to that obtained from vegetable oils in the conventional biodiesel production.

Acknowledgements: Project ENE2017-86864-C2-1-R (AEI/FEDER, UE). María Gallego would like to thank “MICINN” and ESF/UE (Grants Ref. PRE2018-086317)

Two-stage hydrogen and methane production using residual fermented solid obtained after enzymatic biodiesel production

S. Buback dos Santos, M. de Oliveira Faber and E. Cristina Gonçalves Aguieiras, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil; M. Antunes Pereira Langone, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, Brazil; V. Santana Ferreira-Leitão, National Institute of Technology, Rio de Janeiro, Brazil*

The objective of this study was to evaluate the sequential production of H_2 and CH_4 using, as raw material, the residual biocatalyst from enzymatic biodiesel synthesis. The biocatalysts synthesis through solid-state fermentation (SSF) using residual biomass from the vegetable oil industry has been investigated to reduce the costs of enzymatic biodiesel production. This biomass, after SSF, results in a biocatalyst with lipase activity called Fermented Solid (FS). The biodiesel synthesis by esterification reactions of Palm Fatty Acid Distillate (PFAD) and ethanol, using FS as the biocatalyst, produces a high amount of Residual Fermented Solid (RFS). For each 1 L of biodiesel produced, approximately 350 g of RFS is generated. RFS is rich in organic matter, which makes it attractive for sequential biological production of H_2 via dark fermentation and CH_4 via anaerobic digestion. Thus, the integration of these processes is interesting because it adds value to the biodiesel chain and minimizes the environmental problems, besides it is also an alternative for clean and decentralized energy production. For both H_2 and CH_4 productions using RFS, an anaerobic sludge from the biodigester of a sewage treatment plant was used as inoculum. For the hydrogen production the concentration of RFS 31 g_{RDFS}/L, was investigated. The highest hydrogen production (239 ± 44 mL_{H2}/L_{medium}) was observed after 24 h using 31 g_{RDS}/L. Additionaly, the methane production obtained from the Hydrogen Production Liquid Waste was 204 ± 13 mL_{CH4}/g_{COD}, which represented 61% of efficiency.

High-throughput promoter optimization for improved biobutanol *in vivo* biosensor

N.M. Kim, R. Sinnott and N. Sandoval, Tulane University, New Orleans, LA, USA*

Rising costs and environmental stress tied to fossil fuels has motivated industrial interest in butanol as a renewable fuel source and alternative to gasoline. Biobutanol production from renewable feedstocks has been demonstrated, but the production yield remains economically unviable. High-throughput screens on non-growth-related phenotypes and dynamic butanol-dependent regulation represent powerful metabolic engineering strategies that are largely unavailable to these efforts. This capability gap is due to a lack of inducible transcription factor/promoter pairs with user-defined controls. A butanol-responsive transcription factor, BmoR, and its cognate promoter PBMO have been previously described in the native form, but PBMO remains relatively uncharacterized and optimizing its function by sequence modification has not been attempted. In this work, we demonstrate the engineering of the PBMO promoter at the nucleotide level to improve biosensor characteristics, specifically an improved dynamic range, and to generate synthetic promoters. To this end, we use massively parallel reporter assays to study the sequence-function relationship of PBMO using the ‘sort-seq’ method. A mutagenized PBMO library cloned upstream of *gfp* in *E. coli* was induced with butanol and sorted into activity-based (i.e., fluorescence-based) populations. These populations were subsequently sequenced via NGS and their PBMO mutations correlated with changes in *gfp* expression, enabling construction of synthetic promoters with desirable characteristics. Best of the mutated promoters demonstrated over 4-fold increase in dynamic range.

Additionally, sort-seq approach identified sites that are essential to the function of the biosensor and those that increase the sensor output. This work can enable rational strategies to edit the dynamic range of transcription factor-based biosensors.

Towards zero plastic waste: Identifying bioplastic degradation genes and enzymes in *Burkholderia*

Z.L. Yap, H. Tesfu, J.P. Hawkins, I. Oresnik, D.B. Levin and S.T. Cardona, University of Manitoba, Winnipeg, MB, Canada*

Many microorganisms produce polyhydroxyalkanoates (PHAs), which are biological polyesters that are used as intracellular storage compounds. Some PHA polymers have physical properties that are similar to polymers derived from petrochemicals ("petro-plastics"), and thus could displace some petro-plastics in the future. Upon microbial death, PHAs are released to the environment. Thus, microorganisms have evolved to produce extracellular PHA depolymerases to breakdown exogenous PHA and utilize them as a carbon source for growth and metabolism. Most of the characterised PHA depolymerases degrade short chain length (scl)-PHAs. However, a few medium chain length (mcl)-PHA-degrading enzymes that have been identified. Most of the characterized extracellular mcl-PHA depolymerases are from the genus *Pseudomonas.* Bacteria in the genus *Burkholderia* are phenotypically similar to *Pseudomonas* and have extraordinary genomic and metabolic plasticity. We hypothesize that *Burkholderia* species cultured under certain environmental conditions will adapt to the available nutrients and turn-on different metabolic processes, including extracellular mcl-PHA depolymerases activity. Using a method to screen for extracellular mcl-PHA depolymerases, we found *Burkholderia vietanmiensis* LMG 16232 was able to degrade extracellular mcl-PHA. We sequenced the genome of *B. vietanmiensis* LMG 16232 and performed *in silico* analyses to identify potential genes responsible for this activity. We also developed a transposon mutagenesis and screening method for *B. vietanmiensis* LMG 16232 to identify putative genes associated with extracellular mcl-PHA degradation. Once we identify the transposon mutants that have disrupted activity, we will validate the identified genes by using CRISPR-Cas tools. Identification of PHA degradation genetic elements in *Burkholderia* will contribute to the development of industrial bioplastic recycling processes.

Enzymatic Synthesis of Xylan Microparticles with Tunable Morphologies

P. Smith, S. Ziegler and Y. Bomble, National Renewable Energy Laboratory, Golden, CO, USA; T. Curry, W. Barnes, W. York, M. Pena and B. Urbanowicz, University of Georgia, Athens, GA, USA*

Xylans are a diverse family of hemicellulosic polysaccharides found in abundance within the cell walls of nearly all plants and many algal species. Here we demonstrate the ability of a recombinantly expressed xylan synthase(*Kf*XYS1) to synthesize xylan microstructures with well-defined and tunable morphology *in vitro* using UDP-xylose as a donor substrate and short xylan oligosaccharides as acceptor substrates. We have investigated the conditions under which xylan microparticles form, and demonstrate the ability to synthesize structures with unique shapes, morphologies, and compositions influenced by the modification of, for example, the fine structural elements of the oligomeric xylan acceptor substrates used to initiate polymerization. We have investigated the characteristics of the xylan polymers which comprise the microparticles, including their average degree of polymerization and polymer conformation. Furthermore, we have probed the ability of glycosyl hydrolases to degrade xylan microstructures, as well as demonstrated the ability to functionalize xylan microstructures via chemical modification. Together, these results provide a model system to investigate influential factors in polymer-polymer interactions, and suggest a possible synthetic biology based route to new biobased materials with favorable properties for biocompatible and renewable applications.

A flexible kinetic assay efficiently sorts prospective biocatalysts for PET subunit hydrolysis

J. Lusty Beech, R. Clare, D. Kim and J. Dubois, Montana State University, Bozeman, MT, USA; E. Erickson, National Renewable Energy Laboratory, Golden, CO, USA; J. McGeehan, University of*

Portsmouth, Portsmouth, United Kingdom; G. Beckham, National Renewable Energy Laboratory, USA, Golden, CO, USA

Esterases are enzymes with wide-ranging biological, commercial, and biotechnological applications. The canonical esterase activity assay utilizes readily hydrolysable alkyl-*p*NP esters, which react as nearly universal esterase substrates but which are limited in structural variability. The use of pH indicator dyes offers an effective, affordable, and easily quantifiable alternative for monitoring carboxylic esterase reactions in real time. Here, we refined a UV/visible assay using pH-sensitive aryl sulfonate dyes to quantify hydrolysis of the polyethylene terephthalate (PET) plastic subunit *bis*-(2-hydroxyethyl) terephthalate (BHET) in real time, and applied it to a suite of 24 known PETases and diverse commercial esterases in microplate format. This approach quickly identified one known PET and a well-known commercial esterase as effective catalysts against BHET under mild conditions (turnover number ≥100 in 48h, pH 8, 37 °C). The method was shown to be amenable to multiple pH/temperatures, and semi-pure enzymes in solution or freeze-dried cells.

Amazonian açaí (*Euterpe precatoria* Mart.) and Juçara fruit (*Euterpe edulis* Mart.) seeds as a potencial source of mannose

D. Marconi Miranda Carvalho da Silva, R. de Araújo Pontes, I. Santos Miguez and A. Sant'Ana da Silva, National Institute of Technology, Rio de Janeiro, Brazil*

Euterpe precatoria, popularly known as Amazonian açaí palm, is the second most common species among 28 other palms from the genus Euterpe found in the Amazon river delta, while *Euterpe edulis*, known as juçara palm, is one of the main non-timber products in the Atlantic Florest. The *E. precatoria* and *E. edulis* non-edible seeds corresponds to 75%-80% of the total fruit weight, and are discarded as a by-product after pulp extraction. Therefore, this work aimed to characterize the açaí and juçara seeds and explore routes to obtain valuable compounds from these unexplored residues. For the determination of the chemical composition, the whole seeds were analyzed including the core stone and the fibrous layer. Mannose was the major sugar present in the whole seeds, which presented a molar ratio of mannose (man), glucose (glc), xylose (xyl), galactose (gal) and arabinose (ara) of 84:11:2:2:1 for *E. precatória* and 76:17:5:1:1 for *E. edulis*. To access the potential to obtain the mannose from the untreated milled whole açaí seeds, enzymatic hydrolysis assays were performed with mannanases. However, only 15.3% and 16% of mannan were converted into mannose for *E. precatória* and *E. edulis* seed samples, respectively, indicating the need for seed preprocessing prior to enzymatic hydrolysis to reduce the mannan recalcitrance. Mannose has great importance in the market, being used by medicine to treat urinary tract infections, and also in the food and cosmetic industris. Thus, the present data can contribute to the stimulate the valorization of largely unexplored residues from Brazil.

VALORIZATION OF PEACH BYPRODUCT IN A BIOREFINERY CONTEXT: BIOACTIVE PROFILE, ANTIOXIDANT CAPACITY AND ENZYME DIGESTIBILITY

M.P. García-Aparicio, A. Martín-Ortiz and M.L. Marina, University of Alcalá, Alcalá de Henares, Spain*

The aim of this work was to characterize the profile of main components and bioactive compounds, and the antioxidant activity of peach juice byproduct (PJB) originated during fruit concentrate production using discarded/outgrade peaches as feedstock. PJB was subjected to freeze dry and to oven dry treatments for their evaluation as storage treatments. The chemical analysis revealed a composition of aqueous extractives (52.97% from which 35.48% are sugars), organic extractives (6.97%), 11.29% fermentable sugars as structural polysaccharides, total lignin (16.05%), total ashes (1.89%) and protein (5.8%). A set of conventional extraction methods of the extractable and non-extractable bioactive compounds were applied and compared with an enzyme-based extraction process. The phenolic compounds profile of the different extracts was compared. In addition, the impact of the storage treatment on saccharification of the PJB by different commercial enzyme preparations was evaluated. The hydrolysable tannins and other polyphenols linked to dietary fibre and/or protein presented the highest antioxidant activity based on results from DPPH and ABTS assays. Moreover, these antioxidant activities were not significantly

reduced after applying the oven dry process. Information about the composition, bioactive compounds profile and their antioxidant activity after different treatments is essential to guide process and technologies for their exploitation. The high fermentable sugars concentration (about 50%) and the presence of bioactive substances makes the PJB a potential feedstock in biorefineries for production of biofuels, bioproducts or to develop new functional products for the food and/or cosmetic sectors.

On relation between pretreatment conditions, supramolecular properties and efficiency of enzymatic hydrolysis of steam pretreated spruce

F. Caputo, V. Novy and L. Olsson, Chalmers university, goteborg, Sweden; B. Al-Rudainy and O. Wallberg, Lund university, Lund, Sweden*

The development in cellulosic ethanol production has mainly focused on agricultural biomasses. However, woody biomasses remain a very important feedstock for ethanol production even if the process is not yet economically feasible. In order to use lignocellulosic biomasses in a biorefinery, the use of harsh pre-treatment is necessary to overcome the recalcitrance of the biomass. These harsh pre-treatments result in the formation of inhibitors, that decrease the efficiency during the fermentation, and the loss of the hemicellulose, that lowers the total yields of the process.
We aim to increase the saccharification efficiencies of steam-pretreated (STEX) spruce that retains the hemicellulose in the material. We will investigate the different factors that affect the spruce recalcitrance like the accessibility and the diffusion of enzymes into the pretreated spruce, the non-productive binding of the enzymes towards the biomass and the presence of the lignin-carbohydrate bonds (LCs). A cellulolytic enzyme cocktail (Celluclast) was used for the enzymatic hydrolysis and it the role of different accessory enzymes (Xylanase, Mannanase, Laccase, Esterases, LPMOs) on the efficiency of the enzymatic hydrolysis was investigated. Here, we discuss the steam explosion (STEX) pre-treatment performed on spruce in different conditions (180°C/5min-autocatalyzed, 210°C/5min-autocatalyzed, 210°C/5min-SO_2 catalyzed), the determination of structural carbohydrates on the solid fraction, the enzymatic hydrolysis performed on the pre-treated materials using Celluclast and Novozyme 188. Preliminary data on the accessibility of the enzymes to the materials will be discussed.

Bioprospection of CAZYmes coded in the genome of the white-rot basidiomycete *Pycnoporus sanguineus*

M. Garrido, MS, J. Topalian and E. Campos, Ph.D., University of Buenos Aires/ National Institute for Agricultural Technology, Buenos Aires, Argentina; R. Brunecky, Ph.D., National Renewable Energy Laboratory, Golden, CO, USA; M. Landoni, Ph.D., University of Buenos Aires/ Carbohydrate Research Center (CIHIDECAR), Buenos Aires, Argentina; S. Wirth, Ph.D., University of Buenos Aires/ Institute for Biodiversity and Experimental and Applied Biology, Buenos Aires, Argentina*

Existing biomass conversion schemes typically rely on a combination of chemical and enzymatic treatments that include a pretreatment step to reduce recalcitrance exposing the crystalline cellulose core, which is then hydrolyzed by cellulase enzymes. Reducing-end acting cellobiohydrolases (EC 3.2.1.176) are the main enzymes required for cellulose hydrolysis due to their enzymatic proficiency in cellulose depolymerization. The saprotrophic white-rot fungi *Pycnoporus sanguineus* is known as a potent degrader of plant biomass with the ability to degrade all lignocellulose components using a wide diversity of enzymes. After a thorough analysis of genomic and secretomic data available for this fungus, two cellobiohydrolases from family GH7, *Ps*Cel7A and *Ps*Cel7B, were selected for further characterization and recombinantly expressed in *Trichoderma reesei*. The desired proteins were successfully purified from the fungus's culture broth using Fast Purification Liquid Chromatography (FPLC) and the optimal temperature and pH of their enzymatic activity was determined with the commercial substrate *p*NP-lactoside. Pretreated corn stover (PCS) hydrolysis was tested, resulting in approximately 40% glucan conversion after 5 days of incubation with both enzymes. The amount of cellobiose and cellotriose released by *Ps*Cel7B was determined by HPAEC-PAD using Phosphoric Acid Swollen Cellulose (PASC) as substrate. Enzymatic reactions were carried out with *Ps*Cel7B alone or in conjunction with *Ps*AA9A, a previously characterized lytic polysaccharide monooxigenase (LPMO) from the same fungus. LPMOs

have their active site in a flat surface enabling them to access crystalline regions of cellulose and generate new chain ends for glycosyl hydrolases to act upon. The amount of cellobiose detected was not improved by adding *Ps*AA9A to the reaction, but the amount of cellotriose was significantly increased. This work provides valuable information about highly secreted CAZymes by *P. sanguineus* and serves as a starting point for the future development of engineered enzymes with enhanced performance for the deconstruction of cellulose feedstocks.

AA9 LPMOs are differently affected by cellulose ultrastructure.

S. Magri and D. Cannella, Université Libre de Bruxelles, Bruxelles, Belgium*

The boosting effect of Lytic Polysaccharide MonoOxigenases (LPMOs) on hydrolytic depolymerization is commonly ascribed to their ability to attack crystalline regions of the substrate creating new entry sites for glycosyl hydrolases. Variability in substrate specificity within LPMOs family members is known, while their preference among different substrate portion among crystalline and amorphous regions linked to their regioselective mechanism remains poorly characterised. Amorphous (PASC) and crystalline (CNC) cellulose are here used to investigate the constrains posed by the substrate ultrastructure on the activity of four different AA9 LPMOs. All the tested enzymes were active on CNC upon detection of oligosaccharides using HPAEC-PAD. However, X-ray diffraction pattern analysis was used to assess the deconstruction efficacy of the enzymatic treatments and revealed that high released of oligosaccharides did not corresponded to lower crystallinity on the remaining substrate. The effect upon crystallinity of two AA9 LPMOs were in the same degree of that caused by Expansin, a non-catalytic destructive enzyme. Finally, the substrate crystallinity degree did not affect the regioselectivity (C1 or C4) of the tested AA9 but impaired the secondary C4 oxidation in case of one double C1/C4 active AA9 LPMO.

Comparison of conventional carbon sources with dilute acid and steam treated DDGS on the cellulase and hemicellulase production by fungal strains

A. Iram and A. Demirci, PhD, Penn State University, State College, PA, USA; D. Cekmecelioglu, PhD, Middle East Technical University, Ankara, Turkey*

Distillers' dried grains with solubles (DDGS) is the byproduct of first-generation bioethanol production. It has high fiber content which can be used for hydrolytic enzyme production after pretreatment methods such as dilute acid hydrolysis or semi-continuous steam explosion. This study analyzes the effect of such treated DDGS samples with conventional carbon sources such as glucose, Avicel (crystalline cellulose) and untreated DDGS along with other cellulosic materials on the production of cellulases and hemicellulases by fungal strains such as *Aspergillus niger* and *Trichoderma reesei*. The results show that acid hydrolysis is a better treatment than untreated, and steam treated DDGS for both cellulase and hemicellulase production ($p<0.05$). Acid hydrolyzed DDGS has cellulase production similar to Avicel (0.23 IU/ml) but hemicellulase was lower in case of Avicel for two *A. niger* strains. The results show the significant positive effect of dilute acid treatment in making DDGS as the feedstock for hydrolytic enzyme production.

Water Retention Value as a Characterization Approach for Predictive Modeling of Corn Stover Deconstruction

W. Otto, Montana State University, Bozeman, MT, USA and D. Hodge, Montana State University, BOZEMAN, MT, USA*

Chemical and physical heterogeneity in herbaceous biomass feedstocks due to substantial differences in plant tissue types can contribute significant challenges to handling, preprocessing, and conversion in biorefining processes. A second, related challenge is quantifying this heterogeneity within feedstocks as it relates to processing in a biorefinery. Water sorption properties vary significantly with plant tissue and particle properties. In this work, we will apply several techniques for quantifying water-biomass interactions in combination with other characterization approaches for developing predictive models.

These models will predict relative abundance of anatomical fractions and response to processing by pretreatment and enzymatic hydrolysis of air-classified corn stover. One technique, water retention value (WRV) assay, is employed as a tool for screening biomass response to pretreatment and enzymatic hydrolysis in combination with other characterization approaches. We have further developed this assay to increase response sensitivity and identify the operating limits, while the impact of particle size, centrifugation speed and filter loading on WRV were explored.

Characterization of Carpenter Bee Bacterial Isolates for Biodegradation of Pulp Milling Waste

M. Cuebas-Irizarry[*] and A. Grunden, North Carolina State University, Raleigh, NC, USA; S. Sidhu, University of North Carolina, Chapel Hill, NC, USA

Complex polymers represent a challenge for environmental pollution as well as an opportunity for microbial catalyzed conversion to generate valorized chemicals. As an example, black liquor, a by-product from paper milling contains lignocellulose components but is typically burned for energy. Here, we are investigating biodegradation of the lignin and hemicellulose-derived compounds present in black liquor using bacterial strains isolated from carpenter bees. The isolates were identified to be be *Streptomyces spp.* by 16S rDNA sequencing. Growth was assessed for the isolates cultured in minimal media with the lignocellulose constituents (cellulose, xylan, and lignin) or black liquor (pulping waste) added as the only source of carbon. Filter paper deconstruction, dye decolorization assays, and % lignin reduction assays were used to determine the cellulose, hemicellulose, and lignin degradation potential of the isolates, respectively. Strain 2-6 and 2-10 were able to decolorize Congo Red by 60 and 50%, respectively, Tolouidine Blue by 100 and 70%, Remanzol Brilliant Blue by 30 and 32% and Bromocresol Green by 15 and 18% after one week incubation without the addition of a reaction mediator. Cellulose deconstruction experiments showed degradation of up to 30% of the filter paper within 10 days. Growth on lignin revealed that strain 2-6 could degrade up to 24% of the lignin mass within 30 days. Evaluation of enzymes activities that may participate in lignin deconstruction (e.g. laccases, peroxidases, etc.) have been conducted to provide additional insight into the potential of *Streptomyces spp.* isolate 2-6 and 2-10 for lignin degradation and dye decolorization.

3-hydroxypropionic acid metabolism in *Aspergilli*

K. Pomraning[*], Z. Dai, N. Munoz, Y.M. Kim, Y. Gao, S. Deng, J. Kim, B. Hofstad, T. Lemmon, M. Swita, K. Burnum-Johnson and J. Magnuson, Pacific Northwest National Lab, Richland, WA, USA

Biological engineering of microorganisms to produce value-added chemicals is a promising route to sustainable manufacturing. However, overproduction of metabolic intermediates at high titer, rate, and yield from inexpensive substrates is challenging in non-model systems where limited information is available regarding metabolic flux and its control in production conditions. Integrated multi-omic analysis of engineered strains offers an in-depth look at metabolites and proteins directly involved in growth and production of target and non-target bioproducts. Here we applied multi-omic analysis to overproduction of the polymer precursor 3-hydroxypropionic acid (3HP) in the filamentous fungi *Aspergillus pseudoterreus* and *Aspergillus niger*. Metabolic pathways involved in degradation of 3HP were identified, elimination of which improved the yield of 3HP dramatically.

Tuesday, April 27

8:10 AM - 9:00 AM Tuesday Keynote

8:10 AM Welcome.

8:20 AM Driving the Future: Development and Deployment of Advanced Biomass Conversion Technologies at the Joint BioEnergy Institute

B. Simmons, Lawrence Berkeley National Laboratory, CA, USA*

After earning his B.S. in chemical engineering from the University of Washington in 1997, Dr. Simmons continued his studies at Tulane University and received his doctorate in the same field in 2001. Dr. Simmons worked as part of the Senior Management team at Sandia National Laboratories for 15 years, most recently serving as the Senior Manager of Advanced Biomanufacturing as well as the Biomass Program Manager. He joined Lawrence Berkeley National Laboratory in February of 2016 as the Division Director of Biological Systems and Engineering. He is an Adjunct Professor at the University California-Berkeley and the University of Queensland in Australia. He also serves as the Chief Science and Technology Officer and Vice-President of the Deconstruction Division at the Joint BioEnergy Institute (www.jbei.org), a DOE Office of Science funded project tasked with the development and realization of next-generation “drop-in” biofuels and bioproducts produced from sustainable, non-food lignocellulosic biomass. He has over 350 peer-reviewed publications and his work has been featured in the New York Times, BBC, the Wall Street Journal, the San Francisco Chronicle, CNN, Fast Company, and the KQED televised science program Quest.

8:35 AM Q & A

8:40 AM Bioinspired functional protein-based materials for catalysis and beyond

C. Schmidt-Dannert, University of Minnesota, St. Paul, MN, USA*

In biological systems, proteins, nucleic acids and lipids are precisely organized to form higher ordered structures across multiple length scales. We believe that harnessing the principles and mechanisms underlying the assembly and organization of natural bionanomaterials offers tremendous opportunities for the design and scalable fabrication of functional biomaterials with emergent properties. Proteins and peptides provide the greatest versatility for the bottom-up design and low-cost production of such self-assembling supramolecular materials due to the chemical diversity of their amino acid building blocks. They are also genetically encoded, allowing for the genetically programmable production of self-organizing materials using cell factories or in the future, de novo via cell free expression systems. Proteins are also key players in the formation inorganic-organic composite materials with properties unmatched by synthetic properties. Inspired by the spatial organization of enzymes at the subcellular level via different types of protein nanostructures, we are taking advantage of these mechanisms for the design of self-assembling protein-based nano-architectures for different applications, including for *in vitro* biocatalysis and for the fabrication of new types of materials. Of key interest to us is the discovery and design of mechanism with which to interface protein-based materials with biomineralization processes for the production of innovative materials with unique mechanical and other properties. This presentation will discuss possibilities and examples for the design of genetically encoded biomaterials as functional and smart materials for applications, including e.g. for chemical synthesis, as functional coatings and living materials.

8:55 AM Q & A

9:00 AM - 10:00 AM Session: 5: New plastics from renewable monomers

Conveners: **Dr. Tanja Narancic**, University College, Dublin, Ireland and **Robert Allen**, National Renewable Energy Laboratory

9:00 AM Converting waste cooking oil to a biodegradable polymer

K. O'Connor, University College Dublin, Dublin, Ireland*

Humankind's appetite for polymers (plastics, foams, adhesives, fibres) is not abating with worldwide annual production expected to triple by 2050. The resources used to make polymers is a major challenge for society with current production reliant almost entirely on fossil based oil and gas, which is finite and depleting. Polymers can be be made from renewable biological resources such as cellulose, starch, sucrose. Emerging technologies can convert simple sugars into polymers that are chemically identical to fossil based polymers. However these drop in biologically equivalents of the fossil polymers are not biodegradable and this limits the end of life options for these materials where for example composting and anaerobic digestion (AD) are not suitable management tools. Using renewable resources to make biodegradable polymers addresses the start of life and opens up end of life options that allow materials to flow back to nature through compost/AD digestate. The use of waste resources further contributes to resource efficiency in a circular bioeconomy. Waste cooking oils (WCO) are interesting starting materials to make biological polymers called polyhydroxyalkanoates (PHAs). A number of studies have already investigated both virgin sources and waste sources of plant oils to make PHAs. However to date no studies have investigated WCO for high cell density and high PHA productivity. We developed a bioprocess to achieve high biomass and biopolymer (PHA) productivity from hydrolysed waste cooking oil. In fed batch (pulse feeding) experiments *Pseudomonas putida* KT2440 achieved a cell density of >159 g/l with just over a third of the biomass composed of PHA. We achieved a PHA volumetric productivity of >1.9 g/l/h, and a biomass yield of 0.76 g/g. The polymer produced is a medium chain length PHA that was amorphous and tacky with potential use as an adhesive.

9:08 AM Q & A

9:11 AM New Materials based on Biodegradable PHA

K. Brooks, DaniMer Scientific UGA, Athens, GA, USA*

With the current plastics crisis facing the world today, consumers and legislative bodies world-wide are requesting environmentally friendly alternatives to replace petroleum-based single use plastic packaging. Though several biobased alternatives have been presented in the market and in the literature, most of the materials have significant drawbacks, such as performance or end-of-life issues. Polyhydroxyalkanoates (PHAs) are the most promising alternative for petroleum-based plastics, offering diversity in material properties and more attractive end-of-life scenarios. At Danimer, we have developed a method of PHA production through fermentation and resin production through compounding using our patented extrusion process. With these materials and processes, we have been able to make a variety of materials for use in different applications, including straws, cutlery, and films, and these materials can be designed to be industrially compostable, marine biodegradable, and home compostable. These improved replacements offer the ability to address the plastics crisis and provide better alternatives to help protect our environment and planet.

9:19 AM Q & A

9:22 AM Urea as a Monomer for Non-Isocyanate Segmented Polyurea Copolymers

T. Long, Professor and J. Sintas, Student, Arizona State University, Biodesign Institute, Phoenix, AZ, USA; J. Wolfgang, Student, Virginia Tech, Blacksburg, VA, USA*

Biology provides an expanded toolbox of renewable monomers and polymers, and urea now serves as a monomer to enable the solvent-free and isocyanate-free synthesis of polyurea random semi-crystalline copolymers and segmented elastomeric copolymers. Water-soluble polyureas are amenable to enzymatic degradation, which suggests the feasibility of biodegradable agricultural films. Urea readily reacts with a

diversity of diamines and oligomeric diamines in the absence of solvent with the liberation of ammonia. The copolymers exhibit tunable thermomechanical performance and microphase separation that suggest utility as thermoplastic elastomers. Moreover, the synthetic method employs reactor technology that is well established for the melt phase polymerization of polyesters.

9:30 AM Q & A

9:33 AM Bio-upcycling of Polyethylene Terephthalate

T. Tiso and L.M. Blank, RWTH Aachen University, Aachen, Germany; T. Narancic, University College, Dublin, Ireland; R. Wei, University of Greifswald, Greifswald, Germany; E. Pollet and L. Averous, Strasbourg University, Strasbourg, France; S. Kenny, Bioplastech Ltd., Dublin, Ireland; R. Perrin, SOPREMA, Strasbourg, France; N. Wierckx, Institute of Bio and Geoscience, Jülich, Germany; W. Zimmermann, Leipzig University, Leipzig, Germany; K. O'Connor, University College Dublin, Dublin, Ireland*

Converting waste cooking oil to a biodegradable polymer
Humankind's appetite for polymers (plastics, foams, adhesives, fibres) is not abating with worldwide annual production expected to triple by 2050. The resources used to make polymers is a major challenge for society with current production reliant almost entirely on fossil based oil and gas, which is finite and depleting. Polymers can be be made from renewable biological resources such as cellulose, starch, sucrose. Emerging technologies can convert simple sugars into polymers that are chemically identical to fossil based polymers. However these drop in biologically equivalents of the fossil polymers are not biodegradable and this limits the end of life options for these materials where for example composting and anaerobic digestion (AD) are not suitable management tools. Using renewable resources to make biodegradable polymers addresses the start of life and opens up end of life options that allow materials to flow back to nature through compost/AD digestate. The use of waste resources further contributes to resource efficiency in a circular bioeconomy. Waste cooking oils (WCO) are interesting starting materials to make biological polymers called polyhydroxyalkanoates (PHAs). A number of studies have already investigated both virgin sources and waste sources of plant oils to make PHAs. However to date no studies have investigated WCO for high cell density and high PHA productivity. We developed a bioprocess to achieve high biomass and biopolymer (PHA) productivity from hydrolysed waste cooking oil. In fed batch (pulse feeding) experiments Pseudomonas putida KT2440 achieved a cell density of >159 g/l with just over a third of the biomass composed of PHA. We achieved a PHA volumetric productivity of >1.9 g/l/h, and a biomass yield of 0.76 g/g. The polymer produced is a medium chain length PHA that was amorphous and tacky with potential use as an adhesive.

9:41 AM Q &A

9:44 AM Construction of *Pseudomonas putida* cell factories for bioproduction of tailored biopolymers

M.T. Manoli and M. Auxiliadora Prieto, Biological Research Center Margarita Salas (CIB-CSIC), Madrid, Spain; M.P. Mezzina and P.I. Nikel, Systems Environmental Microbiology Group, The Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, Lyngby, Denmark*

Growing environmental worries resulted to an increased interest on the sustainable production of bio-based plastics that could replace the recalcitrant and petrochemical derived materials. Polyhydroxyalkanoates (PHAs) are naturally produced in a variety of bacteria and are non-toxic and biodegradable, attracting considerable attention as a starting point towards renewable and versatile materials. These bacterial polyesters are accumulated in the cell cytoplasm, traditionally under nutrient imbalanced conditions and act as carbon and energy reservoirs. *Pseudomonas putida* KT2440 is a Gram-negative model mcl-PHA producing bacterium well known for its metabolic versatility, resilience to stress and genetic amenability. Its PHA metabolic machinery involves multilevel regulatory circuits consisting of

interactions between polyester granules, enzymes including the granule-associated proteins (GAPs) and PHA-specific or global regulators. We have constructed a PHA-deficient chassis which lacks the native PHA regulatory system as the foundation for implementing an orthogonal PHA production strain based on *Cupriavidus necator*, *Rhodospirillum rubrum* and *Pseudomonas pseudoalcaligenes* PHA machineries. Implementing synthetic biology strategies, we rapidly constructed gene expression systems, containing multiple transcription units following the same brick structure, which enable interchangeability of genetic parts across modules. Different expression systems were tested and a combination of engineering approaches were adopted for identifying the best growth and PHA accumulation conditions with defined monomer composition. This work provides *P. putida* cell factories for the production of tailored biopolymers.

9:52 AM Q & A

9:00 AM - 10:00 AM Session: 6: Advanced biofuels

Conveners: **Scott Baker**, Pacific Northwest National Laboratory and **Aindrila Mukhopadhyay, VP, Biofuels and Bioproducts, JBEI**, Lawrence Berkeley National Laboratory, Berkeley, CA, USA

9:00 AM Stepping on the Gas to a Circular Economy: Accelerating Development of Carbon-Negative Chemical Production from Gas Fermentation

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

Gas fermentation technology offers a path to produce impactful volumes of sustainable products from abundant, low value renewable carbon feedstocks. LanzaTech is pioneering the commercialization of a gas fermentation process that allows the continuous production of sustainable fuels, chemicals and protein from renewable carbon resources at scale. Our first commercial plant in China has produced over 60,000 tons sustainable ethanol averting the emission of over 110,000 tons of CO_2. Further commercial plants are in design or under construction with the process having been demonstrated with waste gas from numerous industries and synthesis gas produced from agricultural and municipal waste sources. Commercial deployment activities will updated and partnerships with consumer-facing companies such as L'Oréal and the Mibelle Group using the ethanol as a "CarbonSmart™" intermediate platform for the production of goods including cleaning products, plastics for packaging, and fibers for clothing will be highlighted.

The development of a comprehensive synthetic biology capability for gas fermenting organisms has further broadened product opportunities. Acetone and isopropanol are important industrial bulk and platform chemicals, exclusively produced from fossil resources today. We have developed a sustainable and commercially relevant route from abundant, low-cost waste gas feedstocks by engineering the biocatalyst. To achieve this, we constructed and screened a combinatorial biosynthetic pathway library using genes derived from a historical industrial strain collection and enzyme engineering. To optimize flux, we performed strain engineering using omics analysis, kinetic modelling, and cell-free prototyping to identify competing interactions between heterologous enzymes and native metabolism. We developed and scaled up a continuous fermentation process in an industrial pilot plant, consistently demonstrating commercial production rates. Life cycle analysis confirmed significant (>165%) greenhouse gas savings. We show that acetogens, despite living on the edge of life, can be efficient cell factories for chemicals production.

9:08 AM Q & A

9:11 AM Towards zero net-emission biohybridfuels: hydroxy-fatty acids as platform molecules

L.M. Blank, RWTH Aachen University, Aachen, Germany*

The increasing availability of non-fossil energy opens unprecedented possibilities to re-design the interface of energy and material value chains towards a sustainable future. The fundamental research in the Cluster of Excellence "The Fuel Science Center – Adaptive Conversion Systems for Renewable Energy and Carbon Sources" (FSC) aims to integrate renewable electricity with the joint utilization of bio-based carbon feedstocks and CO_2 to provide high-density liquid energy carriers ("bio-hybrid fuels"), which enable innovative engine concepts for highly efficient and clean combustion.
In this context, the efficient production of OH-fatty acid esters as platform molecules for the synthesis of advanced biofuels, including bio-hybrid fuels will be presented. The tailoring of the carbon chain length of the OH-fatty acid esters opens design options for the use in combustion engines. The contribution to a close to net zero-emission mobility will be discussed.

9:19 AM Q & A

9:22 AM High titer methyl ketone production with tailored *Pseudomonas taiwanensis* VLB120

S.C. Nies, T.B. Alter and L.M. Blank, RWTH Aachen University, Aachen, Germany; B.E. Ebert, The University of Queensland, Brisbane, Australia*

Methyl ketones (MKs) present a group of platform chemicals industrially produced by oxidation of petroleum-derived hydrocarbons. MKs find applications in the fragrance, flavor, pharmacological, and agrochemical industries and are discussed as biodiesel blends. Microbial production can be a more sustainable alternative and has been enabled in various microbes by re-engineering the fatty acid metabolism. One challenge in the development of highly productive processes is the high demand for reducing power. Here, we tested the potential of *Pseudomonas taiwanensis* VLB120 for MK production as this microbe has been proven to sustain exceptionally high NAD(P)H regeneration rates. The implementation of reported metabolic engineering strategies resulted in 540 mg MKs L^{-1}_{aq} in batch fermentation. We further boosted MK production by the removal of competing reactions predicted by metabolic modeling. The introduction of two computed gene deletions increased the MK production to 70 g L^{-1} in the in-situ organic extractant phase (corresponding to 9.8 g L^{-1} in the aqueous phase). It boosted the yield to 67 % of the theoretical maximum. This represents a 4-fold improvement of the product titer compared with the first-generation strain and is the highest titer of recombinant produced MKs reported to date. This study emphasizes the high potential of *P. taiwanensis* VLB120 to produce methyl ketones and model-guided metabolic engineering to rationalize and accelerate strain optimization efforts.

9:30 AM Q & A

9:33 AM Cost and greenhouse gas implications of advanced bio-jet fuel production

N. Baral, PhD, M. Yang, PhD and C. Scown, PhD, Lawrence Berkeley National Laboratory, Berkeley, CA, USA*

Decarbonizing the air transportation sector remains one of the most challenging hurdles to mitigating climate change. Lignocellulosic biomass-derived jet fuel blendstocks can contribute to the shift toward renewable, low-carbon energy sources for aircrafts. Achieving blends suitable for completely replacing Jet-A requires a not just paraffins, but naphthenes as well. Advanced biological routes offer the potential to produce more energy-dense fuel blendstocks that fill an important role not satisfied by most bio-derived jet fuel blendstocks on the market currently, such as Hydroprocessed Esters and Fatty Acids (HEFA), and

provide advantages in aircraft weight and range relative to petroleum-derived fuels. We explore six different promising bio-jet fuel molecules produced through microbial routes and catalytic upgrading, and quantify the minimum selling price of these blendstocks and their life-cycle greenhouse gas (GHG) footprints. We also explore the impacts of metrics from bench scale research, including titer, rate, and yield, on the eventual economics and GHG footprint at the commercial scale. We find that, when fully optimized, these fuel production pathways do not reach parity with current Jet-A prices but can be competitive with modest credits for GHG mitigation that are well within the range of policy incentives offered in parts of the U.S.

9:41 AM Q &A

9:44 AM Short term adaptation in *Saccharomyces cerevisae* - a key to efficient lignocellulose fermentation

L. Olsson, M. van Dijk, P. Rugbjerg and Y. Nygård, Chalmers university, goteborg, Sweden*

The limited tolerance of *Saccharomyces cerevisiae* to inhibitors is a major challenge in second-generation bioethanol production, and our understanding of the molecular mechanisms providing tolerance to inhibitor-rich lignocellulosic hydrolysates is incomplete. Short-term adaptation of the yeast in the presence of dilute hydrolysate can improve its robustness and productivity during subsequent fermentation. We utilized RNA sequencing to investigate differential gene expression in the industrial yeast strain CR01 during short- term adaptation, mimicking industrial conditions for cell propagation. In this first transcriptomic study of short-term adaption of S. cerevisiae to lignocellulosic hydrolysate, we found that cultures respond by fine-tuned up- and down-regulation of a subset of general stress response genes. Furthermore, time-resolved RNA sequencing allowed for identification of genes that were differentially expressed at 2 or more sampling points, revealing the importance of oxidative stress response, thiamin and biotin biosynthesis. furan-aldehyde reductases and specific drug:H+ antiporters, as well as the down-regulation of certain transporter genes. In this presentation we will discuss, the molecular mechanisms governing short-term adaptation of *S. cerevisiae* to lignocellulosic hydrolysate, and suggest new genetic targets for improving fermentation robustness

9:52 AM Q & A

10:00 AM - 11:00 AM Session: 7: Speeding up synthetic biology

Conveners: **Jay Fitzgerald**, Department of Energy (DOE) - Bioenergy Technologies Office and **Nathan Hillson**, Lawrence Berkeley National Laboratory

10:00 AM Guiding synthetic biology via machine learning

T. Radivojevic, Lawrence Berkeley National Laboratory, Berkeley, CA, USA*

One of the most important challenges in bioengineering is effectively using -omics data to guide metabolic engineering towards higher production levels. Here, we present the Automated Recommendation Tool (ART), a tool that leverages machine learning and probabilistic modeling techniques to guide synthetic biology in a systematic fashion, without the need for a full mechanistic understanding of the biological system. ART provides a set of recommendations for the next engineering cycle, alongside probabilistic predictions of their outcomes. It can be used as a python library or through a web-based graphical frontend that does not require coding expertise.

10:08 AM Q & A

10:11 AM Cell-free prototyping and rapid optimization of biosynthetic enzymes for cellular design

M. Jewett, Northwestern University, Evanston, IL, USA*

The design and optimization of biosynthetic pathways for industrially relevant, non-model organisms is challenging due to transformation idiosyncrasies, reduced numbers of validated genetic parts, and a lack of high-throughput workflows. Here we describe a platform for in vitro prototyping and rapid optimization of biosynthetic enzymes (iPROBE) to accelerate this process. In iPROBE, cell lysates are enriched with biosynthetic enzymes by cell-free protein synthesis and then metabolic pathways are assembled in a mix-and-match fashion to assess pathway performance. In this presentation, I will describe the use of cell-free systems for optimizing the biosynthesis of 3-hydroxybutyrate, acetone, and products from reverse beta oxidation. We then show how iPROBE selected pathways can be used in *Clostridium autoethanogenum* to produce bioproducts solely from carbon waste gas. We expect iPROBE to accelerate design–build–test cycles for industrial biotechnology.

10:19 AM Q & A

10:22 AM Finding the needle in the haystack: leveraging scale for enzyme discovery and optimization

E. Wrenbeck, Head of Protein Engineering, Ginkgo Bioworks, Boston, MA, USA*

At Ginkgo Bioworks our mission is to make biology easier to engineer. To support this mission, we leverage the power of scale in enzyme discovery and optimization campaigns via software, automation, deep knowledge of enzyme families, and protein engineering expertise. This is motivated by the fact that we apply enzymes, nature's exquisite chemical reaction catalysts, to an ever more diverse range of biotechnological applications, often needing to push beyond the functional limits of naturally occurring enzymes. The focus of this presentation will center on the infrastructure of Ginkgo Bioworks's Foundry that simultaneously enables enzyme engineering workflows that are both flexible – in that they support a highly diverse range of assay types and readouts – and large scale – in that they support the routine assay of thousands of unique protein variants per experiment. Case studies will be presented to highlight the delivery of optimized enzymes in low numbers of Design-Build-Test-Learn cycles enabled by this infrastructure.

10:30 AM Q & A

10:33 AM DAMP Lab: Services, Workflows, and Infrastructure for Remote Synthetic Biology

D. Densmore, Boston University, Boston, MA, USA and S. MD Oliveira, DAMP Lab @ Biological Design Center*

In this talk I will outline the current state of the "Design, Automation, Manufacturing, and Prototyping" (DAMP) Lab at Boston University. This includes a software infrastructure for the execution of numerous microbiology services, the analysis of the performance of these services, and a direct connection to the physical fabrication of both DNA and low-cost microfluidics from high level specifications. Also discussed is how to disseminate standardized protocols and liquid handling routines. Current and past projects will be put in the context of how they utilized these services.

10:41 AM Q &A

10:00 AM - 11:00 AM Session: 8: Enzyme discovery and engineering for biomass deconstruction and biofuels and chemical production

Conveners: **Michelle O'Malley**, University of California-Santa Barbara, CA, USA and **Kevin Solomon**, University of Delaware, DE, USA

10:00 AM Shedding light on the function and potential application of microbial expansin-related proteins

M. Monschein, E. Ioannou, T. Koitto and D. Dahiya, Aalto University, Espoo, Finland; E. Master, University of Toronto, Toronto, ON, Canada*

Biotechnologies used for biomass processing have mainly focused on enzymes for biomass deconstruction to commodity fuels and chemicals. By contrast, biotechnologies that upgrade intact biomass structures for more resource-efficient applications are critically needed. Our team is investigating the untapped potential of fungal loosenin-like proteins, and other microbial expansin-related proteins, to control the flexibility and porosity of lignocellulosic materials to permit their broader application in sustainable textiles. In addition to introducing fungal loosenin-like proteins, this presentation will describe the complementary functional screens developed by our team to evaluate the action of fungal loosenins and other microbial expansin-related proteins.

10:08 AM Q & A

10:11 AM Predicting Rates of Cellulose Hydrolysis by Cellulases from Elementary Mechanisms

T. Jeoh, J. Nill, A. Hitomi and L. Knowles, University of California, Davis, Davis, CA, USA*

Despite over seventy years of research, the precise mechanisms by which cellulase enzymes completely breakdown cellulose have been elusive. While recent studies have revealed significant new insights into hydrolysis rate-limiting cellulase-cellulose interactions, long-term cellulose hydrolysis rates have remained challenging to predict. We recently proposed a breakthrough concept that cellulose is not the substrate for cellulase enzymes. Rather, ‘productive binding sites’, i.e. sites on cellulose where cellulases can engage its active site to form active enzyme-substrate complexes are the true substrate of cellulase enzymes. The productive binding capacity of a cellulosic substrate, i.e. the number (moles) of productive cellulase binding sites per mass of cellulose, is a measureable property of the material. We demonstrate that the initial productive binding capacity of a cellulosic substrate limits initial hydrolysis rates and the depletion of available productive binding sites ultimately limits long-term hydrolysis rates. The processing history (e.g. thermochemical pretreatment or wet/dry storage) and origin (e.g. bacterial v. algal) can influence both initial and long term availability of productive binding sites. By incorporating time-dependent productive binding site concentrations with elementary cellulase-productive binding site interactions, we have simulated experimental hydrolysis rates of several cellulosic substrates. Our current work continues to investigate how to maximize productive binding sites on cellulose, and how to predict the availability of productive binding sites during hydrolysis.

10:19 AM Q & A

10:22 AM Discovery of novel aromatic catabolic enzymes from non-model bacteria

G. Presley, M. Allemann, D. Garcia, R. Giannone, J.G. Elkins and J. Michener, Oak Ridge National Laboratory, Oak Ridge, TN, USA; A. Werner, R. Katahira, S. Haugen, K. Ramirez and G. Beckham, National Renewable Energy Laboratory, USA, Golden, CO, USA*

In a typical biorefinery, biomass-derived sugars are fermented to fuels by microorganisms, while residual lignin is burned for process heat. Converting waste lignin into value-added bioproducts offers a potential source of additional revenue to improve the economics of biofuel production. While bacteria have been isolated and engineered to catabolize lignin-derived compounds, economically viable lignin valorization will require further work to identify and optimize pathways for assimilation and conversion of diverse lignin-derived aromatic compounds. We have discovered and characterized multiple novel pathways for assimilation of lignin-derived compounds in the non-model bacterium *Novosphingobium aromaticivorans*, including syringate, guaiacol, and β-1 aromatic dimers. We have also demonstrated that this strain has latent catabolic capabilities that can be activated through mutagenesis, enabling the search for additional enzymes and pathways. Finally, to further broaden the repertoire of potential catabolic pathways, we have isolated and characterized environmental bacterial isolates that degrade a variety of aromatic substrates but lack known catabolic pathways. In combination, these efforts will expand the classes of lignin-derived aromatic compounds that can be converted by engineered microbes as well as provide alternative pathways to aid in strain optimization.

10:30 AM Q & A

10:33 AM Engineering the thermotolerant yeast *K. marxianus* for the conversions of biomass-derived sugars into valuable fuels and chemicals.

I. Wheeldon, University of California, Riverside, Riverside, CA, USA*

A central challenge in chemical bioprocessing is matching the right microbial production host with the right process. Part of the difficulty in doing so is that often a valuable trait exists in a host that is difficult to engineer or lacks sophisticated metabolic engineering tools to improve strains and optimized titers, rates, and yields. In this talk, we advance an argument for the yeast *Kluyveromyces marxianus* as a next-generation microbial host for the conversion biomass-derived sugars into biofuels and biochemicals. *K. marxianus* has a natural capacity to grow at temperatures upward of 50 °C and is commonly described as the fastest growing eukaryote, traits that can benefit high temperature, high rate bioprocesses. The ability to metabolize a range of C5, C6, and C12 sugars, as well as organic acids also makes it well-matched with biomass-derived feedstocks. To better exploit these phenotypes, we have created a set of genome editing technologies, functional genomic screens, standardized promoters, and inducible gene regulation switches. These new metabolic engineering tools have allowed us to create strains that produce high titers of 2-phenylethanol, a valuable flavor and fragrance compound. We have also understood and enhanced the natural ability of *K. marxianus* to produce grams per liter quantities of the short chain volatile ester ethyl acetate. Finally, we show the ability of wild type and engineered strains to grow on sugars extracted from lignocellulosic biomass by fungal cellulases. Together, these new tools and examples of their application in metabolic engineering demonstrate that *K. marxianus* is a viable host for bioprocessing of biomass-derived sugars.

10:41 AM Q &A

Wednesday, April 28

8:00 AM - 9:00 AM Award Presentations and Keynote

8:00 AM Closing Remarks

8:15 AM Awards and Acknowledgement

8:30 AM Break

8:35 AM Metabolic Engineering of Yeast

J. Nielsen, BioInnovation Institute Foundation (Denmark) & Chalmers University of Technology, Denmark*

Metabolic Engineering relies on the Design-Build-Test cycle. This cycle includes technologies like mathematical modeling of metabolism, genome editing and advanced tools for phenotypic characterization. In recent years there have been advances in several of these technologies, which has enabled faster development of metabolically engineered strains that can be used for production of fuels and chemicals, but it is still challenging to perform efficient design. There is therefore in particular a need for advancing our ability to model metabolism, and this can be achieved through integration of systems biology tools. The yeast *Saccharomyces cerevisiae* is widely used for production of fuels, chemicals, pharmaceuticals and materials. Through metabolic engineering of this yeast a number of novel industrial processes have been developed over the last 10 years. In the lecture I will give some recent examples of metabolic engineering of yeast that are in the process of being translated into novel bioprocesses.

8:50 AM Q&A

9:00 AM - 10:00 AM Session: 10: Lignin upgrading

Conveners: **Davinia Salvachua**, National Renewable Energy Laboratory, Golden, CO, USA and **Lindsay Eltis**, University of British Columbia, Vancouver, BC, Canada

9:00 AM Elucidation and engineering of an S-lignin catabolic pathway in Pseudomonas putida KT2440

A. Werner, S. Notonier, E. Kuatsjah, C. Hoyt, A. Amore, K. Ramirez, S. Woodworth, C. Johnson and G. Beckham, National Renewable Energy Laboratory, USA, Golden, CO, USA; L. Dumalo and L. Eltis, University of British Columbia, Vancouver, BC, Canada; P. Abraham, E. Hatmaker, D. Klingeman, R. Giannone, A. Guss and R. Hettich, Oak Ridge National Laboratory, Oak Ridge, TN, USA*

Biological funneling using microbial biocatalysts has emerged as an attractive approach to convert complex mixtures of chemicals from lignin depolymerization to value-added compounds. Ideal biocatalysts will convert aromatic compounds derived from all three of the canonical types of lignin: syringyl (S), guaiacyl (G), and *p*-hydroxyphenyl (H). *Pseudomonas putida* KT2440 (*P. putida*) has been developed as a biocatalyst for lignin valorization owing in part to its native aromatic catabolic capabilities. Here, we first elucidate a catabolic pathway for syringate, a common S-type lignin-derived compound, and then leverage this pathway for simultaneous conversion of S, G, and H-type monomers to 2-pyrone-4,6-dicarboxylate (PDC), a promising bio-based chemical. Specifically, we find syringate is utilized by *P. putida* in the presence of an auxiliary energy source or when *vanAB* is overexpressed. Biochemical characterization of VanAB, a two-component monooxygenase, confirms *O*-demethylation of syringate and 3-*O*-methylgallate to produce gallate, which is further catabolized via extradiol cleavage. We then employ VanAB from *Pseudomonas* sp. HR199 and LigABC from *Sphingobium* sp. SYK-6 to enable conversion of syringate (S), *p*-coumarate (H), and ferulate (G) to PDC at a 93% mol/mol yield, demonstrating the potential of *P. putida* as a robust chassis for lignin valorization.

9:08 AM Q & A

9:11 AM Elucidating metabolic and regulatory networks of microbial aromatic utilization for lignin valorization

T.S. Moon, Washington University in St. Louis, St. Louis, MO, USA*

Lignin represents a renewable resource whose bioconversion could displace petroleum-based processes (1). Research has been conducted to develop a hybrid platform to generate value-added bioproducts from lignin breakdown products (LBPs) obtained by thermo-catalytic depolymerization of waste lignin (2). Traditional model organisms are not well suited for converting LBPs that consist of various toxic aromatic compounds, but non-model organisms have been identified as ideal candidates. One such host is *Rhodococcus opacus*, which has demonstrated high native tolerance to LBPs and the ability to improve its tolerance and consumption through adaptive evolution. However, the key challenges in such lignin upgrading include our limited understanding of microbial utilization of toxic LBPs at gene levels and limited tools to engineer this organism. To understand and maximize its metabolic potential, we have employed multi-omics approaches, providing a systems-level understanding of the complex metabolism of the wild-type and evolved strains (3-5). Additionally, we have developed a genetic toolbox for *R. opacus* engineering (6-8). Despite recent advances in our understanding of its versatile metabolism and available genetic tools, studies of its gene functions at gene levels are still lagging. In this talk, I will discuss our recent efforts to facilitate functional studies of this non-model organism using our genetic toolbox and engineer this promising chassis for lignin valorization.

1. Davis K, Moon TS. 2020. *Current opinion in chemical biology* 59:23-9
2. Chatterjee A, DeLorenzo DM, Carr R, Moon TS. 2020. *Current opinion in biotechnology* 64:10-6
3. Henson WR et al. 2018. *Metabolic engineering* 49:69-83
4. Yoneda A et al. 2016. *Nucleic Acids Res.* 44:2240–54
5. Roell GW et al. 2019. *Metabolic engineering* 55:120-30
6. DeLorenzo DM, Henson WR, Moon TS. 2017. *ACS Synthetic Biology* 6:1973–8
7. DeLorenzo DM, Moon TS. 2019. *ACS Synthetic Biology* 8:1921-30
8. DeLorenzo DM, Rottinghaus AG, Henson WR, Moon TS. 2018. *ACS Synthetic Biology* 7:727-38

9:19 AM Q &A

9:22 AM Bacterial transformation of monomeric aromatic compounds of black liquor

L. Navas, J. Liu, G. Dexter, W. Mohn and L. Eltis, University of British Columbia. Department of Microbiology and Immunology, Vanocuver, BC, Canada; M. Cho, S.K. Jang, S. Mansfield and S. Renneckar, University of British Columbia. Department of Wood Science, Vancouver, BC, Canada*

Lignin is a major component of biomass and its sustainable valorization is essential to the success of next generation biorefineries. Herein, we identified the monoaromatic compounds present in black liquor, an under-utilized, lignin-rich stream generated in the kraft pulping process, and their bacterial transformation. Among four tested solvents, acetone extracted the greatest amount of monoaromatic compounds from a softwood black liquor, with guaiacol, vanillin and acetovanillone present in approximately a 4:3:2 ratio. 4-Ethanol guaiacol (4EG), vanillate, and 4-propanol guaiacol were also present in significant quantities. A number of bacterial strains grew on minimal media supplemented with the black liquor extracts to 1 mM aromatic compounds, including *Pseudomonas putida* KT2442, *Sphingobium* sp. SYK-6, and three strains of *Rhodoccocus rhodochrous*: EP4, GD01, and GD02. Interestingly, the extracts inhibited the growth of *R. jostii* RHA1 and *R. opacus* PD630, two rhodococcal strains studied to valorize lignin. Of the five strains that grew on the extracts, only GD01 and GD02 depleted the six major monoaromatic compounds in the extract. This result was replicated using a mixture of the six compounds and was consistent with the known and predicted catabolic capabilities of each strain. Thus, KT2442 completely consumed vanillin and vanillate, and some of the 4EG. SYK-6 did not degrade guaiacol or 4EG, and EP4 consumed all the compounds except acetovanillone. RT-qPCR analysis of GD02 growing on a mixture of the black liquor compounds identified the relevant catabolic pathways. Overall, our results establish that bacteria are able

to catabolize the major monoaromatic components of black liquor, help define the catabolic capabilities of the different strains, and facilitate the design of biocatalysts to valorize under-utilized components of industrial lignin streams.

9:30 AM Q & A

9:33 AM Engineering *Pseudomonas putida* for Efficient Aromatic Conversion to Bioproduct Using High Throughput Screening in a Bioreactor

A. Mukhopadhyay, VP, Biofuels and Bioproducts, JBEI, Lawrence Berkeley National Laboratory, Berkeley, CA, USA*

Pseudomonas putida KT2440 is an emerging biomanufacturing host amenable for use with renewable carbon streams including *para*-coumarate, a model lignin-derived aromatic. We used a pooled transposon library to characterize this microbe under common stirred-tank bioreactor parameters with quantitative fitness assays. Assessing differential fitness values by monitoring changes in mutant strain abundance identified several genes with improved fitness across multiple stir-tank bioreactor formats. Selected genes from this subset were reconstructed and evaluated for conversion of *para*-coumarate, to a heterologous bioproduct, indigoidine. Several mutants improved fitness in a bioreactor by 35 fold and showed an 8-fold improvement in indigoidine production from *para*-coumarate as the carbon source. Development of aromatic utilization strains that have high fitness in bioreactors as well as high conversion performance to downstream products are ideal candidates for lignin valorization.

9:41 AM Q & A

9:44 AM Production of itaconic acid from alkali pretreated lignin by dynamic two stage bioconversion

J. Elmore, Pacific Northwest National Laboratory, Richland, WA, USA; G. Dexter, J. Martinez-Baird, G. Peabody, E. Hatmaker, J. Huenemann, D. Klingeman and A. Guss, Oak Ridge National Laboratory, Oak Ridge, TN, USA; D. Salvachua, D. Peterson, C. Singer and G. Beckham, National Renewable Energy Laboratory, Golden, CO, USA*

Expanding the portfolio of products that can be made from lignin will be critical to enabling a viable bio-based economy. Here we engineer *Pseudomonas putida* for high-yield production of the tricarboxylic acid cycle-derived building block chemical, itaconic acid, from model aromatic compounds and aromatics derived from lignin. We develop a nitrogen starvation-detecting biosensor for dynamic two-stage bioproduction in which itaconic acid is produced during a non-growth associated production phase. Through the use of two distinct itaconic acid production pathways, the tuning of TCA cycle gene expression, deletion of competing pathways, and dynamic regulation, we achieve an overall maximum yield of 56% (mol/mol) and titer of 1.3 g/L from *p*-coumarate, and 1.4 g/L titer from monomeric aromatic compounds produced from alkali-treated lignin. We also demonstrate that engineered *Pseudomonas putida* can produce itaconic acid using a diverse range of "waste-stream" carbon sources. This work illustrates a proof-of-principle that using dynamic metabolic control to reroute carbon after it enters central metabolism enables production of valuable chemicals from lignin at high yields by relieving the burden of constitutively expressing toxic heterologous pathways.

9:52 AM Q &A

9:00 AM - 10:00 AM Session: 9: Biomaterials

Conveners: **Yasuo Yoshikuni**, DOE Joint Genome Institute and **Seema Singh**, Sandia National Laboratories

9:00 AM Biological Synthesis of Ultrahard Magnetic Composite Materials in a Marine Organism

D. Kisailus, Ph.D.[*] and T. Wang, University of California, Irvine, Irvine, CA, USA; M. Nemoto, Okayama University, Okayama, Japan

Natural systems have evolved efficient strategies to synthesize composites from a limited selection of available materials that often exhibit exceptional mechanical properties that are frequently superior to those exhibited by engineering materials. These systems have accomplished this by establishing controlled synthesis and hierarchical assembly of nano- to micro-scaled building blocks. This requires organic that is used to transport mineral precursors to organic scaffolds, which not only precisely guide the formation and phase development of minerals, but also significantly improve the mechanical performance of otherwise brittle materials.

Here, we investigate the formation of heavily crystallized radular teeth the chitons, a group of elongated mollusks that graze on hard substrates for algae. From the investigation of synthesis-structure-property relationships in these unique organisms, we are now developing and fabricating multifunctional engineering materials for energy conversion and storage.

9:10 AM Q & A

9:15 AM Bacterial Nanofactories for Biomineralization of Metals

C. Grant, S. Krishnapura and A. Komeili[*], University of California, Berkeley, Berkeley, CA, USA; M. Amor, Aix-Marseille University, CEA Cadarache, France

Biomineralization of metals is a widespread phenomenon across the tree of life. In the bacterial world, biomineralization serves a variety of purposes ranging from storage to detoxification of metals. While many bacterial biominerals have been observed through electron microscopy, the molecular and cellular basis for their formation remains unresolved. Here, I present our recent findings that intracellular lipid-based organelles are central to accumulation and mineralization of iron in diverse bacterial species. I will focus on our discovery of ferrosomes which are a broadly conserved organelle for storage of iron under anaerobic conditions. By defining the molecular pathways of ferrosome formation, we have developed an easily transferrable genetic module for storage of iron in lipid-based vesicles. Further engineering of these nanofactories holds promise for biomining of metals and production of novel biomaterials.

9:25 AM Q & A

9:30 AM Programming Bacteria to Grow into Macroscopic Multi-Functional Materials

C.M. Ajo-Franklin[*], S. Molinari and R.F. Tesoriero, Jr, Rice University, Houston, TX, USA

Engineered living materials (ELMs) are emerging technologies that aim to mimic many capabilities of natural biomaterials, while also introducing tailorable non-native functions. Most examples of macroscopic ELMs have introduced novel properties onto naturally-occurring biological materials. However, programming macroscopic material formation de novo from a model organism would offer much greater, user-defined control over material structure and function. In my talk, I will describe how we programmed *Caulobacter crescentus* to grow into a macroscopic, multifunctional material. We hypothesized that we could self-assemble individual cells into a material if the individual cells bound to each other through many, relatively weak interactions. To encode a high density of interacting proteins on the surface of *C. crescentus*, we replaced the crystallization domain of RsaA with a hydrogel-forming polypeptide that is

known to weakly interact with itself. As *C. crescentus* expressing this RsaA-hydrogel fusion protein grows, the cells assemble into a pellicle at the air-water interface. This layer expands in thickness and ultimately sinks into the media to form free-floating, macroscopic materials dubbed ‘cocoons.’ As expected, the cocoons are predominantly composed of cells are bound to each other through the hybrid hydrogel-RsaA protein. Surprisingly, we also observed the RsaA-hydrogel fusion proteins dissociate from the cell surface to form long ~2 micron-thick filaments, which bundle into ~100-micron-thick fibers running throughout the cocoon. Within a cocoon, *C. crescentus* cells remain viable and can re-seed growth of new cocoons even after desiccation. Cocoons can also be processed into different 3D shapes and can be mixed with inorganic materials to form inorganic-organic composites. Taken together, this work provides design rules and a technological platform for programming bottom-up assembly of cells into hierarchically-structured, multi-functional living materials.

9:38 AM Q & A

9:43 AM Living Building Materials: Using Bacteria to Enhance Mechanical Performance

J. Artier, J. Qiu, S. Cook, W.V. Srubar III, M.H. Hubler and J.C. Cameron, University of Colorado Boulder, Boulder, CO, USA*

Living building materials (LBMs) are produced by integrating viable microorganisms into inert structural scaffolds. We developed a novel LBM composed of sand, hydrogel (gelatin) for binding aggregates, and bacteria that induced calcium carbonate precipitation and showed that the biological properties enhance material compression strength and flexural energy. Our framework takes advantage of microbially induced calcium carbonate precipitation (MICP) and has potential for recycling and self-healing, features that depends on the microorganism survival under diverse conditions. By introducing a desiccation protectant, the small sugar trehalose, LBMs cells were able to survive in the dried state for several days, with no negative effect on mechanical properties. Comparison between biotic and abiotic MICP show greater structural properties, especially superior fracture energy for biotic MICP samples. Both autotrophic and heterotrophic bacterial MICP pathways were successful in mechanically enhancing the LBM. We also tailored several LBM design factors in an effort to expand the multifunctionality of our building materials. This study shows that LBMs are an exciting new class of materials with a range of features that can be tailored for adaptation to the in-service application.
This project is funded by DARPA: Engineering Living Materials Program.

9:53 AM Q &A

10:00 AM - 11:00 AM Session: 11: Waste valorization

Conveners: **Violeta Sanchez i Nogue**, National Renewable Energy Laboratory, USA and **Steve Singer**, Lawrence Berkeley National Lab

10:00 AM Engineering Microbial Consortia from the Herbivore Rumen for Waste Valorization

M. O'Malley, University of California-Santa Barbara, CA, USA*

Anaerobic microbes work together in complex communities that decompose and recycle carbon biomass throughout the Earth – from our guts to landfills and compost piles. Despite their importance, little information exists to parse the role of each microbial member within their dynamic community. To address these knowledge gaps, we pioneered new techniques to isolate anaerobes from biomass-rich environments (e.g. guts and fecal materials of herbivores), characterize their shared metabolism, and build synthetic microbiomes to drive biomass to renewable chemicals. Herbivore fecal samples were

challenged by different types of biomass during cultivation to identify important microbial partnerships; 10 billion metagenomic reads spread across 402 enrichment samples tracked biological diversity as the cultures converged to a set of stable microorganisms. Nearly 200,000 carbohydrate active enzymes were identified from the fecal samples, and 724 genomes were assembled for previously uncultured microbes. Surprisingly, consortia dominated by anaerobic fungi generated more than twice the amount of methane compared to prokaryotic consortia, suggesting that fungi accelerate biomass breakdown and methane release in herbivores. Overall, our analysis points to natural compartmentalization between anaerobes as a means to degrade crude biomass, which can be exploited to harness nature's microbes for sustainable chemical production using synthetic systems.

10:08 AM Q & A

10:10 AM Valorization of high-strength organic wastewater streams by arrested methanogenesis

M. Urgun-Demirtas, H. Wu, R. Dalke, J. Mai and P. Thai, Argonne National Laboratory, Lemont, IL, USA*

The potential of arrested methanogenesis (AM) to treat high-strength wastewater while producing carboxylic acid as a valuable bioproduct was investigated. High titer, yield and productivity of carboxylic acids from complex organic waste streams have been challenging due to lack of resiliency and robustness of microbial community structures and product and salt toxicity. To overcome these challenges, we selectively established a resilient and robust acidogenic consortium from saline soil, yogurt, kefir and probiotics to regulate metabolism towards sustainable organic production. A blend stream of cheese whey wastewater and brewery wastewater at high COD concentration (>70 g/L) was treated by AM to produce high organic acid concentrations at bench-scale (0.5 liter) and pilot-scale (14 liter) fermenters. Experimental results show that AM technology has the potential to be scaled up to field-scale applications.

10:18 AM Q & A

10:20 AM Production of volatile fatty acids from lignocellulose using anaerobic fungal-bacterial consortia

C. Lawson, Lawrence Berkeley National Laboratory, Emeryville, CA, USA*

Anaerobic microbial communities sourced from the rumen and anaerobic digesters can be harnessed to sustainably produce short and medium-chain fatty acids from lignocellulosic and waste feedstocks. However, detailed knowledge of the interwoven metabolic networks controlling feedstock deconstruction and conversion is missing, and strategies for optimizing volatile fatty acid yields and chain-length selectivity are limited. Here, we enrich parallel fungal and bacterial microbiomes sourced from rumen and digester sludge inocula that anaerobically convert lignocellulose into volatile fatty acids via chain elongation. We reconstruct the metabolism and interactions of key organisms involved in lignocellulose deconstruction and conversion using metagenomic and metatranscriptomic analysis. Subsequently, we develop a novel approach that integrates high-throughput screening with machine learning to optimize fermentation conditions for improving volatile fatty acid production. Our results offer detailed insights on the metabolic networks and activity of uncultivated fungi and bacteria in anaerobic microbiomes and presents a systematic approach for optimizing microbiome function. These results are used to develop strategies for constructing synthetic microbiomes for converting lignocellulose and wastes to biofuels and bioproducts.

10:28 AM Q & A

10:30 AM Composition of inoculum microbiome impacts fatty acids produced from cellulose

S. De Long, PhD, K. Reardon, PhD and J. Rico, M.S.E., Colorado State University, Fort Collins, CO, USA*

Conversion of organic wastes to fatty acids rather than methane through anaerobic digestion-based technologies has considerable promise. However, the relationships between microbiome structure and the specific fatty acids produced from cellulosic feedstocks are not well understood. This study investigated the nature of those relationships for three inoculum sources – anaerobic digester sludge, bison rumen, and cattle rumen – grown on cellulose. To promote fatty acid accumulation, methanogenesis was inhibited with iodoform. By comparing fatty acid concentrations, production rates and proportions for the different inocula, and tracking the relative abundance of microbial taxa present via 16S rRNA gene amplicon sequencing, statistical analyses could be used to associate certain taxa with production of specific acids. Acetic acid production was highest in anaerobic sludge reactors, while propionic acid production was highest in cattle rumen reactors. Butyric and pentanoic acid were produced at low levels (<300 mg/L) by all inocula but were produced at the highest rates in bison rumen before Day 5. Reactor microbiomes remained phylogenetically distinct over time among the inoculum sources, despite identical operating conditions. Fatty acids produced correlated with higher relative abundance of expected taxa (e.g., *Clostridium*, *Bacteroides*, *Fibrobacter*, and *Prevotella*); novel associations linked *Alistipes* with butyric acid production and *Eubacterium nodatum group* and *Clostridiales bacterium DJF_VP35* with pentanoic acid production. This study provides new insights into the ability of different microbiomes to convert cellulose to different fatty acid mixtures and demonstrates that bison rumen inoculum has significantly different structure and metabolic capabilities than cattle rumen. This knowledge supports development of innovative microbial management and monitoring methods. These results provide further impetus for rewiring anaerobic digestion to generate high-value products.

10:38 AM Q &A

10:40 AM Integrated Biorefinery for Chemicals and Fuels Production from Waste Biomass

D.B. Visolis, PhD, visolis, Hayward, CA, USA*

One promising technology to reduce and reuse organic waste including food waste, agricultural residues and municipal solid waste is anaerobic digestion, whereby a community of microbes breaks down complex organic molecules into biogas. Biogas, however, is of limited valued, which prevents anaerobic digestion from being widely deployed.

Visolis aims to develop a novel hybrid process to rewire anaerobic digestion by arresting methanogenesis (ADAM) to produce short chain organic acids (SCOAs) instead of volatile biogas. The SCOAs will then be used as a feedstock to upgrade to a range of high value bioproducts and renewable fuels.

The key technology that will be developed under the grant is a process to selectively concentrate the SCOAs from the ADAM effluent. This concentrated SCOAs will then be used as feedstock for Visolis' proprietary engineered microbes that will convert it into a Platform molecule (PM1) at high titers. We will then catalytically upgrade PM1 into a variety of valuable chemicals including polymers and 2nd generation biofuels.

We have been able to produce high titers of the liquid intermediate in the ADAM process and developed a process for filtering and clarifying the ADAM effluent as well as optimizing the process for selective concentration of the SCOAs from the ADAM effluent. We have also hit target titers for PM1 production from the SCOA feed derived from the ADAM effluent. The major challenge that we will be tackling next will be integrating all the different unit operations at the project site.

10:48 AM Q & A

10:50 AM Conversion of Organic Waste Streams through Bioprocessing

N. Sun, Lawrence Berkeley National Laboratory, Emeryville, CA, USA*

This presentation will discuss projects related to converting landfill designated municipal solid waste (MSW, paper-rich, food-rich, and absorbent hygiene products) to sugars and bioethanol through integrated bioprocessing, also a way of improving the MSW diversion rate to reduce environmental stress. Compositional analysis, bacterial community profiling, homogenization, enzymatic hydrolysis, and yeast fermentation were performed on the collected MSW samples. Results show that these types of MSW streams are potential feedstock sources for biorefineries based on their abundance, carbohydrate composition, and bioconversion yield. Addition of selected enzyme cocktails to the homogenized substrates resulted in coproduction of C6/C5 sugars and organic acids in the hydrolysates. The sugars were readily fermentable without detoxification and solid separation.

10:58 AM Q & A

10:00 AM - 11:00 AM Session: 12: Feedstock Variability and Impacts on Conversion

Conveners: **Sunkyu Park**, North Carolina State, NC, USA and **Beau Hoffman**, Department of Energy - Bioenergy Technologies Office DOE-BETO, NC, USA

10:00 AM Seeing something that others cannot see -- Mesoscale packing of cellulose inside biomass

S.H. Kim, Pennsylvania State University, University Park, PA, USA*

Lignocellulosic biomass is basically cell walls of plants produced for their own life. Since plants are living in non-equilibrium environments, their products (biomass) are also in non-equilibrium states. This means that its structure will vary depending on species and their growth histories, which complicates designing efficient ways to utilize it. We have demonstrated that non-linear optical spectroscopy technique called sum frequency generation (SFG) can be utilized to characterize the nano-, meso-, micro-scale physical structures of crystalline cellulose in plant cell walls (biomass) in their native state with minimum to no disruption of the sample status and without interference from amorphous matrix polymers. This talk will review a few critical examples of using SFG to unveil structural packing of cellulose microfibrils in plant cell walls and how this knowledge is related to biomass degradation / conversion processes.

10:08 AM Q & A

10:11 AM Lignin variability induced by biological degradation during storage and its impact on mechanical properties, pretreatment, hydrolysis, and upgrading

B. Donohoe, Y. Zeng and R. Happs, National Renewable Energy Laboratory, Golden, CO, USA; A. Hoover and A. Ray, Idaho National Laboratory, Idaho Falls, ID, USA; J. Leal and T. Semelsberger, Los Alamos National Laboratory, Los Alamos, NM, USA*

Biomass feedstocks are highly variable in their chemical, physical, and mechanical properties and different from other commodities. Intrinsic and introduced variability in biomass resources is a key challenge underlying the often inconsistent and unreliable handling and conversion experienced in integrated biorefineries. The DOE-BETO-led Feedstock Conversion Interface Consortium (FCIC) has

investigated the range and sources of feedstock variability. One of the sources of introduced variability is biological degradation that can impact biomass during field-side storage.
We have investigated how degradation during storage modifies surface properties (texture, energy, chemistry) and internal properties (porosity, composition, structure) of different corn stover anatomical fractions. One of the surprising findings was evidence that lignin's structure was being altered by biological degradation during storage. Spectroscopic, microscopic, and NMR analyses were performed to elucidate the extent and potential mechanisms of the changes to lignin structure and its environment. We further explore the implications of the variability in lignin structure and degradation in general on downstream enzymatic hydrolysis performance and sugar and lignin upgrading.

10:19 AM Q & A

10:22 AM Time Domain-NMR for resolution of bound and free water in anatomical fractions of pine residues and corn stover as functions of biological degradation

L. Ding, Idaho National Laboratory, Idaho Falls, USA, A. Ray, Idaho National Laboratory, Idaho Falls, ID, USA and B. Donohoe, National Renewable Energy Laboratory, Golden, CO, USA*

Many quality attributes of biomass are influenced by water status and location. Water status, water distribution, and its interactions within biomass microstructure can influence the physical and chemical changes during storage and preprocessing. Time-domain nuclear magnetic resonance has been used to examine the relationship between water constraints within lignocellulosic biomass microstructure. The Carr-Purcell-Meiboom-Gill (CPMG) sequence, when combined with knowledge of chemical composition and physical structure of pine residues and corn stover anatomical fractions, gives an accurate measurement of the state and location of bound and free water. In this work , the impacts of storage and biological degradation were investigated in order to elucidate the distribution of water and status within distinct plant tissues. we also investigate how degradation during storage affects the location and states of water of different pine residue anatomical fractions (e.g. cambium, bark, branch, needle, and whitewood) and whole materials as well as corn stover anatomical fractions (e.g. leaf, stalk, cob) using transvers relaxation times (T_2). The results of this study show that the time-domain NMR method can precisely provide the quantitative information of water status inside biomass anatomical fractions and can be used to investigate the effect of preprocessing on product quality. Our findings suggest that biological heating enhanced the interactions between biomass and bound and free water. The method provided in this study can be used to evaluate water status and distribution in distinct anatomical fractions of corn stover and pine residues to enhance understanding of optimal bioprocessing strategies.

10:30 AM Q & A

10:33 AM Variability in corn stover feedstock affects downstream biological lignin conversion to muconate

I. Ruhl, S. Haugen, N. Cleveland, D. Peterson, X. Chen and D. Salvachua, National Renewable Energy Laboratory, Golden, CO, USA*

The variability of corn stover feedstocks has been raised as a potential issue that may affect biocatalyst performance during sugars and lignin conversion to bioproducts and biofuel precursors. To understand the criticality of this variability, this study specifically examines the conversion of lignin-rich black liquors from eight corn stover batches differing in moisture and ash content or anatomical fraction distribution when utilized as the sole carbon source by *Pseudomonas putida* CJ781, an engineered, muconate-producing strain. Conversion efficiency was tracked by analyzing bacterial growth, muconate titer, yield, and productivity. Bacterial cultivations in four batches varying in moisture and ash content resulted in a maximal difference of 31% in final cell density, 19% in growth rate, and a 96% and 38% difference in muconate titer and yield, respectively. Bacterial cultivation in four batches varying in anatomical fraction

content (cobs, husks, stalks, or unfractionated whole stover) resulted in a maximal difference 31% in final cell density and a 73% difference in muconate titer. Observed growth differences are likely due to distinct carboxylate concentrations in the black liquor (i.e., acetate, formate), and muconate titer and yield differences to varying aromatic acid concentrations in the black liquor (i.e., ferulate, *p*-coumarate). These results suggest that common sources of variability in corn stover feedstocks, such as moisture, ash, and anatomical fraction content, can have statistically significant impacts on downstream biological lignin conversion efficiency. The data from this study will be used to build predictive models to ultimately mitigate the risks associated with feedstock variability in lignocellulosic biorefineries.

10:41 AM Q &A

10:44 AM Investigation of lignin variability and associated genetic control in a diverse switchgrass population - CANCELLED

N. Bryant, University of Tennessee Knoxville, Knoxville, TN, USA; H. Chhetri, Y. Pu, D. Kainer, D. Jacobson and A.J. Ragauskas, Oak Ridge National Laboratory, Oak Ridge, TN, USA; T. Pendergast IV and K. Devos, University of Georgia, Athens, GA, USA*

The variability in the cell wall structure of lignocellulosic biomass has been identified as a barrier to the development of commercial scale biorefineries. It is therefore highly desirable to understand and potentially modify the abundance of certain components of the cell wall. This especially applies to lignin, a heterogeneous biopolymer that is known to hinder enzymatic conversion of biomass to biofuels. To this effect, a diverse panel of switchgrass was established for the purpose of analyzing lignin variation via a genome wide association study. Lignin of over 100 unique genotypes was analyzed by HSQC NMR for fourteen lignin phenotypes, including monolignol abundance and interunit linkage composition. Associations between lignin phenotypes and SNPs were then examined to identify potential causal mutations impacting lignin structure. A select subset of genotypes was subjected to additional lignin analyses for a more comprehensive understanding of lignin variation. This analysis across a diverse population of switchgrass establishes the foundation for a greater understanding of the genetic control of lignin variability.

10:52 AM Q & A

ePosters

High Throughput expression and characterization of laccases in *Sacchromyces cerevisiae*

P. Wolski and A. Lopes, Joint Bioenergy Institute and Sandia National Laboratories, CA, USA; K. Deng, B.A. Simmons, S. Singer and K.L. Sale, Joint BioEnergy Institute, Emeryville, CA, USA; A. Mukhopadhyay, VP, Biofuels and Bioproducts, JBEI, Lawrence Berkeley National Laboratory, Berkeley, CA, USA*

Laccases are oxidative enzymes containing 4 conserved copper heteroatoms. Laccases catalyze cleavage of bonds in lignin using radical chemistry, yet their exact specificity for bonds (such as the b-O-4 or C-C) in lignin remains unknown and may vary with the diversity of laccases across fungi, plants and bacteria. Bond specificity may perhaps even vary for the same enzyme across different reaction conditions. Determining these differences has been difficult due to the fact that heterologous expression of soluble, active laccases has proven difficult. Here we describe the successful heterologous expression of functional laccases in two strains of *Sacchromyces cerevisiae*, including one we genetically modified with CRISPR. We phylogenically map the enzymes that we successfully expressed, compared to those that did not express. We also describe differences protein sequence differences and pH and temperature

profiles and their ability to functionally express, leading to a potential future screening platform for directed evolution of laccases and other ligninolytic enzymes such as peroxidases.

Two-stage pretreatment of cotton microdust with alkali and acid for ethanol production at high solids loading.

V. Natarajan, Indian Institute of Technology Madras, CHENNAI, India*

Cotton is the principal source of natural fiber in various textile products. The processing of cotton in textile industry generates surplus amount of wastes. An enormous quantity of waste named as cotton microdust (CMD) is discharged in the spinning processes. The CMD was composed of 37% cellulose as the predominant component. The high concentration of cellulose in the CMD and its low cost are desirable for its use as a feedstock for the production of fuels and biomaterials. However, CMD was associated with other non-cellulosic components like lignin, extractives and ash. Besides, the enzymatic hydrolysis of CMD was inefficient due to its fluffy nature and poor dispersion in buffer. Therefore, the conversion of CMD to value added products required a suitable pretreatment to facilitate further biochemical process. In the case of single stage pretreatment of CMD using either alkali or acid, a considerable improvement in the fermentable sugar production was observed at solids loading <10%. Interestingly, the two stage pretreatment comprising of alkali and acid pretreatments in sequence resulted in higher enzymatic digestibility of CMD for solids loading above 10%. More importantly, the two stage pretreatment facilitated hydrolysis of CMD up to 30% solids loading and further fermentation resulted in production of high ethanol concentration. Hence, the results indicated the possibility of developing an economical process for the valorization of CMD to cellulosic ethanol.

Bioethanol production from eucalyptus sawdust at high solid enzymatic hydrolysis loading: combined alkali impregnation with steam explosion pretreatment evaluation

V. Scutari, E. Rochón, M.D. Ferrari and C. Lareo, Facultad de Ingeniería, Universidad de la República, Montevideo, Uruguay*

Eucalyptus sawdust represents an important residue from local pulp and paper industries which is little used to obtain valuable products. The aim of this work was to evaluate bioethanol production from steam-exploded *Eucalyptus grandis* sawdust at high solid enzymatic hydrolysis loading. To maximize obtaining a glucose concentration with a high hydrolysis efficiency, the effect of solid loading and enzyme dosage on the enzymatic hydrolysis performance was evaluated using a central composite rotatable design with central point. The pretreated solid (200°C, 1.5 MPa, 10min) was enzymatically hydrolyzed using Cellic CTec2 at 15-25% solid loadings and 10-25FPU/g_{glucan} enzyme dosages. The assays were performed in 250-mL flasks at 50°C, pH4.85, 150rpm. Results showed that both enzyme dosage and solid loading presented a significant effect on glucose concentration (p<0.05). As expected, higher hydrolysis efficiencies were observed for lower solid loadings and higher enzyme dosages. However, a relatively high hydrolysis efficiency (72%) and a high glucose concentration (125 g/L) were obtained for a high solid loading (25%) when high enzyme dosage was used (25 FPU/g_{glucan}). Once enzymatic hydrolysis conditions defined, the effect on ethanol production of carrying out separate enzymatic hydrolysis and fermentation (SHF) or simultaneous enzymatic fermentation (PSSF and SSF) was evaluated using *Saccharomyces cerevisiae.* Fermentation assays were performed in 250-mL flasks containing 100 mL of enzymatic hydrolysate for SHF and the medium with pretreated sawdust (solid concentration: 27%(w/w)) for PSSF (24h pre-hydrolysis) and SSF processes. For SHF, a glucose concentration of 134±5 g/L was effectively achieved which was within the range predicted by the model with a hydrolysis efficiency of 74±3% after 48h. However, the SSF process configuration showed the best performance in terms of ethanol concentration, volumetric productivity and yield, 75.6 g/L, 2.1 g/Lh, and 259 L/$t_{dry\ sawdust}$, the microorganisms not showing difficulties to ferment in conditions of high solid concentration.

Adaptive laboratory evolution of *Eubacterium limosum* ATCC 8486 on carbon monoxide

S. Kang, Y. Song, J. Shin, S. Jin and J. Bae, Korea Advanced Institute of Science and Technology (KAIST), Daejeon, Korea, Republic of (South); S. Cho and B.K. Cho, Korea Advanced Institute of Science and Technology, Daejeon, Korea, Republic of (South)*

Acetogens are naturally capable of metabolizing carbon monoxide (CO), a component of synthesis gas (syngas), for autotrophic growth in order to produce biomass and metabolites via the Wood–Ljungdahl pathway. However, the autotrophic growth of acetogens is often inhibited by the presence of high CO concentrations because of CO toxicity, thus limiting their biosynthetic potential for industrial applications. Herein, we implemented adaptive laboratory evolution for growth improvement of *Eubacterium limosum* ATCC 8486 under high CO conditions. The strain evolved under syngas conditions with 44% CO, resulting in a significant increased growth rate by 1.44 folds. In addition, the evolved populations were capable of proliferating under CO concentrations as high as 80%. These results suggest that cell growth is enhanced as beneficial mutations are selected and accumulated, and the metabolism is altered to facilitate the enhanced phenotype. To identify the causal mutations related to growth improvement under high CO concentrations, we performed whole genome resequencing of each population. Interestingly, we found a key mutation in CO dehydrogenase/acetyl-CoA synthase (CODH/ACS) complex coding gene, *acsA*. To characterize the mutational effects on growth under CO, we isolated single clones and confirmed that the growth rate and CO tolerance level of the clone were comparable to those of the evolved populations and wild type strain under CO conditions. Furthermore, the evolved strain produced 1.34 folds acetoin when compared to the parental strain while introducing the biosynthetic pathway coding genes to the strains. Consequently, this study demonstrates that the mutations in the CODH/ACS complex affect autotrophic growth enhancement in the presence of CO as well as the CO tolerance of *E. limosum* ATCC 8486. This work was supported by the C1 Gas Refinery Program (2018M3D3A1A01055733 to B.-K.C.) through the NRF funded by the MSIT.

Comparative Genomics Determines Strain-Dependent Secondary Metabolite Production in *Streptomyces venezuelae* Strains

W. KIM, N. Lee, S. Hwang, Y. Lee, J. Kim, S. Cho and B.K. Cho, Korea Advanced Institute of Science and Technology, Daejeon, Korea, Republic of (South); B. Palsson, University of California San Diego, La Jolla, CA, USA*

Streptomyces venezuelae is well known to produce various secondary metabolites, including chloramphenicol, jadomycin, and pikromycin. Although many strains have been classified as *S. venezuelae* species, only a limited number of strains have been explored extensively for their genomic contents. Moreover, genomic differences and diversity in secondary metabolite production between the strains have never been compared. Here, we report complete genome sequences of three *S. venezuelae* strains (ATCC 10712, ATCC 10595, and ATCC 21113) harboring chloramphenicol and jadomycin biosynthetic gene clusters (BGC). With these high-quality genome sequences, we revealed that the three strains share more than 85% of total genes and most of the secondary metabolite biosynthetic gene clusters (smBGC). Despite such conservation, the strains produced different amounts of chloramphenicol and jadomycin, indicating differential regulation of secondary metabolite production at the strain level. Interestingly, antagonistic production of chloramphenicol and jadomycin was observed in these strains. Through comparison of the chloramphenicol and jadomycin BGCs among the three strains, we found sequence variations in many genes, the non-coding RNA coding regions, and binding sites of regulators, which affect the production of the secondary metabolites. We anticipate that these genome sequences of closely related strains would serve as useful resources for understanding the complex secondary metabolism and for designing an optimal production process using *Streptomyces* strains. This work was supported by Bio & Medical Technology Development Program (2018M3A9F3079664 to B.-K.C.) through the National Research Foundation of Korea (NRF) funded by the Ministry of Science and ICT (MSIT). This work also supported by a grant from the Novo Nordisk Foundation (NNF10CC1016517).

Engineering *Bacteroides thetaiotaomicron* to produce butyrate based on a genome-scale metabolic model-guided design

K. Kim, Y. Song, D. Choe, M. Kang and B.K. Cho, Korea Advanced Institute of Science and Technology (KAIST), Daejeon, Korea, Republic of (South)*

Abstract

Bacteroides thetaiotaomicron represents a major symbiont of the human gut microbiome that is increasingly viewed as a prospective candidate strain for microbial therapeutics. This chiefly owes to several intrinsic features of *B. thetaiotaomicron* including the prevalence in the human gut, ability to stably colonize and modify the intestinal niches, and prospective role in the alleviation of gut inflammations. To streamline design and manipulation of this strain, we reconstructed a genome-scale metabolic model (GEM) using the previous GEM *i*AH991 as the template. Validation of the updated model *i*KS1119 revealed that the *in silico* predictions were in close agreement with the experimentally measured parameters, suggesting that the model can be employed for the reliable prediction of *B. thetaiotaomicron* phenotypes. We used butyrate as a proof-of-concept to test whether *B. thetaiotaomicron* harbors the capacity for the heterologous production of biomolecules. Genomic integration of butyrate biosynthetic pathway from *Clostridium acetobutylicum* into a wild-type *B. thetaiotaomicron* yielded around 30 mg/L of butyrate. The GEM-guided rational strain design improved the final titer by up to 3-folds. This study shows that *B. thetaiotaomicron* may serve as an effective strain for live microbial therapeutics in human.

[This project was supported by the Korea Bio Grand Challenge (2018M3A9H3024759 to B.-K.C.), the Basic Science Research Program (2018R1A1A3A04079196 to S.C.), the Bio & Medical Technology Development Program (2018M3A9F3079664 to B.-K.C.) from the Ministry of Science and ICT (MSIT) through the National Research Foundation (NRF) of Korea.]

Deletion of genes with predicted function in the N-glycosylation pathway of Aspergillus nidulans negatively impact enzymes secretion

J.A. Gerhardt, M.V. Rubio, C.R. Terrasan, F.L. Figueiredo, N. Sayuri Wassano, E.P. Antoniel, A.C.P. Oliveira and A. Ricardo de Lima Damasio, Institute of Biology, University of Campinas (UNICAMP), Campinas, Brazil; F.J. Contesini, Technical University of Denmark (DTU), Lyngby, Denmark*

Filamentous fungi are essential as cell factories to maintain the global enzyme market representing billions of dollars a year to serve end-use industries such as food and beverage, biopolymers, biofuel, and animal feed. Improving the yield of enzyme production by genetic engineering of fungal strains is an important step to maintain this growth market. In addition to the high level of enzymes secretion, filamentous fungi have the ability to perform post-translational modifications (PTMs) such as N-glycosylation. Recently, we demonstrated the influence of N-glycosylation sites on the thermal stability, secretion, folding, and efficiency of a target GH3 β-xylosidase (BxlB) of *Aspergillus nidulans*. Based on that, we hypothesized that the single deletion of genes involved in the N-glycan pathway may interfere in the secretion of recombinant proteins. For this purpose, CRISPR/Cas9 technology was applied to knockout 15 genes involved in the assembly of N-glycans (*alg7*, *alg13*, *alg1*, *alg2*, *alg11*, *rft1*, *alg3*, *alg9*, *alg12*, *alg6*, *mns1a*, and *mns1b*) and glycoprotein quality control (*clxA*, *gtb1*, and *uggt*) resulting in the successful deletion of 9 targets. The deletion of genes at the early stages of N-glycan assembly into the ER outer membrane such as *alg7*, *alg13*, *alg1* and *alg11* was lethal for *A. nidulans*, and the deletion of *alg2* and *rft1* resulted in very sick strains. We are still trying to obtain viable mutants by the deletion of *alg12* and *uggt*. The 7 viable knockout strains (*alg3*, *alg9*, *alg6*, *mns1a*, *mns1b*, *clxA*, and *gtb1*) were then transformed with the *bxlB* gene (AN8401) for constitutive expression and evaluation of its secretion and enzymatic activity. By combining protein quantification, fungal biomass measurement, β-xylosidase activity, and Western blot we concluded that the deletion of any of the target genes reduced not only the secretion of recombinant BxlB but negatively impact the entire *A. nidulans* secretome.

A novel multi-step enzymatic process for the isolation of nanocellulose from organosolv pretreated hardwood biomass: insights into the key role of a newly discovered AA9 LPMO

K. Chorozian[*] and E. Topakas, Industrial Biotechnology & Biocatalysis Group, School of Chemical Engineering, National Technical University of Athens, Athens, Greece; A. Karnaouri, D. Zouraris and A. Karantonis, Laboratory of Physical Chemistry and Applied Electrochemistry, School of Chemical Engineering, National Technical University of Athens, Athens, Greece

Nanocellulose is one of the most important lignocellulose-derived value added products in the emerging market of biobased polymers. Its isolation upon employment of milder, environmentally friendly processes is particularly attractive. Biocatalysis is a promising approach due to targeted and substrate-specific activity, selectivity, mild and non-toxic chemistry. Endoglucanases are the most exploited enzymes for the production of nanocellulose due to their potential to remove the less ordered amorphous regions from cellulose fibers, leaving intact the more organized, crystalline areas, thus facilitating the nanocellulose isolation without altering the cellulose surface chemistry. Moreover, accessory activities including xylanases and other hemicellulose-acting enzymes hold a key role for the isolation of nanocrystalline cellulose. The newly discovered lytic polysaccharide monooxygenases (LPMOs) are also gaining attention due to their implication in nanocellulose production. Within this context, we report the heterologous expression and production of a novel fungal C1-acting AA9 LPMO from *Thermothelomyces thermophila*. The enzyme was biochemically characterized for its activity on different polysaccharide substrates, while different electrochemically active compounds were tested as electron donors. The formal potential of the of the Cu(II) center in the active site of the LPMO was determined with the use of large amplitude Fourier Transform alternating current cyclic voltammetry (FTacV). Finally, the application of the enzyme together with other glycoside hydrolases on nanocellulose isolation from beechwood was studied. Starting with an initial mild oxidative organosolv pretreatment, efficient delignification was achieved, leaving behind a cellulose-rich solid fraction. An enzymatic treatment step with LPMO was then applied, followed by different sequential hydrolysis steps with cellulolytic and hemicellulolytic enzymes, thus enabling a fine-tuning of all activities with the aim to shed light on the contribution of different enzymes in each step. Both commercially available enzyme cocktails and a combination of different monoenzymes with specific activities were tested in order to assess their individual effects.

Extraction of Waxes and Lipids from Sorghum Using Green and Renewable Solvents Followed by Conversion to Biofuels Using GVL Pre-Treatment

M. Gallmeyer[*], Michigan Technological University, White Lake, MI, USA

Plant matter contains waxes and lipids that can be extracted and used or sold as value-added product prior to conversion of the remaining plant material to biofuels. Wax and lipid extractions are currently performed using volatile, non-renewable hydrocarbons, primarily hexane. As part of this study, several extractions will be completed on the sorghum using hexane, methyl-tetrahydrofuran (MeTHF), diethoxymethane, and ɣ-valerolactone (GVL) followed by an acid-GVL pretreatment solution in the same reactor. The initial extractions will be carried out at 20C, 40C, 60C, and 80C. The lipids will be collected and analyzed using GC-MS. The remaining cellulose can then be reacted, solubilized, and converted to biofuels.

Genetic manipulation of transcription factors involved in the secretion of recombinant enzymes in *Aspergillus nidulans*

E. Paschoal Antoniel[*], J. Aline Gerhardt, M. Alexandrino de Assis and N. Sayuri Wassano, University of Campinas (UNICAMP), Campinas, Brazil; A. Ricardo de Lima Damasio, Institute of Biology, University of Campinas (UNICAMP), Campinas, Brazil

Filamentous fungi are capable of secreting a significant amount of proteins and are used as a platform for the production of industrially important enzymes, such as hydrolytic enzymes used in the production of

bioproducts such as biofuels. However, there is still potential for progress in terms of recombinant protein production. In this regard, many techniques have been used to boost fungal cell factories production capacity; however, as consumer demand grows and productivity remains poor, identifying new genetic targets to promote increased enzyme secretion becomes increasingly important. Therefore, our aim was to evaluate the production/secretion of enzymes in strains of *Aspergillus nidulans* in which target transcription factors have been genetically manipulated. To identify these transcription factors, we used RNA-seq data from *A. nidulans* strains overexpressing three heterologous enzymes and we performed differential expression and GO enrichment analysis. Next, we used CRISPR-Cas9 to knock out these transcription factors in *A. nidulans* and analyzed their phenotype to see whether they have any effect on enzyme secretion. Based on RNA-seq data, we identified five transcription factors candidates – AN8772, AN9373, AN7331, AN7913, and AN7592 – with predict function in golgi vesicle transport, protein refolding, small molecule catabolic process and proteolysis. We found that the strain ΔAN7592 had decreased sporulation and slight resistance to osmotic stress, while the strains ΔAN9373, ΔAN7331, and ΔAN7913 had increased protease secretion. Taken together, our results suggest that these transcription factors may be involved in a variety of biological processes associated with the protein secretion pathway. We concluded that transcriptomic data could be used to identify transcription factors with possible involvement in the protein secretion pathway of *A. nidulans*, and we plan to continue our research by performing additional experiments to determine the role of these transcription factors in the secretion of biotechnologically relevant recombinant enzymes.

Inversion of sucrose in sweet sorghum syrup followed by fermentation to produce succinic acid

K.T. Klasson, PhD and M. Sturm, USDA, Agricultural Research Service, New Orleans, LA, USA*

The inversion of sucrose in sweet sorghum syrup was investigated at 65, 75, and 85°C using 0.0862, 0.136, 0.273, 0.409, and 0.545 M HCl followed by neutralization and fermentation by Escherichia coli AFP 184 to produce succinic acid from the glucose and fructose. The sucrose inversion rate was measured using HPLC for the disappearance of sucrose and the appearance of the glucose and fructose. The calculated rate constant was found equal to previously published reports. The fermentation results showed that succinic acid was produced at an average concentration of 27 g/L in approximately 94 hours with a yield of 0.36 g succinic acid/g sugar.

Storage and bioethanol fermentation of sweet sorghum syrups

K.T. Klasson, PhD and S. Boone, USDA, Agricultural Research Service, New Orleans, LA, USA*

Sweet sorghum represents a readily available renewable carbohydrate feedstock for biofuel production. Generally, the sweet sorghum growing season and window for processing is short and it is often desirable to store a sugar-rich material for future processing. Therefore, we investigated the possibility of storing three different sweet sorghum sugar syrups under a layer of soybean oil; then we investigated subsequent yeast bioethanol fermentation. A layer of oil on the surface of the syrup reduced sugar loss in 30 and 40 °Bx syrups, but the loss was still 21-36 %. Storage of 50 °Bx syrup resulted in an 11-18 % loss of sugars with or without a surface layer of oil. Elevated levels of lactic and acetic acids, caused by bacterial contamination, almost completely inhibited yeast fermentation; however, when ethanol-producing microorganisms were part of the contamination, stored syrups produced a bioethanol at a yield of 83-91%, compared to theoretical.

Characterizing fungal inhibitors from drought-stressed switchgrass

S. Chipkar, K. Smith, M. Gallmeyer and R. Ong, Michigan Technological University, Houghton, MI, USA; A.D. Jones, Michigan State University, East Lansing, MI, USA*

Development of economically viable and greener pathways to synthesize renewable energy has been an important research theme in recent years. Lignocellulosic biomass is a major resource that can be used for biofuel production. Recent research has shown that biomass characteristics are altered by

environmental growth conditions, and directly influence the extent of biomass conversion to fuels. Previously it was reported that drought experienced during the growth of switchgrass led to complete inhibition of yeast growth during fermentation. In this project, we characterized specific compounds that led to this inhibition. Switchgrass harvested in drought and non-drought years were pretreated using Ammonia Fiber expansion (AFEX). Untreated and AFEX processed samples were separately extracted using solvents (i.e., water, ethanol, and ethyl acetate) to selectively remove potential inhibitory compounds and determine whether pretreatment affects the inhibition. A key goal of the project was to determine whether the microbial-inhibitors are plant-generated compounds, by-products of the pretreatment process, or a combination of both. High solids loading enzymatic hydrolysis was performed on all samples followed by fermentation using genetically modified, xylose consuming yeast strain *Saccharomyces cerevisiae* Y330. Cell growth (OD_{600}), sugar consumption, and ethanol production were used to evaluate fermentation performance. Extracts were analyzed using liquid chromatography-mass spectrometry (LC-MS) to identify potential inhibitory compounds. We identified numerous saponins, a class of plant-generated triterpene glycosides, which were significantly more abundant in the drought-year (inhibitory) switchgrass water extracts and potential microbial inhibitors. Tandem MS analysis on the unknown characterized inhibitors was conducted to annotate their identities. Add-back fermentation experiments in non-inhibitory hydrolysates for the identified inhibitors will be conducted to replicate inhibition.

Lactate overloading phenomenon in carboxylate platform: stable caproate and hydrogen co-production

F. Brodowski, M. Lezyk, N. Gutowska and P. Oleskowicz-Popiel, Poznan University of Technology, Poznan, Poland*

The emerging carboxylate platform, producing medium chain carboxylates (MCC) during the chain elongation process in an open culture fermentation, is an alternative to popular biotechnology technologies producing biogas and ethanol. The most popular MCC is caproate produced in ethanol-based or lactate-based carboxylate platform. The conversion of lactate to caproate is becoming more and more popular, however there are many limitations affecting production stability, e.g. lactate overloading. Lactate overloading not only affects caproate production, but can also activate propionate producers that can outcompete chain elongators. The main objective of the study was to investigate the effect of external acetate addition (electron acceptor) on lactate overloading in the continuous caproate production process. The process was carried out in two 1L continuous stirred-tank reactors. In the first one denoted as B1, lactate conversion to caproate was carried out without the external acetate, and in the second one denoted as B2, with the addition of acetate. The concentration of lactate in the feedstock (inflow) was increased until lactate overloading occurred. In B1, fluctuations in caproate production were observed as a result of the appearance of residual lactate, however no propionate production was observed. The addition of external acetate to lactate-overloaded B1 recovered stable caproate production and induced hydrogen co-production. In B2, the external acetate influenced the shift of the lactate overloading limit (the lactate overloading occurred at a higher concentration of lactate in the feedstock than in B1) and despite the presence of residual lactate, a stable caproate production was achieved. Additionally, hydrogen production in lactate-overloaded B2 was observed. Thus, the addition of external acetate was proposed as a viable strategy to stabilize caproate production in lactate-based carboxylate platform and recover production stability in the case of lactate overloading.

Glucose Transport Systems for Upgrading Farm Residue-Derived Hydrolysates to Fine Chemicals via Shikimate Pathway Biosynthesis

K. Draths, Y. Jadidi, C. Saffron, H. Frost and W. Liao, Michigan State University, East Lansing, MI, USA*

Examination of Glucose Transport Systems for Upgrading Farm Residue-Derived Hydrolysates to Fine Chemicals

Yasheen Jadidi, Henry Frost, Chris Saffron, Wei Liao and Karen Draths

The natural product shikimic acid (SA) is the starting material for synthesis of oseltamivir phosphate (Tamiflu®), an antiviral agent effective against influenza. SA may be isolated from plants; however, methods for biosynthesizing SA from glucose via fed-batch fermentation have also been developed. Our research aligns with the developing trend to utilize organic farm waste in place of corn-derived feedstocks for microbial synthesis of chemicals. Data presented will encompass an effort to add to the "agricultural residue economy", utilizing mixed sugars derived from lignocellulosic corn stover for SA biosynthesis. *Escherichia coli* K-12 strains expressing different glucose transport systems – native phosphotransferase (PTS), heterologously expressed *Z. mobilis* GIF and GIK, and an uncharacterized evolved uptake mechanism – were examined in a fed-batch fermenter for their abilities to co-utilize glucose and xylose for SA synthesis. Strain YJ1.144/pSC6.090B expressing GIF and GIK performed better than a strain expressing the native glucose PTS and another PTS^- strain evolved to take up glucose. YJ1.144/pSC6.090B achieved an SA titer of 94.9 g L^{-1} when grown on a 70:30 (*w*/*w*) mixture of glucose/xylose. CO_2 emission data collected from the fed-batch fermentations were used to perform a comparative life-cycle assessment (LCA) and techno-economic analysis (TEA). In the LCA/TEA, a fair techno-economic and life cycle impact (LCI) comparison was made between use of corn-derived glucose and stover-derived glucose/xylose mixtures for SA production. These results will be presented.

Evaluation of a bioaugmentation technique for biochemicals production

A. Duber, N. Gutowska and P. Oleskowicz-Popiel, Poznan University of Technology, Poznan, Poland*

Open (mixed) culture fermentation is a bioprocess where a naturally enriched microbial consortia are able to convert different waste streams into industrially valuable products and biofuels precursors, such as short and medium chain carboxylic acids or molecular hydrogen. However, it is still unravelled how to lead and control the process for stable and efficient production. Recently bioaugmentation was described as a biological control method to be applied in an open culture fermentation. Bioaugmentation is a microbial-based strategy for bioprocess control based on introduction of a selected strain or defined mixture of selected microorganisms responsible for final products generation to improve the process performance. The aim of the study was to investigate the impact of bioaugmentation with selected strains, i.e. *Megasphaera elsdenii* and *Eubacterium limosum*, on conversion of organic compounds that naturally occur in waste streams to important biochemicals. The experiments were conducted in batch mode under anaerobic conditions with lactate and lactose (LL, main compound of whey wastewater), and glycerol (G, by-product from biodiesel production) used as substrates. The results showed that bioaugmentation with *M. elsdenii* as well as *E. limosum* increased iso-valeric and valeric acid production from LL and G against the control enrichment trials. Among all trials, the highest caproic acid production was recorded in reactors inoculated with pure strain of *M. elsdenii* grew on glycerol. Even though, the strain did not improve the caproic acid production in bioaugmented trials, simultaneous bioaugmentation with both mentioned strains enhanced caproic acid production from glycerol when compared to non-bioaugmented trials. Here, we demonstrated that the bioaugmentation had a positive impact on open culture fermentation processes for short and medium chain carboxylic acid production.
The study was funded by the National Science Centre (Poland), contract no.: 2017/25/N/ST8/01795.

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

S. Nakagame, Kanagawa Institute of Technology, Atsugi, Japan and J. Saddler, University of British Columbia, Vancouver, BC, Canada*

The growth of six oleaginous yeasts were compared that were inoculated in corn steep liquor (CSL) medium with 5-hydorxymethy furfural (HMF) and furfural, which were inevitably produced by SO_2-catalyzed steam pretreatment of lignocellulosic biomass from hexose and pentose, respectively. Of the six oleaginous yeasts, *R. graminis* showed the highest tolerant to HMF (0.2%) or furfural (0.1%) in the CSL medium. But, the presence of both HMF (0.2%) and furfural (0.1%) in the CSL medium inhibited the growth of *R. graminis*. Evaporation of the CSL medium successfully removed furfural from the CSL medium and increased the sugar concentrations, but the concentrated HMF (0.4%) in the CSL medium inhibited the growth of *R. graminis*. To improve the tolerance of *R. graminis* to HMF, a wild type of *R.*

graminis was inoculated in the CSL medium containing HMF (0.4%) for acclimation. As the result, the 5-HMF (0.4%) tolerance of *R. graminis* was obtained after 140 h incubation. The lipids productivity by the HMF(0.4%) tolerant *R. graminis* incubated in the CSL medium with HMF(0.4%) was 1.8 g/L after 96h, which was almost same as that by wild type of *R. graminis* incubated in the CSL medium without HMF and furfural. This result suggests that the production of lipids using HMF tolerance *R. graminis* is a promising way for producing lipids from the hydrolyzates of SO_2-catalyzed steam pretreated lignocellulosic substrates.

Conversion of herbaceous biomass to single cell lipids using a low-moisture ammonium pretreatment with *in situ* ball milling

B. Dien, Ph.D., L. Iten, P. Slininger, Ph.D. and S. Thompson, NCAUR-ARS-USDA, Peoria, IL, USA; M.H. Chen, Ph.D. and V. Singh, University of Illinois Urbana Champaign, Urbana, IL, USA; R. Mitchell, Ph.D. and G. Sarath, Ph.D., ARS-USDA, Lincoln, NE, USA*

Switchgrass is a native warm season perennial grass with high productivity that has proven to be a suitable biomass crop to cultivate in northern United States. Conversion of switchgrass into sugars and subsequent microbial biofuels/bioproducts requires an intensive pretreatment step. Dilute-acid pretreatments are often used but are problematic because the hydrolysate is inhibitory in microbial production cultures. A novel pretreatment was developed where the biomass is reacted with pressurized ammonia at low-moisture with *in situ* ball milling. Following pretreatment, ammonia was evaporated, and the pretreated biomass hydrolyzed using commercial cellulases and hemicellulases (CTEC3/HTEC3) at 20%w/w biomass solids. The optimal ammonia loading (3%v/v) yielded 121 g/L monosaccharides. Without ball milling the sugar yield was reduced to 104 g/L. The effects of reduced ammonia loading (2%v/v) and milling times (1, 2, and 3 hr) were explored and it was observed that reducing the reaction/milling time from 3 to 2 hours did not affect the sugar yield. The sugar concentration was further increased to 170 g/L by increasing the hydrolysis solids loading from 20 to 30%w/w. Experiments are currently underway comparing hydrolysates prepared using this new pretreatment to that of dilute-acid for producing lipids in oleaginous yeast cultures.

Effect of cultivation conditions on the bioconversion of 4-hydroxybenzoic acid in two white-rot fungi

T. Kijpornyongpan, C. del Cerro, K. Ramirez and D. Salvachua, National Renewable Energy Laboratory, Golden, CO, USA; S.O. Purvine, H.D. Mitchell, L.M. Markillie and M.C. Burnet, Pacific Northwest National Laboratory, Richland, WA, USA*

White-rot fungi (WRF) are the most efficient organisms for lignin degradation in nature. A recent study has also shown the ability of WRF in funneling lignin-derived aromatics, including 4-hydroxybenzoic acid (4HBA) and vanillic acid, to central metabolism. However, it is unknown how the cultivation conditions affect the conversion of lignin-derived aromatic compounds. To address this, we performed multi-omic analyses and tracked the conversion of 4HBA in different cultivation conditions in two white-rot fungi: *Trametes versicolor* and *Ceriporiopsis subvermispora*. Specifically, we evaluated the effect of static and agitation cultivation conditions in the absence and the presence of antioxidants on fungal performance. We found that the comparison between static and agitation has the higher number of differentially expressed genes and protein abundances at the transcriptomic and proteomic levels, respectively, compared to the presence or the absence of antioxidants. Gene mapping on the enzymatic steps of the proposed 4HBA conversion pathway revealed that agitation causes upregulation of several oxidase genes such as aldehyde dehydrogenases, aldehyde oxidases, and hydroxylases in both fungi. Time-course experiments including 4HBA and cellobiose as carbon sources showed that *T. versicolor* exhibited higher 4HBA conversion levels in agitation (~110% more conversion, compared to static cultivation conditions) and in the presence of antioxidants (~10% more conversion, compared to treatments without antioxidants). Interestingly, these trends were not found in *C. subvermispora*, suggesting different carbon metabolism in both species. Our findings are fundamental to better understand carbon sequestration from lignin in WRF, which will be key for future applications in the lignin valorization field.

Mining subsurface microbial genetic traits to develop robust cell factories for high-toxic waste valorization

L. Dissanayake[*]*, I. Shaw, P. Langridge, J. Zefo, R. Ligon, S. Kayastha, S. Hamilton-Brehm and L. Jayakody, Southern Illinois University, Carbondale, IL, USA*

Developing robust microbial cell factories to tolerate chemical stressors is vital to enhance the desire product titer, rate, and yield to enable an economically viable process for making fuels, chemicals, and materials from biomass and unconventional substrates such as synthetic polymers and industrial wastewater. Subsurface microbes' deal with multiple extreme environmental conditions, including heat, pressure, nutrient starvation, radiation, and chemical stressors. Consequently, these microbes have developed genetic stress tolerances that are useful to synthetic biology. We explore the genetic traits from the novel strictly anaerobic, thermophilic, subsurface bacterium, *Caldiatribacterium inferamans* strain SUIC1, the first cultured subsurface member from the candidate phylum Atribacteria OP9. Here we demonstrated successful heterologous expression of SIUC1, alcohol dehydrogenase (ADH_{SIUC}) in the domesticated potent industrial workhorse *Pseudomonas putida* KT2440 and novel catabolic powerhouse *Erwinia aphidicola* LJJL01. The expression of ADH_{SIUC} remarkably enhances tolerance of engineered strains to several high-toxic aliphatic and aromatic compounds, including methanol and ethanol catechol, and aldehydes such as glycolaldehyde and furfural. The biochemical characterization of the substrate specificity of the enzymes is underway. Additionally, we are currently exploring *C. inferamans* SIUC1 chaperone machinery in *P. putida* KT2440 to enhance thermophilic enzymes' expression, such as plastic degrading enzymes. In summary, we demonstrated the synthetic expression of genes from the extreme subsurface bacterium *C. inferamans*, SIUC1, in industrial cell factories for the first time. The engineered strains can be employed to valorize high toxic compounds in biomass-hydrolysate and industrial wastewater streams.

Keywords: subsurface microbes, alcohol dehydrogenase, chemical stress, *P. putida* KT2440, *E. aphidicola*.

Microbiome Analysis of Iodoform Effects on Advanced Anaerobic Digestion Processes for Production of Fatty Acids

J.L. Rico Reyes[*]*, R. Daly, K. Wrighton, PhD, K. Reardon, PhD and S. De Long, PhD, Colorado State University, Fort Collins, CO, USA*

Advanced anaerobic digestion (AD) technologies have the potential to convert organic residues into added-value chemicals and fuel precursors. This rewired AD (RWAD) approach requires selectively directing feed carbon into fatty acids (FA) instead of methane. For such an approach, studies in RWAD have used methanogen inhibitors, like iodoform, and demonstrated success in arresting methanogenesis while producing FA. However, RWAD is a complex biological system. A single perturbation can significantly impact the microbiome structure and its metabolic pathways. To date, there is a lack of understanding of how iodoform impacts the microbiome in RWAD. This knowledge gap is one of the limitations in the field for optimizing process performance. This study investigated the effects of iodoform addition in RWAD of food waste using anaerobic wastewater sludge as a microbial inoculum. Microbiome reactors were acclimated to operating parameters for one month, followed by a five-day experimental run with five reactor replicates per treatment (with and without iodoform addition). Significant differences were observed in the production of gases and the FA product spectrum. As expected, methane was produced in controls but not in iodoform-treated reactors. Interestingly, acetic and propionic acid production was highest in controls, while butyric and hexanoic acids were highest in iodoform treated reactors. Comparative microbiome analysis via 16S rRNA gene and metagenomic sequencing revealed differences in the microbiome structure and the metagenome. These differences were driven by *Prevotella* and *Peptostreptococcacea*, which dominated iodoform reactors, and *Alcaligenes* and *Rombustia*, which dominated in controls. Metagenome assembled genomes were reconstructed and linked to specific taxa, genes, and metabolic pathways. This is the first comparative microbiome and metagenomic analysis of iodoform effects on RWAD and provides new insights into microbiomes' genetic potential to transform

organic residues into FA. Further, this study demonstrates the value of integrated meta-omics studies for advancing knowledge of RWAD systems.

Detecting Physiological Status of Microbial Cultures, In-situ.

H. Teel, K. Likit-anurak, P. Satjaritanun and S. Shimpalee, University of South Carolina, Columbia, SC, USA; C. Turick, Savannah River National Laboratory, Aiken, SC, USA*

Detecting Physiological Status of Microbial Cultures, In-situ.

Kris Likit-Anurak[1], Hunter R. Teel[1], Pongsarun Satjaritanun[1], Sirivatch Shimpalee[1], Charles E. Turick[2,3]

[1]University of South Carolina, Columbia, SC, USA, [2]Savannah River National Laboratory, Aiken, SC, USA, [3]ElectroBioDyne, Aiken, SC, USA

For bioconversion of organic feedstocks to fuels and chemicals to be cost-effective, bioprocesses need to operate at near optimum conditions with sufficient chemical, biochemical and microbial monitoring. To avoid conventional time and labor-intensive monitoring, a new paradigm is required for in-situ, real time analysis. Since bioconversion of organic is accomplished by microorganisms through the oxidation of feedstocks linked to the reduction of electron acceptors, microorganisms can be viewed as electrochemical catalysts. In this regard, following electron flow through well-established electrochemical techniques offers a novel and inexpensive approach to real time monitoring with the advantage of abundant data.

Here we demonstrate the use of electrochemical techniques of cyclic voltammetry (CV) and electrochemical impedance spectrometry (EIS) for monitoring microbial metabolic activity in real time, in-situ. CV provides precise information regarding extracellular electron transfer throughout growth and EIS offers a data rich platform for evaluation of microbial physiological status in real time. In addition, the problem of electrode fouling is managed with voltammetric stripping, an established electrochemical technique used to clean and condition electrodes in-situ.

The effect of organic electron donors as a function of concentration to the physiological status of *Shewanella oneidensis* was determined. In this study, the Gram-negative, pyomelanin overproducer (*S. oneidensis ΔhmgA*) and the pyomelanin deficient mutant (*S. oneidensis ΔmelA*) were chosen due to different surface electrochemical characteristics along with relative degrees of oxygen utilization efficiency. Electrochemical properties changed with growth status and correlated with electron flow from organic carbon sources and terminal electron acceptor availability. These results are compared with those of the Gram-positive *Clostridium phytofermentans* from previous studies.

Non-oxidative thermal analysis and kinetics of humin based by-products from agroindustrial wastes processing to levulinic acid

J.C. de Jesus Gariboti, M. Gontijo Souza Macedo, E. Ladeia Gomes, R. Fernandez Felisbino and L. Plazas Tovar, Federal University of São Paulo, Diadema, Brazil; E. Savioli Lopes, University of Campinas, Campinas, Brazil; Y. Camacho Ardila, Pedagogical and Technological University of Colombia, Tunja, Colombia*

Humins obtained from the sugarcane bagasse and rice husk biorefining in three stages (H-SCB and H-RH, respectively) during the production of levulinic acid are examined in this study as a potential raw material for pyrolysis. Proximate and ultimate analyses and the determination of higher heating value confirmed their potential as an alternative and renewable source of energy in the thermochemical conversion process. The non-isothermal thermogravimetric analysis (TGA) revealed that both humins had a significant mass loss (57,6%-84.2%) during thermal decomposition. The derivative thermogravimetric curves exhibited that the thermal decomposition occurred into three stages: I (20-120ºC), II (300-600°C) and III (600-800°C) explained by dehydration, decarboxylation, decarbonylation reactions together with the decomposition of the network structure. Kinetics and mechanism of the thermal decomposition of H-SCB and H-RH were examined by a one-step global reaction. The activation energies using integral isoconversional methods of Ozawa–Flynn–Wall, Starink, and Vyazovkin showed a strong dependency with the conversion evolution. Vyazovkin's method stated that this approach leads to an accurate and reliable estimate of kinetic parameters. Isoconversional activation energy varied significantly from 156.9 to 274.1 kJ mol^{-1} to H-SCB and from 108.6 to 39.6 kJ mol^{-1} to H-RH, with the increase in conversion degree ($0.10<\alpha<0.70$). The master-plot approach suggested that the thermal degradation of H-SCB and H-RH could be probably described by a chemical reaction and a three-dimensional diffusion model, respectively. These results were interpreted through thermodynamic parameters, revealing that the process of decomposition is endothermic and favorable for the formation of the products, with the thermal decomposition of H-SCB being more reactive (ΔS=-0.08 kJ mol^{-1} K^{-1}) compared to H-RH (ΔS=0.24 kJ mol^{-1} K^{-1}). The additional challenges in the technology of carbonaceous materials for energy and hydrocarbon generation lead to great opportunities for innovation and growth through integrated platforms, leveraging by-products such as humins.

Continuous fermentation of methanotrophic culture at lab scale prototype for bioprotein production process

I. Oshkin and D. Chernushkin, Federal Research Centre "Fundamentals of Biotechnology", Moscow, Russian Federation; I. Nizovtseva and I. Starodumov, Ural Federal University named after the first President of Russia B.N. Yeltsin, Ekaterinburg, Russian Federation*

The modern level of biotechnology, the requirements for its economic parameters, a high level of industrial and environmental safety that must be ensured at biotechnological enterprises, presuppose the use of the latest methods of controlling the biosynthesis process based on a deep, comprehensive understanding of the process. The biotechnology industry development plan indicates an increased demand for innovation and development in the field of biotechnology and the need to promote scientific research and building science-intensive production and use of materials obtained on a biological basis. Protein production from natural gas is based on the unique properties of methanotrophic bacteria to use methane as the only source of carbon and energy. Within the presented research we share on results of the lab scale study which was performed in order to achieve the key parameters of the bioprotein production process. Continuous fermentation was carried out for 30 days in the stirred-tank bioreactor with a working-volume of 2 l. Equal volumes of methane and air were supplied to the bioreactor as it was previously used in industrial bioprotein production process. Concentration of mineral nutrients was monitored and controlled during the whole period of bacterial growth. The biomass production rate was stable in a continuous fermentation mode and constituted 4 g/l/h. The protein content of microbial biomass reached 70% of the total dry weight. Contents of key amino acids lysine and methionine were relatively high and accounted for 0.9 and 0.2 g per 100 g of dry biomass, respectively. Fermentation conditions applied in the study resulted in obtaining the required values of biomass production rate and

protein content. Thus, lab model of 2 l bioreactor can be used as a small-scale prototype for bioprotein production process.

Engineering *Streptomyces* to Capture Value from Lignocellulosic Biofuel Conversion Residue

C. Wadler, K. Throckmorton and M. Thomas, University of Wisconsin, Madison, Madison, WI, USA*

Current methods of switchgrass hydrolysate fermentation to bioethanol leave behind about 60% of the organic material in the hydrolysate after ethanol distillation. This material is referred to as conversion residue (CR). To increase the economic viability of lignocellulosic biofuels, we are engineering *Streptomyces* species to maximize the metabolism of CR carbon into valuable bioproducts. From a library of 120 phylogenetically distinct *Streptomyces* isolates, we generated a collection of *Streptomyces* that produce lycopene from CR as a reporter for their potential to produce isoprenoids. The genetic element used in constructing this reporter is mobilizable between *Streptomyces* species and we have constructed further plasmids using a combination of traditional cloning techniques and Golden Gate assembly that allow for rapid alterations in expression levels and the generated bioproduct.
Initial screens of the engineered *Streptomyces* reporter strains showed a wide range of lycopene production levels. We were able to further increase lycopene production by introducing two different pathways that produce isoprenoid precursors: an optimized version of the native methylerythritol phosphate (MEP) pathway or the mevalonate (MEV) pathway with a constitutive promoter. These strains also showed differences in carbon utilization of the CR, suggesting other avenues for engineering to increase bioproduct formation while we expand our repertoire of target compounds for bioproduct generation.

Deployment of an engineered methanotroph as a novel platform for biomanufacturing chemicals from methane and xylose

E.Y. Lee, A.D. Nguyen and T.H.T. Chau, Kyung Hee University, Yongin, Korea, Republic of (South)*

Although methane, with its abundance and low price, is considered a potential carbon source for industrial biotechnology, low productivity of methane bioconversion in methanotrophs has limited its applications in industrial biomanufacturing. In this research, a novel methanotrophic platform was developed to achieve highly efficient bioconversion, with the production of shinorine – a sunscreen material- as a model compound. *Methylotuvimicrobium alcaliphilum* 20Z has an intrinsic high carbon flux through the non-oxidative branch of pentose phosphate pathway in ribulose monophosphate (RuMP) cycle. Thus, we have introduced the biosynthesis pathway of shinorine, a non-oxidative RuMP cycle-derived secondary metabolite, into *M. alcaliphilum* 20Z, which resulted in an initial titer of 0.52 mg/L. The xylose utilization pathway was also introduced into *M. alcaliphilum* 20Z to reinforce the RuMP cycle, which resulted in a significant increase in the growth rate and shinorine production of the xylose-utilizing recombinant cultured in methane and xylose. With additional enhancements by re-designing a synthetic operon of the shinorine biosynthesis pathway and supplying the micromineral in the culture medium, a shinorine titer of 17.13 mg/L was achieved in the final engineered strain cultured in methane and xylose, which was an increase of 33 fold compared to the titer of initial strain. The final titer, productivity, and yield of shinorine synthesized from this novel methanotrophic biocatalyst are comparable to those in industrial workhorses. The versatile applicability of this novel platform was demonstrated by remarkably enhancing the production of 2,3-butanediol, acetoin, and 3-hydroxybutyric acid as well.

Metabolic engineering of type II methanotroph, *Methylosinus trichosporium* OB3b for production of cadaverine from methane

E.Y. Lee, O.K. Lee, T.T. Nguyen and S. Naizabekov, Kyung Hee University, Yongin, Korea, Republic of (South)*

Cadaverine is an important C5 platform chemical with a wide range of potential applications in industry, agriculture, and medicine. Bio-based production of cadaverine from methane, attractive low-priced feedstock is a promising and sustainable alternative to the petroleum-based chemical synthesis. In this study, we report development of metabolically engineered strain of a type II methanotroph, *Methylosinus trichosporium* OB3b, capable of producing lysine and cadaverine from methane. A genome-scale metabolic model (GEM) of *M. trichosporium* OB3b was developed, which was used to predict the metabolic fluxs leading to lysine and cadaverine, and employed to find metabolic engineering targets for improved production of lysine and cadaverine. First, L-lysine decarboxylase, which directly converts L-lysine to cadaverine, was introduced into *M. trichosporium* OB3. Next, the lysine biosynthetic pathway was reconstructed, and the CO_2 assimilation efficiency was improved through overexpression of pyruvate carboxylase, and the antiporter was overexpressed. The final engineered strain was able to produce 283.63 mg/L of cadaverine with a volumetric productivity of 6.52 mg/g DCW per d using a gas bioreactor system. This work demonstrates the production of C5 diamine compound for bio-based polyamides from methane using engineered type II methanotrophic strains.

Bioethanol production from sugarcane hydrolysates using SHF strategy in a continuous process

C.M. Chavez Rodríguez, M.T. Ponce Noyola, A.C. Ramos Valdivia and H.M. Poggi Varaldo, CINVESTAV, Mexico City, DF, Mexico*

Biofuels are those fuels that are produce mainly from lignocellulosic biomass. Nowadays, bioethanol is one of the most produced biofuels globally.

Along with sugarcane bagasse, sugarcane pith is one of the most broadly available lignocellulosic biomass sources, but unlike the former one its lignin content is lower which makes it a promising raw material to produce bioethanol because of its high cellulose and hemicellulose content.

Four strategies have been documented to produce bioethanol from cellulosic hydrolysates, these are: (a) sequential hydrolysis and fermentation (SHF), (b) simultaneous saccharification and fermentation (SSF), (c) simultaneous saccharification and co-fermentation (SSCF), (d) consolidated bioprocess (CBP). In SHF process, hexoses and pentoses are generated during enzymatic hydrolysis of pretreated lignocellulosic biomass; then these sugars are consumed by the microorganism during fermentation. The main advantage of this strategy is that both hydrolysis and saccharification are carry out in their respective temperature and pH optimal conditions.

In this work we use a recombinant *Saccharomyces cerevisiae* RP2-BGL that expresses an extracellular b-glucosidase from *Cellulomonas flavigena* PR-22 strain that makes it capable of using cellobiose besides glucose as carbon source. Additionally, we will evaluate the feasibility and efficiency of bioethanol production by *Saccharomyces cerevisiae* RP2-BGL in a continuous sequential hydrolysis (SHF) as fermentation strategy.

Development of an electro-transformation protocol for genetic manipulation of *Bacillus coagulans*

L. Chen and M. Tumen-Velasquez, Oak Ridge National Laboratory, Knoxville, TN, USA; A. Guss and J.G. Elkins, Oak Ridge National Laboratory, Oak Ridge, TN, USA*

Microbial cells with robust substrate utilization capabilities and high tolerances to end-products and inhibitors are urgently needed to produce renewable platform bio-chemicals. *Bacillus coagulans* is a Gram-positive thermophile of industrial interest and has received renewed attention as a possible producer of lactic acid. *B. coagulans* can sustain growth within a broad range of temperatures while utilizing various carbon sources, including pentoses, hexoses, and oligomeric polysaccharides. They are also resistant to inhibitors from lignocellulosic hydrolysates. Considering their highly efficient sugar metabolism, if carbon flux is redirected towards other pathways instead of lactate pathway after further strain development by genetic and evolutionary engineering, *B. coagulans* strains hold immense promise as a starting point for the development of consolidated bioprocess from lignocellulosic biomass. *B. coagulans* strains have many appealing characteristics, but unfortunately, its genetic and metabolic

engineering has been fundamentally impeded. A prerequisite for genetic studies of *B. coagulans* is a high-efficiency transformation system that allows for DNA transfer. This study develops a detailed electroporation method through a systematic examination of the entire electroporation process factors. Key features of this procedure include selecting growth media and electroporation/recovery buffer, pre-treatment of wall-weakening agents, and DNA concentration. The transformation rate also varied depending on the pulse parameters. Using current strategies, we generally achieved a maximum efficiency of 10^4 transformants per microgram of plasmid pMTV80.

Ethanol organosolv delignification of spruce biomass for biorefinery applications

M.M. Martins, F. Carvalheiro and F. Gírio, National Laboratory of Energy and Geology, Lisboa, Portugal*

Lignin constitutes the largest renewable source of aromatic building blocks on earth and a valuable alternative source to produce fuels, chemicals and, materials in the near future. It can be obtained from different lignocellulosic materials, namely wood species. Norway spruce (*Picea abies* (L.) Karst.) is one of the most important coniferous species in Europe both for economic and ecological aspects, and with a long tradition of cultivation. This softwood contains around 30% of lignin and 40% of cellulose in its composition, which, together with its high availability, makes spruce an attractive feedstock for biorefinery applications.

Organosolv treatments are among the most promising processes for lignocellulosic biomass fractionation. Contrarily to the traditional pre-treatment methods, organosolv can efficiently separate the main biomass components (cellulose, hemicellulose, and lignin) into fractions with high valorization potential. Furthermore, organosolv lignins are usually recovered in high yields and good quality, providing adequate substrates for high-value applications.

In this work, spruce biomass was subjected to organosolv fractionation using ethanol/water mixtures (50:50) at 190 ºC (no-catalyst added), for a reaction time ranging from 60 to 120 minutes. The highest delignification yield was obtained for the longest pre-treatment time that also led to significant hydrolysis of hemicellulose. The solid fraction obtained, contained near to 60% cellulose, which makes it particularly suitable for saccharification. These solids were subjected to enzymatic hydrolysis with 15, 30, and 60 FPU/g_{solids}. Soluble lignin was recovered by water precipitation with a yield of 8.3 g/100 g of biomass. The yields of hemicellulose-derived products, (glucose, xylose, mannose, acetic acid) and degradation compounds are presented, as well as the saccharification yields. The purity and composition of lignins obtained were assessed by gravimetric methods, elemental analysis, and infrared spectroscopy which enable to elucidate their valorization potential.

This work was funded by OXYMOD project (Forskerprosjekt - BIOTEK2021; Application Number: ES583910).

RuBisCO engineering and RNA-sensor based RuBisCO assay development for sustainable production of essential amino acids

A.P. Sarnaik, A. Mhatre and A.M. Varman, Arizona State University, Tempe, AZ, USA; M. Faisal, University Institute of Biochemistry and Biotechnology, Rawalpindi, Pakistan; R. Davis, Sandia National Laboratories, Livermore, CA, USA*

Undernourishment is a global issue, prevailing since decades. Hence, availability of nutritive, affordable food becomes necessary. Plant-based diet is sustainable and affordable, but there are certain essential amino acids which cannot be obtained from natural plant sources and need to be resourced from animal-based diet only.

We strategized to enrich RuBisCO, a common and abundant protein across all phototrophs, with essential amino acids, lysine and methionine. Based on Grantham's distance, the RuBisCO polypeptide sequence in *Synechocystis* sp. PCC6803 was modified. Four different RuBisCO (large subunit) variants were created, where replacements were performed distant from (rbcL1 and rbcL3) or around (rbcL2 and rbcL4) the active site, thereby increasing Lys or Met content by two-fold. *In silico* structural comparison was performed to ensure protein folding is unaffected, before analysing their *in vivo* performances.

We introduced the gene isoforms at natural *rbcL* locus in *Synechocystis* (WT) through homologous recombination. Comparative growth studies confirmed there was no effect on growth of mutants over WT, indicating neutral mutations. In parallel, we developed a sophisticated biochemical activity assay and RNA sensor-based fluorescence assay for quantitative and semi-quantitative estimation of RuBisCO activity. Results exhibited that all the isoforms of RuBisCO displayed uniform functionality, indicating successful protein engineering.

Simultaneously, genes for the RuBisCO variants were overexpressed in *E. coli* to experimentally quantify lysine/methionine levels, and to verify if the cells were able to cope up with the increased demand for lysine and methionine without further pathway engineering. GC-MS analysis of the engineered *E. coli* cell lysates established, ~75% increase in Met, and ~65% increase in Lys content in the corresponding variants relative to WT.

As RuBisCO is an integral enzyme in plant photosynthesis, prospectively these modifications can be safely translated to the food crops, seeds, legumes ultimately improving their nutritive value.

Discarded vegetal residues as low-cost carbon source for lipid production

M. Gallego-García[*]*, D. Moreno, A. González, R. Iglesias and M.J. Negro, CIEMAT, Madrid, Spain*

In South-western Europe, the horticultural intensive type systems dedicated to the production of greenhouse vegetables represents one of the main industries generating organic waste. For instance, the fruits that do not meet the required quality standards for sale are removed during harvesting and are considered as waste. These residues may, however, represent an interesting resource to use in different bioprocesses as a low-cost raw material.

Carbohydrates from discarded vegetable products can be easily extracted by collecting the corresponding juice after a crushing procedure. These carbon sources might be used, for example, in the production of single cell oils using oleaginous yeasts, to later convert these microbial oils into biofuels. This is possible because oleaginous yeasts have the ability to accumulate more than 20% lipids to their dry weight. However, with high carbon/nitrogen (C/N) ratios they can achieve a lipid accumulation of more than 70% with respect to their cellular biomass.

In this work, discarded vegetables (tomato, pepper and watermelon) were employed as raw material for microbial oil production. The liquid fraction of this residue was mainly composed by carbohydrates (including glucose, fructose and sucrose), and were used as a culture media for lipid production using the yeast *Cryptococcus curvatus*. Depending on the studied residue, the sugar content varies from 30 g/L up to 65 g/L.

Different C/N ratios (15, 30 and 50) were tested to evaluate the percentage of lipids and the profile of fatty acids accumulated by yeast under fruit-derived media. The best result was achieved when using the discarded pepper medium with a C/N ratio of 50, obtaining a 40% accumulation of lipids and showing a fatty acid profile similar to that obtained from vegetable oils in the conventional biodiesel production.

Acknowledgements: Project ENE2017-86864-C2-1-R (AEI/FEDER, UE). María Gallego would like to thank “MICINN” and ESF/UE (Grants Ref. PRE2018-086317)

Two-stage hydrogen and methane production using residual fermented solid obtained after enzymatic biodiesel production

S. Buback dos Santos[*]*, M. de Oliveira Faber and E. Cristina Gonçalves Aguieiras, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil; M. Antunes Pereira Langone, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, Brazil; V. Santana Ferreira-Leitão, National Institute of Technology, Rio de Janeiro, Brazil*

The objective of this study was to evaluate the sequential production of H_2 and CH_4 using, as raw material, the residual biocatalyst from enzymatic biodiesel synthesis. The biocatalysts synthesis through solid-state fermentation (SSF) using residual biomass from the vegetable oil industry has been investigated to reduce the costs of enzymatic biodiesel production. This biomass, after SSF, results in a biocatalyst with lipase activity called Fermented Solid (FS). The biodiesel synthesis by esterification

reactions of Palm Fatty Acid Distillate (PFAD) and ethanol, using FS as the biocatalyst, produces a high amount of Residual Fermented Solid (RFS). For each 1 L of biodiesel produced, approximately 350 g of RFS is generated. RFS is rich in organic matter, which makes it attractive for sequential biological production of H_2 via dark fermentation and CH_4 via anaerobic digestion. Thus, the integration of these processes is interesting because it adds value to the biodiesel chain and minimizes the environmental problems, besides it is also an alternative for clean and decentralized energy production. For both H_2 and CH_4 productions using RFS, an anaerobic sludge from the biodigester of a sewage treatment plant was used as inoculum. For the hydrogen production the concentration of RFS 31 g_{RDFS}/L, was investigated. The highest hydrogen production (239 ± 44 mL_{H2}/L_{medium}) was observed after 24 h using 31 g_{RDS}/L. Additionaly, the methane production obtained from the Hydrogen Production Liquid Waste was 204 ± 13 mL_{CH4}/g_{COD}, which represented 61% of efficiency.

From sugarcane bagasse-derived humins towards porous materials with high-energy potential

M. Gontijo Souza Macedo, J.C. de Jesus Gariboti, E. Ladeia Gomes, R. Fernandez Felisbino and L. Plazas Tovar, Federal University of São Paulo, Diadema, Brazil; E. Savioli Lopes, University of Campinas, Campinas, Brazil; Y. Camacho Ardila, Pedagogical and Technological University of Colombia, Tunja, Colombia*

Humins (HUs) valorization, from economic and environmental perspectives toward improving the lignocellulosic biomass conversion into value-added products, is an urgent action concerning to *triple bottom line* model inner the circular economy concept. In this work, two HUs derived from the catalytic upgrading of sugarcane bagasse, via platform chemical (Levulinic Acid), studied in our previous work [https://doi.org/10.1007/s12155-020-10124-9], were investigated: HUs-1: material obtained by three-step acid catalyzed treatment and HUs-2: material obtained in one-pot catalytic biorefining process. HUs characterization using proximate and ultimate analysis confirms that volatile matter (57.00% and 45.04%) and fixed carbon (38.68% and 51.91%) of HUs-1 and HUs-2, respectively, supplies most of their gross energy (22.4 and 19.00 MJ kg^{-1}, respectively). The higher heating values of HUs make them attractive feeds for thermochemical routes. The elemental composition reported a high Carbon (60.61 and 68.55%) and Oxygen (29.55 and 23.50%) contents and a lower Hydrogen content (<5%). The moisture content of these materials was relatively low. In the case of ash content, both materials had a low content (<4.3%) and a high H/C ratio: 0.95 ± 0.02 for HUs-1 and 0.75 ± 0.01 for HUs-2. The O/C ratios were relatively low: 0.37 for HUs-1 and 0.26 for HUs-2. Minor structural differences were observed through the empirical formula $CH_{0.95}O_{0.37}$ and $CH_{0.75}O_{0.26}$ of HUs-1 and HUs-2, respectively. The N_2 sorption isotherms reported a noticeable absorption of N_2, being typical of microporous structures, at low relative pressures (p/p_0<0.15) followed by an approximately linear region (0.15< p/p_0<0.75) and ending with adsorption increased when p/p_0≈1.0. The possible presence of macropores or probable clusters was revealed by the slight hysteresis at p/p_0 of 0.75−1.00 for both HUs. Therefore, both carbon-rich materials can be recognized as valuable porous materials in the production of promising carbon-based chemical building blocks within the concept of biorefinery.

Synthesis, characterization, and utilization of aminated CELF lignin

X. Meng and A. Ragauskas, University of tennessee Knoxville, Knoxville, TN, USA; B. Scheidemantle and C. Cai, University of California, Riverside, Riverside, CA, USA*

In this study, Co-solvent Enhanced Lignocellulosic Fractionation (CELF) pretreatment and Mannich reaction were combined to generate aminated CELF lignin.The physicochemical, morphological, and thermal properties of the aminated CELF lignin were characterized to confirm the successful grafting of amines onto the lignin.The obtained aminated CELF lignin is subsequently applied for removal of azo dye from the aqueous solution. The aminated CELF lignin proved to be an effective azo dye-adsorbent, demonstrating considerably enhanced dye decolorization with a maximum adsorption capacity of Direct blue dye around 502.7 mg/g. The kinetic study suggested the adsorption process conformed to a pseudo-second-order kinetic model and the isotherm results showed that the modified lignin-based adsorbent exhibited monolayer adsorption. We also demonstrated a novel strategy that uses this aminated CELF

lignin to produce bio based non-isocyanate polyurethane (NIPU) by reacting it with bicyclic carbonates. The thermal stabilities of NIPUs were improved because of the addition of aminated lignin, and NIPU containing 47 wt% lignin showed the highest tensile strength (1.2 MPa) and lowest elongation at break (20%).

High-throughput promoter optimization for improved biobutanol *in vivo* biosensor

N.M. Kim, R. Sinnott and N. Sandoval, Tulane University, New Orleans, LA, USA*

Rising costs and environmental stress tied to fossil fuels has motivated industrial interest in butanol as a renewable fuel source and alternative to gasoline. Biobutanol production from renewable feedstocks has been demonstrated, but the production yield remains economically unviable. High-throughput screens on non-growth-related phenotypes and dynamic butanol-dependent regulation represent powerful metabolic engineering strategies that are largely unavailable to these efforts. This capability gap is due to a lack of inducible transcription factor/promoter pairs with user-defined controls. A butanol-responsive transcription factor, BmoR, and its cognate promoter PBMO have been previously described in the native form, but PBMO remains relatively uncharacterized and optimizing its function by sequence modification has not been attempted. In this work, we demonstrate the engineering of the PBMO promoter at the nucleotide level to improve biosensor characteristics, specifically an improved dynamic range, and to generate synthetic promoters. To this end, we use massively parallel reporter assays to study the sequence-function relationship of PBMO using the 'sort-seq' method. A mutagenized PBMO library cloned upstream of *gfp* in *E. coli* was induced with butanol and sorted into activity-based (i.e., fluorescence-based) populations. These populations were subsequently sequenced via NGS and their PBMO mutations correlated with changes in *gfp* expression, enabling construction of synthetic promoters with desirable characteristics. Best of the mutated promoters demonstrated over 4-fold increase in dynamic range. Additionally, sort-seq approach identified sites that are essential to the function of the biosensor and those that increase the sensor output. This work can enable rational strategies to edit the dynamic range of transcription factor-based biosensors.

Potential redox-dependent regulation in oleaginous *Rhodococcus sp.* during lignin conversion

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

Cysteine-based redox modification of proteins plays an essential role in redox cellular signaling and regulation, which alters protein structures and activities, thus modulates a variety of pathways and biological processes. A system-level profiling of protein redox state and modification will help deepen understanding of regulatory event of lipogenesis, which is proposedly impacted by using lignin as a carbon source. In this study, a mass spectrometry-based direct detection workflow was developed and applied to investigate conversion of glucose or corn stover alkali lignin by *Rhodococcus sp.* under nitrogen-limiting condition. Results showed that a great number of proteins were differentially oxidized using lignin or glucose as the sole carbon source, affected by cellular redox state and independent from protein expression level. During lignin fermentation, oxidative stress response such as catalases and peroxiredoxins were significantly up-regulated, suggesting a cellular oxidative environment. However, while a group of proteins involved in carbohydrate metabolism (e.g. fructose-bisphosphate aldolase, pyruvate kinase), fatty acid synthesis (e.g. 3-oxoacyl-[acyl-carrier-protein] reductase, acetyl/propionyl-CoA carboxylase) tended to be more oxidized, certain proteins involved in lignin/aromatic degradation (e.g. 3-ketoacyl-CoA thiolase, dihydrolipoamide dehydrogenase) and fatty acid degradation (e.g. acyl-CoA dehydrogenase) were less oxidized during lignin conversion. We herein propose a redox-dependent regulation that specifically modifies protein thiols in a targeting manner to modulate protein activities in order to fulfill the carbon, energy and redox metabolism requirement while using different carbon sources during lipogenesis.

Towards zero plastic waste: Identifying bioplastic degradation genes and enzymes in *Burkholderia*

Z.L. Yap, H. Tesfu, J.P. Hawkins, I. Oresnik, D.B. Levin and S.T. Cardona, University of Manitoba, Winnipeg, MB, Canada*

Many microorganisms produce polyhydroxyalkanoates (PHAs), which are biological polyesters that are used as intracellular storage compounds. Some PHA polymers have physical properties that are similar to polymers derived from petrochemicals ("petro-plastics"), and thus could displace some petro-plastics in the future. Upon microbial death, PHAs are released to the environment. Thus, microorganisms have evolved to produce extracellular PHA depolymerases to breakdown exogenous PHA and utilize them as a carbon source for growth and metabolism. Most of the characterised PHA depolymerases degrade short chain length (scl)-PHAs. However, a few medium chain length (mcl)-PHA-degrading enzymes that have been identified. Most of the characterized extracellular mcl-PHA depolymerases are from the genus *Pseudomonas.* Bacteria in the genus *Burkholderia* are phenotypically similar to *Pseudomonas* and have extraordinary genomic and metabolic plasticity. We hypothesize that *Burkholderia* species cultured under certain environmental conditions will adapt to the available nutrients and turn-on different metabolic processes, including extracellular mcl-PHA depolymerases activity. Using a method to screen for extracellular mcl-PHA depolymerases, we found *Burkholderia vietanmiensis* LMG 16232 was able to degrade extracellular mcl-PHA. We sequenced the genome of *B. vietanmiensis* LMG 16232 and performed *in silico* analyses to identify potential genes responsible for this activity. We also developed a transposon mutagenesis and screening method for *B. vietanmiensis* LMG 16232 to identify putative genes associated with extracellular mcl-PHA degradation. Once we identify the transposon mutants that have disrupted activity, we will validate the identified genes by using CRISPR-Cas tools. Identification of PHA degradation genetic elements in *Burkholderia* will contribute to the development of industrial bioplastic recycling processes.

Enzymatic Synthesis of Xylan Microparticles with Tunable Morphologies

P. Smith, S. Ziegler and Y. Bomble, National Renewable Energy Laboratory, Golden, CO, USA; T. Curry, W. Barnes, W. York, M. Pena and B. Urbanowicz, University of Georgia, Athens, GA, USA*

Xylans are a diverse family of hemicellulosic polysaccharides found in abundance within the cell walls of nearly all plants and many algal species. Here we demonstrate the ability of a recombinantly expressed xylan synthase(*Kf*XYS1) to synthesize xylan microstructures with well-defined and tunable morphology *in vitro* using UDP-xylose as a donor substrate and short xylan oligosaccharides as acceptor substrates. We have investigated the conditions under which xylan microparticles form, and demonstrate the ability to synthesize structures with unique shapes, morphologies, and compositions influenced by the modification of, for example, the fine structural elements of the oligomeric xylan acceptor substrates used to initiate polymerization. We have investigated the characteristics of the xylan polymers which comprise the microparticles, including their average degree of polymerization and polymer conformation. Furthermore, we have probed the ability of glycosyl hydrolases to degrade xylan microstructures, as well as demonstrated the ability to functionalize xylan microstructures via chemical modification. Together, these results provide a model system to investigate influential factors in polymer-polymer interactions, and suggest a possible synthetic biology based route to new biobased materials with favorable properties for biocompatible and renewable applications.

Aspergillus awamori endoglucanases-rich fraction enhances liquefaction during high solids enzymatic hydrolysis

R. Pereira Espinheira and A. Sant'Ana da Silva, National Institute of Technology, Rio de Janeiro, Brazil; V. Alves Lima Rocha, T. Martins Guimarães, L.F. Costa Ramos, Y. Martins da Silva, G. B. Domont, F.C. Sousa Nogueira, R. Sposina Sobral Teixeira and E. Pinto da Silva Bon, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil; C. Amorim Oliveira, Federal Institute of Education, Science, and Technology of Rio de Janeiro, Rio de Janeiro, Brazil; M. Fernandes de Souza, Ghent University, Ghent, Belgium*

Enzymatic hydrolysis of lignocellulosic biomass at high-solids load is a required condition for economic feasibility. However, the enzymatic hydrolysis during the liquefaction stage is lessened by the deficiency of free water in the reaction medium, hindering the depolymerizing activity of the endoglucanases. This drawback could be improved by the use of endoglucanases with higher efficiency. In this study, it was evaluated the effectiveness of an endoglucanases-rich fraction produced by a particular strain of *Aspergillus awamori*. Hydrolysis assays were conducted using 30% (dry weight) solids, at 50°C and 200 rpm for 24 h. Experiments were carried out using the commercial enzymes, CT2 and CEL188, and these enzymes supplemented with the *A. awamori* culture supernatant (CT2Aa and CEL188Aa). The supplementation decreased the media viscosity by 10-fold and improved glucose release by 20% after 24 h. The *A. awamori* culture supernatant was fractionated by size-exclusion chromatography. An endoglucanases-rich fraction was characterized by mass spectrometry. This fraction was supplemented to CT2 (CT2F3) and used to high-solids load enzymatic hydrolysis in comparison to CT2 and CT2Aa. Results for 6 hours hydrolysis showed for CT2F3 and CT2Aa equivalent profiles for glucose release and viscosity decrease. These data indicate that *A. awamori* endoglucanases are efficient for the liquefaction phase of high-solids enzymatic hydrolysis.

A flexible kinetic assay efficiently sorts prospective biocatalysts for PET subunit hydrolysis

J. Lusty Beech, R. Clare, D. Kim and J. Dubois, Montana State University, Bozeman, MT, USA; E. Erickson, National Renewable Energy Laboratory, Golden, CO, USA; J. McGeehan, University of Portsmouth, Portsmouth, United Kingdom; G. Beckham, National Renewable Energy Laboratory, USA, Golden, CO, USA*

Esterases are enzymes with wide-ranging biological, commercial, and biotechnological applications. The canonical esterase activity assay utilizes readily hydrolysable alkyl-*p*NP esters, which react as nearly universal esterase substrates but which are limited in structural variability. The use of pH indicator dyes offers an effective, affordable, and easily quantifiable alternative for monitoring carboxylic esterase reactions in real time. Here, we refined a UV/visible assay using pH-sensitive aryl sulfonate dyes to quantify hydrolysis of the polyethylene terephthalate (PET) plastic subunit *bis*-(2-hydroxyethyl) terephthalate (BHET) in real time, and applied it to a suite of 24 known PETases and diverse commercial esterases in microplate format. This approach quickly identified one known PET and a well-known commercial esterase as effective catalysts against BHET under mild conditions (turnover number ≥100 in 48h, pH 8, 37 °C). The method was shown to be amenable to multiple pH/temperatures, and semi-pure enzymes in solution or freeze-dried cells.

Formulation of fish waste as a low-cost fermentative nutrient for lactic acid production by *Lactobacillus pentosus*

S. Shi, J. Li and D. Blersch, Auburn University, Auburn, AL, USA; M. Johnson, Morehouse College, Atlanta, GA, USA*

The microbial fermentation process for lactic acid requires certain types and amounts of nutrient. Our previous studies showed that fish waste could be used as an inexpensive nutrient source for lactic acid bacteria, but a relatively high dosage of fish waste is needed. The introduction of high loading of fish waste brought in impurities which complicated the downstream processing. In this study, the formulation of fish waste with the supplement of small amounts of yeast extract was investigated to reduce the impurity input. Three levels of fish waste (10, 15, and 30 g/L) were investigated with 1-5 g/L yeast extract for the lactic acid fermentation using 100g/L glucose as substrate. Satisfactory results were obtained with the optimal formulation of 10 g/L fish waste + 5 g/L YE, which gave 99.5% lactic acid yield with productivity of 2.764 g/L/h. Experimental data showed good fitness to an empirical kinetic model with regression coefficient (r^2) in the range of 0.931 to 0.997.

Understanding Effects of Plant Mineral/Ash in Biomass on Pretreatment and Enzymatic Hydrolysis of Corn Stover

F. Fnu[*]*, Washington State University, Richland, WA, USA, J. Liu, Pacific Northwest National Laboratory, Richland, WA, USA and B. Yang, Washington State University, RICHLAND, WA, USA*

The minerals/ash in lignocellulosic biomass have major effects on both the biorefinery feedstock quality control and biofuel production yield. This is especially true for current thermochemical conversion processes. However, the roles of plant minerals in biological conversion processes have not been thoroughly studied. In this study, both high-ash (hypothetical, 20% addition of ash) and low-ash (without additional ash) corn stover samples were tested by using several current biomass pretreatment methods, i.e., liquid hot water, dilute acid, alkaline, tetrahydrofuran, γ-valerolactone, and ionic liquid pretreatments. Results showed that high ash content led to decreased sugar yields up to 14.61% across different pretreatments. However, the sugar yield from sequential enzymatic hydrolysis was not correlated with ash content. Mineral/ash content and its distribution in the pretreated samples, the enzyme specific surface area of cellulose, and enzyme activities were investigated to better understand mineral's role in the pretreatment and enzymatic hydrolysis of biomass.

Effect of ash in paper sludge on enzymatic hydrolysis and dehydration to produce 5-hydroxymethylfurfural

H. Park[*]*, D. Cruz, P. Tiller, R. Venditti, H. Jameel and S. Park, Department of Forest Biomaterials, Raleigh, NC, USA; D. Johnson, National Renewable Energy Laboratory, Golden, CO, USA; A. Mittal, Chemical and Biosciences Center, Golden, CO, USA*

Paper sludge is a fiber-rich waste material obtained from pulp and paper mills. Paper sludge has been suggested as a potential source to convert into value-added products such as bioethanol and levulinic acid since cellulose and other polysaccharides can be efficiently recovered from the sludge. However, a considerable amount of ash has been proposed as a major barrier to valorize the sludge making it necessary to remove ash previous to the enzymatic and chemical conversion of carbohydrates. 5-hydroxymethylfurfural (HMF), which is a dehydration product of hexoses, has been known as a renewable and essential intermediate chemical. In this study, the enzymatic hydrolysis and dehydration of de-ashed sludge were optimized for high yields of HMF and the impact of ash on both reactions was studied. Enzymatic hydrolysis of de-ashed sludge resulted in 92% of glucose and xylose conversion at 5 FPU cellulase/g o.d. sludge. Calcium carbonate played an important role in changing and managing the pH environment for cellulase due to reacting with hydrochloric acid in the first 6 hours of reaction. Dehydration, which followed enzymatic hydrolysis, was performed in a microwave reactor with 7.5 mM of aluminum chloride as a catalyst and a mixture of dioxane and D.I. water as the solvent. HMF yield showed 62% when the sludge-derived hydrolysate was dehydrated under these conditions. The effect of impurities in the hydrolysate, which were calcium carbonate and calcium chloride, was further investigated to understand impacts on HMF yield. Compared to the pure glucose solution, calcium chloride in the glucose solution had a significant influence in increasing HMF yield. The present work highlights the importance in evaluating the degree of impurities in sludge for high yields of carbohydrates and HMF.

Conversion of cheese whey to lactobionic acid using an engineered strain of Neurospora crassa

A. Poltorak, C. Mojica and J.Z. Fan[*]*, University of California, Davis, Davis, CA, USA; T. Kasuga, University of California, Davis, Department of Plant Pathology,, Davis, CA, USA*

Lactobionic acid (LBA) is a versatile, high-value, low-volume chemical with numerous applications in the food, pharmaceutical, cosmetic, and chemical industries. LBA is also biocompatible, biodegradable, and has advanced cellular recognition properties. Microbial production of LBA has the advantages of high selectivity and the ability to use cheese whey, a waste stream from the dairy industry, which is rich in

lactose and is a strong candidate for sustainability efforts. However, microbial LBA production at an industrial level has not yet been developed as successfully as other organic acids, despite attempts being made to use wild type bacteria, fungi and yeast strains. In this research, an engineered *Neurospora crassa* strain was used to produce LBA using sustainable cellulosic biomass as a substrate to convert lactose sourced from low value deproteinized cheese whey powder to LBA. Our results show that the engineered strain achieved an LBA yield of 87% and an average productivity of 0.77 g/L/h over a 187-hour fermentation period.

CLIMATE RESILIENCE: MODELING THE IMPACTS OF YIELD VARIABILITY ON SWITCHGRASS SUPPLY CHAINS

H. Stauffer, Penn State University, State College, PA, USA, T. Richard, PhD, The Pennsylvania State University, University Park, PA, USA and E. Webb, Oak Ridge National Laboratory, Oak Ridge, TN, USA*

The successful commercialization of advanced lignocellulosic biofuels in the United States will require understanding and management of uncertainty and risk along the feedstock supply chain. Uncertainty in the biofuel industry is perpetuated by climate, weather, and market demand/price fluctuations. These factors greatly affect crop yield and yield stability and have downstream risks, especially for feedstocks like switchgrass that have few alternative markets and thus limited reserve capacity for years when yield is low. Little is known about the impacts of yield variability on the switchgrass bioenergy supply chain from a sustainability and economic perspective. This study attempts to address this gap by measuring the impacts of a genetically improved drought-tolerant switchgrass variety (10% increase in photosynthetic rate and 10% decrease in tissue death) on a standardized biomass supply chain (BSC). Harvesting, on-site storage, and transportation to a biorefinery were modeled in the supply chain through an ExtendSim Pro application for Iowa (IA), Tennessee (TN), and Pennsylvania (PA). Simulated field-level yield values were collected from the daily time-step biogeochemical model, DayCent, over a span of 30 years (2020-2050) and the area weighted county average yield was applied to 5-25% of designated abandoned land within the supply shed. The counties selected in these states for switchgrass production were chosen using the Department of Energy's (DOE) 2016 Billion Ton Report yield density maps. Preliminary results from the Iowa case study show that in projected low yielding, 'bad weather' years, the drought tolerant switchgrass variety was higher yielding than the base case variety and required less land, resulting in a potentially smaller economic burden on the supply chain compared to the excess land needed to satisfy the biorefinery in the base case scenarios. The conclusions gathered from these integrated models will enable greater informed decision making regarding the U.S. switchgrass BSC for all stakeholders.

Bioprospection of CAZYmes coded in the genome of the white-rot basidiomycete *Pycnoporus sanguineus*

M. Garrido, MS, J. Topalian and E. Campos, Ph.D., University of Buenos Aires/ National Institute for Agricultural Technology, Buenos Aires, Argentina; R. Brunecky, Ph.D., National Renewable Energy Laboratory, Golden, CO, USA; M. Landoni, Ph.D., University of Buenos Aires/ Carbohydrate Research Center (CIHIDECAR), Buenos Aires, Argentina; S. Wirth, Ph.D., University of Buenos Aires/ Institute for Biodiversity and Experimental and Applied Biology, Buenos Aires, Argentina*

Existing biomass conversion schemes typically rely on a combination of chemical and enzymatic treatments that include a pretreatment step to reduce recalcitrance exposing the crystalline cellulose core, which is then hydrolyzed by cellulase enzymes. Reducing-end acting cellobiohydrolases (EC 3.2.1.176) are the main enzymes required for cellulose hydrolysis due to their enzymatic proficiency in cellulose depolymerization. The saprotrophic white-rot fungi *Pycnoporus sanguineus* is known as a potent degrader of plant biomass with the ability to degrade all lignocellulose components using a wide diversity of enzymes. After a thorough analysis of genomic and secretomic data available for this fungus, two cellobiohydrolases from family GH7, *Ps*Cel7A and *Ps*Cel7B, were selected for further characterization and recombinantly expressed in *Trichoderma reesei*. The desired proteins were successfully purified from the fungus's culture broth using Fast Purification Liquid Chromatography (FPLC) and the optimal temperature and pH of their enzymatic activity was determined with the commercial substrate *p*NP-

lactoside. Pretreated corn stover (PCS) hydrolysis was tested, resulting in approximately 40% glucan conversion after 5 days of incubation with both enzymes. The amount of cellobiose and cellotriose released by *Ps*Cel7B was determined by HPAEC-PAD using Phosphoric Acid Swollen Cellulose (PASC) as substrate. Enzymatic reactions were carried out with *Ps*Cel7B alone or in conjunction with *Ps*AA9A, a previously characterized lytic polysaccharide monooxigenase (LPMO) from the same fungus. LPMOs have their active site in a flat surface enabling them to access crystalline regions of cellulose and generate new chain ends for glycosyl hydrolases to act upon. The amount of cellobiose detected was not improved by adding *Ps*AA9A to the reaction, but the amount of cellotriose was significantly increased. This work provides valuable information about highly secreted CAZymes by *P. sanguineus* and serves as a starting point for the future development of engineered enzymes with enhanced performance for the deconstruction of cellulose feedstocks.

The Cellulose unraveling mechanism of family 48 glycoside hydrolases

R. Brunecky, Ph.D., B. Donohoe, J. Yarbrough, A. Tucker, M. Himmel and Y. Bomble, National Renewable Energy Laboratory, Golden, CO, USA*

The Family 48 glycoside hydrolase family is one of the most abundant glycoside hydrolase family in cellulolytic bacteria. For example, Cel48S is the most abundant enzyme in the *C. thermocellum* secretome and deletion of Cel48S from the *C. thermocellum* genome dramatically impairs growth of *C. thermocellum* on biomass[3]. Classically, these enzymes were originally thought to be bacterial analogs to the better studied family 6 and 7 exo-cellulases found in fungal biomass degrading systems which are the “heavy lifters” in fungal cellulase systems. To date, all family 48 cellulases characterized are inverting exo-cellulases and their active sites are in a tunnel similar to all other known exo-cellulases. Despite their low activity when measured in classical sugar release assays, GH48s are critical components for achieving high activity as part of synergistic bacterial enzyme mixtures. GH48 enzymes were tested for their activity on both Avicel and Cladophora cellulose as representative cellulose fibril model substrates. As previously reported by our group and others the overall activity of the GH48s was quite low when measured by soluble sugar release. However, GH48s appear to have a unique mechanism to unravel and defibrillate crystalline cellulose. This mechanism may not be their inherent catalytic activity, but maybe the mechanism by which they synergize with other bacterial enzymes. This relationship can be thought of as the opposite of the classical endo-exo systems wherein endoglucanases create new reactive sites for tunnel based cellobiohydrolases such as Cel7s to work on. Here the tunnel based GH48s appear to create more accessible or more easily cleavable surfaces for bacterial processive endoglucanases to operate on.

Expression of fungal laccase in *Pichia pastoris* and characterization of their activity using nanostructure-initiator mass spectrometry assay

L.T.M. Pham, K. Deng, S. Singer, B. Simmons and K.L. Sale, Joint BioEnergy Institute, Emeryville, CA, USA*

Lignin is the most abundant renewable source of aromatics on earth and conversion of it to valuable products is paramount to building an economically viable renewable biofuels industry. Potential biological routes to lignin conversion include depolymerization of lignin using enzyme cocktails derived from heterologously expressed ligninolytic enzymes such as laccases. However, studies of laccases have been limited by difficulties in expressing soluble, active enzyme and by a lack of high-throughput assays for quantifying catalysis of specific bond breaking reactions under different reaction conditions. The objective of this work was to generate fundamental understanding of the factors controlling laccase catalyzed depolymerization of lignin and bond breaking specificity. Two laccases from a basidiomycete fungus in the Polyporaceae family that causes white rot on the surface of hardwood (Cer_lc1, Cer_Lc2), and one laccase from a *Vitis vinifer* pathogenic fungus *Hymenochaetales,* which is the first representative of this order to have its genome sequenced (Fom_lac), were studied. These laccases were expressed in *Pichia pastoris* and characterized for their catalytic performance, thermal stability and solvent stability. To improve our understanding of the mechanism(s) underlying laccase catalyzed degradation of lignin, we quantified b-aryl ether, Ca-Cb, and Ca-Caryl bond cleavage in model lignin-like dimers synthesized

synthesized for use in a nanostructure-initiator mass spectrometry (NIMS) assay. We present detailed studies of the effect of pH and of the natural mediator syringaldehyde on catalysis of the three types of bond cleavage. This study also provides a comprehensive understanding of the structure-function and the structure-stability relationship of these novel fungal laccases, which will facilitate developing this important class of enzymes for applications in the conversion of lignin to valuable products and their use in biorefinery implementations.

Different Transcriptome and Translatome Alteration of *Synechocystis* sp. PCC 6803 in Response to Photosynthetic Inhibitory Conditions

S.H. Cho, Y. Jeong, S. Cho and B.K. Cho, Korea Advanced Institute of Science and Technology, Daejeon, Korea, Republic of (South); S.J. Hong and C.G. Lee, Inha University, Incheon, Korea, Republic of (South); H. Lee, College of Pharmacy, Gachon University, Incheon, Korea, Republic of (South); H.K. Choi, Chung-Ang University, Seoul, Korea, Republic of (South); D.M. Kim, Chungnam National University, Daejeon, Korea, Republic of (South)*

Cyanobacteria are promising industrial platforms owing to their ability to produce diverse natural secondary metabolites and non-native value-added biochemicals from CO_2 and light. To fully utilize their industrial potency, it is critical to understand their photosynthetic efficiency under various environmental conditions. Here, we elucidated the inhibitory mechanisms of photosynthesis under high light and low temperature stress conditions in the model cyanobacterium *Synechocystis* sp. PCC 6803. Under each stress condition, the transcript abundance and translation efficiency were measured using RNA-seq and ribosome profiling, and the genome-wide transcription unit architecture was constructed by data integration of transcription start sites and transcript 3'-end positions obtained from differential RNA-seq and Term-seq, respectively. Our results suggested that the mode of photosynthesis inhibition differed between the two stress conditions; high light stress induced photo-damage responses, while low temperature stress impaired the translation efficiency of photosynthesis-associated genes. In particular, the poor translation of photosystem I resulted from ribosome stalling at the untranslated regions, affecting the overall photosynthetic yield under low temperature stress. Our comprehensive multi-omics analysis with transcription unit architecture provides foundational information on photosynthesis for future industrial strain development. [Supported by the Korea Bio Grand Challenge (2018M3A9H3024759 to B.-K.C.)]

Developing CRISPR interference and knockout tools to produce muconate from lignin-derived aromatic compounds using *Rhodococcus opacus*

D. DeLorenzo, J. Diao, Y. Hu, R. Carr and T.S. Moon, Washington University in St. Louis, St. Louis, MO, USA*

Adipic acid, a monomer for nylon production, is currently produced from petroleum derivatives, requiring an alternative process for its sustainable production. Muconate can be converted into various chemicals, including adipic acid. Using non-model organisms, multiple labs have demonstrated muconate production from lignin-derived aromatic compounds, with glucose used as a growth substrate. *Rhodococcus opacus* is well suited for valorizing lignin (1-8), but developing this promising chassis had been challenging due to limited genetic engineering tools. To address this issue, we have developed various synthetic biology tools (9-12), including a gene repression system based on CRISPR interference (CRISPRi) and a knockout method. In this presentation, we discuss our CRISPRi tool's utility by demonstrating the inducible accumulation of muconate from aromatics. Additionally, its inducibility and partial repressibility are discussed compared to gene knockout's complete metabolic flux blocking. We also provide a cloning strategy that enables constructing multiple CRISPRi plasmids without any PCR step, facilitating this GC-rich organism's engineering. Our tools will be useful to engineer this chassis for lignin conversion into plastic monomers.

1. Chatterjee A, DeLorenzo DM, Carr R, Moon TS. 2020. *Current opinion in biotechnology* 64:10-6
2. Davis K, Moon TS. 2020. *Current opinion in chemical biology* 59:23-9

3. Henson WR et al. 2018. *Metabolic engineering* 49:69-83
4. Yoneda A et al. 2016. *Nucleic Acids Res.* 44:2240–54
5. Henson WR, Hsu F-F, Dantas G, Moon TS, Foston M. 2018. *Biotechnology for biofuels* 11:339
6. Roell GW et al. 2019. *Metabolic engineering* 55:120-30
7. Hollinshead WD, Henson WR, Abernathy M, Moon TS, Tang YJ. 2016. *Biotechnology and bioengineering* 113:91-100
8. Anthony WE et al. 2019. *Biotechnology for biofuels* 12:192
9. DeLorenzo DM, Henson WR, Moon TS. 2017. *ACS Synthetic Biology* 6:1973–8
10. DeLorenzo DM, Moon TS. 2018. *Scientific reports* 8:6019
11. DeLorenzo DM, Moon TS. 2019. *ACS Synthetic Biology* 8:1921-30
12. DeLorenzo DM, Rottinghaus AG, Henson WR, Moon TS. 2018. *ACS Synthetic Biology* 7:727-38

Unraveling the extracellular polysaccharide utilization system of *Cellulomonas* sp.

J. Topalian, O. Ontañon, Ph.D., M. Garrido, MS and E. Campos, Ph.D., Instituto de Agrobiotecnología y Biología Molecular (IABIMO), Instituto Nacional de Tecnología Agropecuaria (INTA), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina; P. Valacco, Ph.D, Centro de Estudios Químicos y Biológicos por Espectrometría de Masa (CEQUIBIEM-FCEN); Departamento de Química Biológica Facultad de Ciencias Exactas y Naturales Universidad de Buenos Aires (UBA-IQUIBICEN); CONICET, Buenos Aires, Argentina*

One of the main features of species from *Cellulomonas* genus is their ability to secrete (hemi)cellulolytic enzymes. We have studied the exo-proteome of two *Cellulomonas* isolates, *Cellulomonas* sp. B6, and *C. fimi* B-402 when grown on different lignocellulosic carbon sources and the genetic distribution of the genes encoding for the secreted proteins. Both isolates presented mainly xylanase activity, although the highest activity was observed by growth on different substrates: wheat bran (WB) for *Cellulomonas* sp. B6 (3.5 IU_{xyn}/ml) and waste paper (WP) for *C. fimi* (1.5 IU_{xyn}/ml). By mass spectrometry analysis of the extracellular fractions, we identified 31 and 26 GHs (out of 195 and 348 total proteins detected) in *Cellulomonas* sp. B6 and *C. fimi*, respectively. In the *Cellulomonas* sp. B6 WB extracellular extract, 2 GH10 xylanases and a GH62 alpha-L-arabinofuranosidase were amongst the most abundant proteins. In *C. fimi*, a single GH10 xylanase was the most abundant protein. In both extracts, a high abundance of extracellular proteins from ABC sugar transport systems was observed. Most CAZymes were encoded in distant regions of the genome, with few exceptions. In *Cellulomonas* sp. B6, we identified a cluster encoding a GH43 and a GH10 and both enzymes were detected in WB-secretome. In *C. fimi*, a GH10 and a GH62 identified in the secretome were encoded along with the sequence for a GH43. A common feature observed was the presence of coding sequences for sugar transporters (mainly ABC family) and transcriptional regulators along with genes encoding CAZymes. In summary, *Cellulomonas* sp. can secrete xylanases and other glycosyl-hydrolases with different relative abundance when cultured in lignocellulosic substrates. The coding sequences for these proteins are distributed along the genome, mostly surrounded by sequences encoding different transcriptional regulators and transporter systems.

Characterization of a β-xylosidase consisting of a glycoside hydrolase family 43 and a novel carbohydrate-binding module from *Paenibacillus xylaniclasticus*

D. Ito, E. Nakano and S. Karita, Mie University, Mie, Japan; C. Tachaapaikoon and K. Ratanakhanokchai, King Mongkut's University of Technology Thonburi, Bangkok, Thailand*

Paenibacillus xylaniclasticus strain TW1, a Gram-positive facultative anaerobic bacterium, has many genes encoding carbohydrate-active enzymes, including xylanolytic enzymes, for example glycoside hydrolase (GH) family 10 and 11 xylanases. TW1 has an enzyme consisting of GH43 β-xylosidase and a unknown region(Unk2). To characterize the enzyme (*Px*GH43), recombinant *Px*GH43 and Unk2 were expressed heterologously in *E. coli* strain BL21(DE3), and the ability of hydrolysis of *Px*GH43 and the binding of Unk2 to substrates were investigated. *Px*GH43 had activities against *p*-nitrophenyl-β-D-

xylopyranoside(*p*NPX) and *p*-nitrophenyl-α-L-arabinoside, 1.3U/μg and 0.8U/μg, respectively. The optimal pH value was 7 and the optimal temperature was 54°C, and it had a K_m of 0.23mM and a V_{max} of 0.013μmol/min/μg for *p*NPX. Tolerance to xylose of *Px*GH43 was found to be high because *Px*GH43 showed about 70% of its maximum activity in 200 mM D-xylose solution. Unk2 bound to birchwood xylan and oat spelt xylan. The absorption constant, K_a, and maximum amount of the bound protein, $[PC]_{max}$, for 0.05% oat spelt xylan were 2.0×10^5 M^{-1} and 10.1 μmol/g, respectively. The result indicated that Unk2 was a novel carbohydrate-binding module because a measured K_a value was within the K_a range of it. Based on the above, it was suggested that the enzyme hydrolyzed xylooligosaccharides released from xylan by xylanases.

Process Intensification and Scale-up of a Continuous Enzymatic Hydrolysis and Separation Process

D. Sievers, PE, J. Lischeske and J. McMillan, National Renewable Energy Laboratory, Golden, CO, USA*

Combining separate unit operations into one where the best of each part can be maximized is one of the benefits of process intensification. An example is the combination of lignocellulosic biomass enzymatic hydrolysis with the downstream solid-liquid separation step to produce clarified sugars ready for fermentation or catalytic upgrading. The productivity and endpoint yield of enzymatic hydrolysis both enjoy the benefits of reduced feedback inhibition through the continuous removal of sugars by incorporating separations into the reactor. Likewise, the efficiency of recovering clarified sugars from the enzymatic hydrolysis slurry can be enhanced by operating separations equipment at steady-state conditions simultaneously with the continuously fed hydrolysis process. A key parameter that enables greater processing capacity while also raising the risks of failure is the solids loading or concentration. Higher solids loading allows for smaller reactor vessels and results in clarified sugars of higher concentration; however, required pumping power increases, reactor agitation may become ineffective, and membrane flux suffers. Feedstock material attributes influenced by upstream pretreatment must also be scrutinized more carefully: dilute-acid pretreated and deacetylated-and-disc-refined feedstocks exhibit different characteristics that affect agitation and pumping. The authors invite you to further explore the process science enabling the scale-up of this technology from conceptual work at the bench to pilot-scale industrially-relevant equipment where the challenges and solutions of integration and process optimization are expounded upon.

Effect of Dilute Acid Pretreatment and Lignin Extraction Conditions on Lignin Properties for the Creation of Phenol Formaldehyde Resins

B. Saulnier and D. Hodge, Montana State University, BOZEMAN, MT, USA; M. Siahkamari and M. Nejad, Michigan State University, East Lansing, MI, USA*

The type of feedstock used, pretreatment employed, and method of lignin extraction/purification all influence the properties of lignins extracted from a biorefinery process. Structural and chemical properties of a specific lignin such as phenolic hydroxyl content and molecular weight effect the ability of a lignin to be incorporated into phenol formaldehyde (PF) resins for wood adhesives, and the subsequent material properties of the formulated adhesives. Our previous studies have shown that lignin generated from NaOH extraction of dilute acid pretreated corn stover can be used to formulate PF resins with 100% of phenol replaced which exhibit material properties that exceed industrial resin standards. We have previously hypothesized that the improved lignin incorporation in this study is largely due to high amounts of *p*-coumarate (*p*CA) present within grass lignins presenting additional phenolic hydroxyls for reaction with formaldehyde. In a previous study we have identified dilute acid pretreatment conditions that result in high hydrolysis yields while retaining high *p*CA levels within the biomass. In this study we expand on those finding by using corn stover to investigate a wide range of dilute acid pretreatment conditions, varying enzymatic hydrolysis conditions, and two different lignin extraction processes using sodium hydroxide and formic acid. These varied conditions were used to create a panel of lignins with varying properties which were completely characterized and most promising lignins were chosen for scaled up production and PF resin adhesive formulation. Using optimized dilute acid pretreatment conditions and

formic acid extraction we were able to formulate lignin-formaldehye resins which exceeded properties of previously formulated resins.

On relation between pretreatment conditions, supramolecular properties and efficiency of enzymatic hydrolysis of steam pretreated spruce

F. Caputo, V. Novy and L. Olsson, Chalmers university, goteborg, Sweden; B. Al-Rudainy and O. Wallberg, Lund university, Lund, Sweden*

The development in cellulosic ethanol production has mainly focused on agricultural biomasses. However, woody biomasses remain a very important feedstock for ethanol production even if the process is not yet economically feasible. In order to use lignocellulosic biomasses in a biorefinery, the use of harsh pre-treatment is necessary to overcome the recalcitrance of the biomass. These harsh pre-treatments result in the formation of inhibitors, that decrease the efficiency during the fermentation, and the loss of the hemicellulose, that lowers the total yields of the process.
We aim to increase the saccharification efficiencies of steam-pretreated (STEX) spruce that retains the hemicellulose in the material. We will investigate the different factors that affect the spruce recalcitrance like the accessibility and the diffusion of enzymes into the pretreated spruce, the non-productive binding of the enzymes towards the biomass and the presence of the lignin-carbohydrate bonds (LCs). A cellulolytic enzyme cocktail (Celluclast) was used for the enzymatic hydrolysis and it the role of different accessory enzymes (Xylanase, Mannanase, Laccase, Esterases, LPMOs) on the efficiency of the enzymatic hydrolysis was investigated. Here, we discuss the steam explosion (STEX) pre-treatment performed on spruce in different conditions (180°C/5min-autocatalyzed, 210°C/5min-autocatalyzed, 210°C/5min-SO_2 catalyzed), the determination of structural carbohydrates on the solid fraction, the enzymatic hydrolysis performed on the pre-treated materials using Celluclast and Novozyme 188. Preliminary data on the accessibility of the enzymes to the materials will be discussed.

Amazonian açaí (*Euterpe precatoria* Mart.) and Juçara fruit (*Euterpe edulis* Mart.) seeds as a potencial source of mannose

D. Marconi Miranda Carvalho da Silva, R. de Araújo Pontes, I. Santos Miguez and A. Sant'Ana da Silva, National Institute of Technology, Rio de Janeiro, Brazil*

Euterpe precatoria, popularly known as Amazonian açaí palm, is the second most common species among 28 other palms from the genus Euterpe found in the Amazon river delta, while *Euterpe edulis*, known as juçara palm, is one of the main non-timber products in the Atlantic Florest. The *E. precatoria* and *E. edulis* non-edible seeds corresponds to 75%-80% of the total fruit weight, and are discarded as a by-product after pulp extraction. Therefore, this work aimed to characterize the açaí and juçara seeds and explore routes to obtain valuable compounds from these unexplored residues. For the determination of the chemical composition, the whole seeds were analyzed including the core stone and the fibrous layer. Mannose was the major sugar present in the whole seeds, which presented a molar ratio of mannose (man), glucose (glc), xylose (xyl), galactose (gal) and arabinose (ara) of 84:11:2:2:1 for *E. precatória* and 76:17:5:1:1 for *E. edulis*. To access the potential to obtain the mannose from the untreated milled whole açaí seeds, enzymatic hydrolysis assays were performed with mannanases. However, only 15.3% and 16% of mannan were converted into mannose for *E. precatória* and *E. edulis* seed samples, respectively, indicating the need for seed preprocessing prior to enzymatic hydrolysis to reduce the mannan recalcitrance. Mannose has great importance in the market, being used by medicine to treat urinary tract infections, and also in the food and cosmetic industris. Thus, the present data can contribute to the stimulate the valorization of largely unexplored residues from Brazil.

VALORIZATION OF PEACH BYPRODUCT IN A BIOREFINERY CONTEXT: BIOACTIVE PROFILE, ANTIOXIDANT CAPACITY AND ENZYME DIGESTIBILITY

M.P. García-Aparicio, A. Martín-Ortiz and M.L. Marina, University of Alcalá, Alcalá de Henares, Spain*

The aim of this work was to characterize the profile of main components and bioactive compounds, and the antioxidant activity of peach juice byproduct (PJB) originated during fruit concentrate production using discarded/outgrade peaches as feedstock. PJB was subjected to freeze dry and to oven dry treatments for their evaluation as storage treatments. The chemical analysis revealed a composition of aqueous extractives (52.97% from which 35.48% are sugars), organic extractives (6.97%), 11.29% fermentable sugars as structural polysaccharides, total lignin (16.05%), total ashes (1.89%) and protein (5.8%). A set of conventional extraction methods of the extractable and non-extractable bioactive compounds were applied and compared with an enzyme-based extraction process. The phenolic compounds profile of the different extracts was compared. In addition, the impact of the storage treatment on saccharification of the PJB by different commercial enzyme preparations was evaluated. The hydrolysable tannins and other polyphenols linked to dietary fibre and/or protein presented the highest antioxidant activity based on results from DPPH and ABTS assays. Moreover, these antioxidant activities were not significantly reduced after applying the oven dry process. Information about the composition, bioactive compounds profile and their antioxidant activity after different treatments is essential to guide process and technologies for their exploitation. The high fermentable sugars concentration (about 50%) and the presence of bioactive substances makes the PJB a potential feedstock in biorefineries for production of biofuels, bioproducts or to develop new functional products for the food and/or cosmetic sectors.

The Impact of High Solids Loadings of Poplar Solids Produced by Co-solvent Enhanced Lignocellulosic Fractionation (CELF) on Deconstruction by *C. thermocellum* Consolidated Bioprocessing (CBP)

P. Singh, C. Alcaraz, C. Cai and C. Wyman, UC Riverside, Riverside, CA, USA; M. Pena, University of Georgia, Athens, GA, USA; E. Holwerda and L. Lynd, Dartmouth College, Hanover, NH, USA; Y. Zeng and Y. Bomble, National Renewable Energy Laboratory, Golden, CO, USA*

Co-solvent Enhanced Lignocellulosic Fractionation (CELF) of lignocellulosic biomass produces solids that are highly enriched in glucan and a hydrolysate stream containing most of the hemicellulose and lignin. Furthermore, deconstruction of CELF solids by consolidated bioprocessing (CBP) using *C. thermocellum* rapidly and nearly completely deconstructs all of the glucan in the CELF solids within 24-48 hours at 5-10 g/L glucan loadings. However, for commercial viability, operation of CBP at higher solids loadings is desirable to reach sugar concentrations that allow more cost effective product recovery. To gain a better understanding of how CELF pretreatment of poplar affects conversion by wild type *C. thermocellum* and performance at high solids levels, glucan rich solids were produced by CELF application to poplar for 5-30 minutes at 140 and 150 degrees C. Then, the resulting CELF pretreated solids were fermented by *C. thermocellum* for solids loadings of 50 g/L and higher. In this presentation, glucan solubilization, changes in solids composition, and structural properties of the solid residues following CELF application to poplar and *C. thermocellum* fermentations of the pretreated solids with increasing solids loading will be reported over a range of CELF reaction times to identify key factors that most impact the rate and extent of solubilization of CELF pretreated solids by the wild type *C. thermocellum* at high solids loadings.

AA9 LPMOs are differently affected by cellulose ultrastructure.

S. Magri and D. Cannella, Université Libre de Bruxelles, Bruxelles, Belgium*

The boosting effect of Lytic Polysaccharide MonoOxigenases (LPMOs) on hydrolytic depolymerization is commonly ascribed to their ability to attack crystalline regions of the substrate creating new entry sites for glycosyl hydrolases. Variability in substrate specificity within LPMOs family members is known, while their preference among different substrate portion among crystalline and amorphous regions linked to their regioselective mechanism remains poorly characterised. Amorphous (PASC) and crystalline (CNC) cellulose are here used to investigate the constrains posed by the substrate ultrastructure on the activity of four different AA9 LPMOs. All the tested enzymes were active on CNC upon detection of oligosaccharides using HPAEC-PAD. However, X-ray diffraction pattern analysis was used to assess the deconstruction efficacy of the enzymatic treatments and revealed that high released of oligosaccharides

did not corresponded to lower crystallinity on the remaining substrate. The effect upon crystallinity of two AA9 LPMOs were in the same degree of that caused by Expansin, a non-catalytic destructive enzyme. Finally, the substrate crystallinity degree did not affect the regioselectivity (C1 or C4) of the tested AA9 but impaired the secondary C4 oxidation in case of one double C1/C4 active AA9 LPMO.

Design of a hemicellulolytic enzimatic cocktail production process using sugarcane bagasse as a carbon source and inducer

Ú.F. Rodríguez Zúñiga, Associate Professor and S.E. Tapia Lishner, Undergraduate Student, Universidad de Ingeniería y Tecnología - UTEC, Lima, Peru*

Hemicelullolytic enzymes, commonly known as xylanases, are industrial biocatalysts with an increasing demand in the second – generation ethanol production, since their use in the biomass saccharification step to degrade the hemicelullose chains results in an increased reducing sugars release and a subsequent cheaper fuel production. Due to this, major advances in recent years regarding its cheaper and more efficient production from a lab - scale point of view have been developed; however, little has been investigated about the actual economic performance of these innovations after their scale - up has taken place. In order to face this problem, the following research project comprises the design of a process to produce a hemicellulolytic enzymatic cocktail using plant biomass waste sources as a carbon source. To do this, a methodology proposal was developed and executed considering the use of residual bagasse from the processing of sugarcane as a raw material, and taking into account the analysis of the kinetics of enzymatic secretion by Trichoderma harzianum P49P11 using a mathematical model and numerical simulation techniques. This project also featured the scale – up of the unit operations required in the sugarcane bagasse pretreatment step, as well as in the fermentation and enzyme recovery steps, the implementation of an optimal task scheduling to accomplish a reduction in the production costs per batch produced and the estimation of the resulting profitability indexes. As a result, it was determined that, by processing 272 batches per year with a process production capacity of 374.33 kg of sugarcane bagasse per batch, it was possible to obtain an enzymatic cocktail with a total protein content of 180 g/L and an enzymatic activity of 15900.87 U/mL of xylanases. Moreover, this project resulted in a NPV of around 39.58 $MM and an IRR of 45.06%.

Comparison of conventional carbon sources with dilute acid and steam treated DDGS on the cellulase and hemicellulase production by fungal strains

A. Iram and A. Demirci, PhD, Penn State University, State College, PA, USA; D. Cekmecelioglu, PhD, Middle East Technical University, Ankara, Turkey*

Distillers' dried grains with solubles (DDGS) is the byproduct of first-generation bioethanol production. It has high fiber content which can be used for hydrolytic enzyme production after pretreatment methods such as dilute acid hydrolysis or semi-continuous steam explosion. This study analyzes the effect of such treated DDGS samples with conventional carbon sources such as glucose, Avicel (crystalline cellulose) and untreated DDGS along with other cellulosic materials on the production of cellulases and hemicellulases by fungal strains such as *Aspergillus niger* and *Trichoderma reesei*. The results show that acid hydrolysis is a better treatment than untreated, and steam treated DDGS for both cellulase and hemicellulase production ($p<0.05$). Acid hydrolyzed DDGS has cellulase production similar to Avicel (0.23 IU/ml) but hemicellulase was lower in case of Avicel for two *A. niger* strains. The results show the significant positive effect of dilute acid treatment in making DDGS as the feedstock for hydrolytic enzyme production.

Water Retention Value as a Characterization Approach for Predictive Modeling of Corn Stover Deconstruction

W. Otto, Montana State University, Bozeman, MT, USA and D. Hodge, Montana State University, BOZEMAN, MT, USA*

Chemical and physical heterogeneity in herbaceous biomass feedstocks due to substantial differences in plant tissue types can contribute significant challenges to handling, preprocessing, and conversion in biorefining processes. A second, related challenge is quantifying this heterogeneity within feedstocks as it relates to processing in a biorefinery. Water sorption properties vary significantly with plant tissue and particle properties. In this work, we will apply several techniques for quantifying water-biomass interactions in combination with other characterization approaches for developing predictive models. These models will predict relative abundance of anatomical fractions and response to processing by pretreatment and enzymatic hydrolysis of air-classified corn stover. One technique, water retention value (WRV) assay, is employed as a tool for screening biomass response to pretreatment and enzymatic hydrolysis in combination with other characterization approaches. We have further developed this assay to increase response sensitivity and identify the operating limits, while the impact of particle size, centrifugation speed and filter loading on WRV were explored.

Nutrient Recovery and Fuel Precursor Production from Extracted Algae Residues Using Mild Oxidative Treatment

J. Kruger, T. Hull, E. Christensen, T. Dong and N. Nagle, National Renewable Energy Laboratory, Golden, CO, USA; K. Adams, Old Dominion University, Norfolk, VA, USA; P. Pienkos, Polaris Renewables, Potsdam, NY, USA*

Valorization of algal biomass to fuels and chemicals frequently requires pretreatment to lyse cells and extraction to recover lipids, producing an aqueous lysate and an extracted solid residue as intermediates. Mild Oxidative Treatment (MOT) is a promising wet oxidation route to simultaneously convert nitrogen contained in the lysate and residues to easily-recyclable ammonia and to convert carbon in the same fractions to carboxylic acids that can be converted to biofuels by ketonization, condensation, and hydrodeoxygenation. We show that for a *Scenedesmus* algae under certain oxidation conditions, nearly all of the nitrogen in the residues can be converted to ammonia and recovered by cation exchange, while almost half of the carbon can be converted to carboxylic acids. At the same time, we also show that soluble phosphorus in the form of phosphate can be selectively recovered by anion exchange, leaving a clean aqueous carboxylic acid stream for upgrading to fuels.

Butanol as a value-added fuel additive to inhibit microbial degradation of stored gasoline

J.G. Elkins, M. Rodriguez Jr., O.C. Cannon, R.M. Connatser, M. Kass, B. West and B.H. Davison, Oak Ridge National Laboratory, Oak Ridge, TN, USA; G. Oguntimein, Morgan State University, Baltimore, MD, USA*

Biodeterioration of gasoline during storage is caused by the ability of microorganisms to inhabit the fuel-water interface that forms at the bottom of large tanks due to the absorption of moisture. Fungi and bacteria form a biofilm by metabolizing fuel compounds that partition into the aqueous phase thus producing organic acids and sulfides resulting in degradation of the fuel and decreased fuel quality. Fuel additives are present in gasoline blends to prevent fouling but are relatively expensive, not always effective against biofilms, and do not contribute to the combustibility of gasoline. Bio-iso-butanol is an approved, certified advanced biofuel at up to “iBu16” [16% (*v/v*) *iso*-butanol in gasoline]; *n*-butanol blends are currently under review. Microorganisms are inhibited by *n*-butanol and *iso*-butanol when the aqueous concentration reaches >2-3% (*v/v*). The toxicity of these C4 alcohols stems from their aliphatic properties that allow the solvents to partition into cellular membranes and disrupt their function, as well as chaotropic effects where essential enzymes are denatured. While the well-documented toxicity of *n*- and *iso*-butanol are problematic to the production of these fuels from carbohydrates, the inhibitory properties may be exploited as a gasoline preservative. Here we provide results on initial studies to determine the partitioning coefficients of 4-carbon alcohols into a small aqueous phase present in a gasoline sample and demonstrate whether the accumulation of the alcohols is sufficient to inhibit a collection of representative microorganisms. Our findings indicate that *n*- and *iso*-butanol could serve as effective microbial inhibitors to prevent fouling in gasoline blends.

Bioprocessing and techno-economic analysis of 2'-fucosyllactose enriched DDGS production using genetically engineered *Saccharomyces cerevisiae.*

C. Kurambhatti, Y.S. Jin, K. Rausch, M. Tumbleson and V. Singh, University of Illinois Urbana Champaign, Urbana, IL, USA; D. Kumar, State University of New York College of Environmental Science and Forestry, Syracuse, NY, USA*

2'-fucosyllactose is one of the most abundant human milk oligosaccharides and has been linked with reduced risks of intestinal dysfunction and fatality in infant piglets. However, high costs associated with synthesis and purification restrict its commercial production. Distillers dried grains with solubles (DDGS) is a post-fermentation coproduct of corn dry grind ethanol process that is used in swine diet formulations. Genetically engineered *Saccharomyces cerevisiae* strains are capable of producing 2'-fucosyllactose using glucose and lactose as substrates. Enriching DDGS in 2'-fucosyllactose by using these genetically engineered strains for fermentation in dry grind ethanol process would be a cost-effective option to supplement swine diets with 2'-fucosyllactose. The objectives of our study were to optimize dry grind ethanol process using genetically engineered *Saccharomyces cerevisiae* strain in terms of ethanol and 2'-fucosyllactose yields and evaluate techno-economic feasibility of the process for commercial scale application. Dry grind ethanol process modified using the genetically engineered strain produced ethanol titers of 133 g/L and 2'-fucosyllactose yields of 3.9 g/dry kg DDGS. 2'-fucosyllactose yields increased to 7.2 g/dry kg DDGS with sequential lactose (supplied as whey) and glucoamylase addition in the fermentation step. The starch-ethanol conversion in the modified process (78.2%) was lower than conventional dry grind process (95.3%) and a residual glucose concentration of 44 g/L was observed at the end of fermentation. Thus, co-fermentation experiments using the genetically engineered and commercial yeast strains were conducted to improve ethanol yields and utilize all residual sugars. A detailed techno-economic analysis will also be performed to estimate the associated costs, potential benefits and overall process feasibility of the optimized modified process.

CRISPR-based transcriptional control in solventogenic *Clostridium* species using d*Fn*Cas12a

R. Joseph and N. Sandoval, Tulane University, New Orleans, LA, USA*

Although solventogenic *Clostridium* species show promise in the production of advanced biofuels such as butanol, their product yields and titers are not viable. Despite efforts to engineer *Clostridium*, a lack of tools for genetic manipulation and a lack of genetic parts has affected progress. Strain engineering with existing tools is time-consuming, labor-intensive and does not enable high-throughput screening. To achieve optimal production yields in *Clostridium*, new tools must be developed for tight and predictable control of protein expression, ensuring maximum carbon flux through desired pathways by eliminating competing reactions.

Here we have developed a system for CRISPR interference (CRISPRi) based repression in *Clostridium* using a nuclease deactivated Cas12a effector protein from *Francisella novicida* (d*Fn*Cas12a). d*Fn*Cas12a facilitates simultaneous repression of multiple genes through the expression of a single CRISPR array. Its recognition of a T-rich protospacer adjacent motif (PAM) site makes it well suited for use in organisms with A/T-rich genomes like *Clostridium*. We demonstrate single- and multi-plexed repression in solventogenic *Clostridium* species including *Clostridium acetobutylicm* and *Clostridium pasteurianum* using a d*Fn*Cas12a CRISPRi system and demonstrate its applicability by altering the carbon flux of feedstocks, resulting in altered metabolite profiles. We show greater than 95% reduction in gene expression through transcription levels, as wells as changes in metabolite profiles through liquid chromatography, including the elimination of solvent production when relevant pathways are targeted. This system represents a valuable addition to the metabolic engineering toolkit for *Clostridium* and will allow further progress in the optimization of biobutanol production by this species.

Novel low-temperature pretreatment method to reduce inhibitor formation and enhance sugar recovery

S. Maitra, University of Illinois at Urbana-Champaign, Urbana, IL, USA and V. Singh, University of Illinois at Urbana-Champaign, Urbana, NY, USA*

Pretreatment of lignocellulosic biomass at high temperatures or with oxidizing chemicals generate various inhibitors that restrict the efficient bioconversion of sugars in subsequent steps. The present study systematically investigates individual and combinatorial effects of pretreatment parameters on the generation of inhibitors. A plot between pretreatment temperature and inhibitor revealed optimum pretreatment temperature for energycane bagasse i.e., 170 ºC beyond which total inhibitor production increased exponentially. No inhibitor production was observed on mechanical processing i.e., disk milling/cryogenic grinding of biomass. Evaluation of response surface regression exhibited that biomass solids loading has a significant effect on inhibitor generation at higher temperatures. The concentrations of certain inhibitors such as acetic acid, furfurals, and HMF increased more than 3-folds on doubling the solids loading. Furthermore, a novel low-severity approach of low-temperature hydrothermal pretreatment coupled with cryogenic grinding for lignocellulosic biomasses has been introduced which improved sugar yields while maintaining a low inhibitor concentration.

Opportunity areas for perennial energy crops: converting multifunctional buffers and marginal land for profits and environmental quality

S. Herbstritt, Penn State, University Park, PA, USA and T. Richard, PhD, The Pennsylvania State University, University Park, PA, USA*

For over a decade, there has been concern about land trade-offs between food production and bioenergy feedstock production. This concern assumes that all agricultural cropland does and should go toward food production and that food production will always be more profitable and environmentally friendly than bioenergy crops. However, there are economically marginal agricultural land areas; these areas are often also hotspots on the landscape for nutrient pollution. This study challenges the paradigm that food production should always take precedence over bioenergy feedstock production on agricultural land, using modern technology, data, and field study. We identified opportunity land areas for bioenergy crops in Pennsylvania, including unprofitable annual cropland, by analyzing satellite imagery obtained from Google Earth Engine and agronomic crop budgets. Converting unprofitable land, along with other opportunity areas like barren, fallow/idle cropland, and portions of riparian buffers to perennial bioenergy crops like switchgrass or other polycultures, represents a large opportunity for cellulosic biofuel feedstock production. Stakeholders are already working to plant perennial systems in buffers per regulatory requirements to improve Chesapeake Bay water quality. Converting these opportunity land areas to well-managed and marketable perennial crops that can reduce erosion and nutrient loss and sequester carbon also represents a market-based solution to improving environmental quality. This strategy was demonstrated by establishing perennial crops on two sites, both unprofitable strips at the downslope of annual crop fields, and one was in a riparian zone. Both sites were outfitted with surface water monitoring equipment and used to quantify water quality improvements and profit of switchgrass and two other native warm-season polycultures that farmers could harvest as energy crops. This presentation will share a methodology for identifying opportunity areas for bioenergy feedstock production and the potential impacts of perennial bioenergy crops on this opportunity land to improve water quality and farm profits.

PRODUCTION OF ETHANOL FROM TEAK WOOD DETOXIFIED HYDROLYZATES

E. Sierra, J. Alcaraz, A. Rosas, Á. Valdivia, M. Hernández-Luna and E. Vivaldo, Universidad Nacional Autónoma de México, Ciudad de México, DF, Mexico; A. Vargas and A. Martínez, Universidad Nacional Autónoma de México, Cuernavaca, MR, Mexico*

Teak wood residues (TWR) were subjected to thermochemical pretreatment, enzymatic saccharification and detoxification, to obtain syrups with high amount of fermentable sugars for ethanol production with *Escherichia coli* MS04. Being teak a hardwood, a robust deconstructive pretreatment was applied. Milled TWR were thermochemical pretreated (140°C, 450 rpm, 90 min) in a 5-L parr-type reactor containing SO_2 (7% w/w), H_2O and teak wood (18% w/w).

As hardwoods are rich in crystalline cellulose (up to 50%), an enzymatic saccharification was performed in a peg-mixer reactor with 15 $FPU_{cellulases}$-g_{glucan}^{-1} (50°C, pH 4.5, 200 rpm, 36 h). This syrup contained 60 g-L^{-1} glucose, 18 g-L^{-1} xylose, 2 g-L^{-1} arabinose, 6 g-L^{-1} acetate, less than 0.1 g-L^{-1} of total furans, and 16 g-L^{-1} of soluble phenolic lignin-derivatives. Since 16 g-L^{-1} of phenolic lignin derivatives are toxic to *E. coli*, two detoxification strategies were assayed: 1) treatment with 510 U_{ABTS}-mL^{-1} of *Coriolopsis gallica* laccase (30°C, 500 rpm, 1 vvm, 120 min), followed by addition of activated carbon (5% w/v); and 2) overliming, adjusting the pH to 11 with $Ca(OH)_2$ (30°C, 500 rpm, 40 min). Both reduced the phenolic compounds by 50 and 90%, respectively.

The detoxified syrups were centrifuged and fermented (37°C, pH 7, 150 rpm, without-aeration) with *E. coli* MS04. Cultivations with the overlimed hydrolysate showed a 50% higher volumetric productivity (0.34 g_{ETOH}-L^{-1}-h^{-1}). For both strategies, the bioethanol/sugars yield was over 90%. According to these results, from 1 ton of teak wood residues, 570 L of ethanol could be produced.

Acknowledgements: PAPIIT-DGAPA-UNAM Grant IV100119

Depolymerization of lignin for biological conversion through sulfonation and a chelator-mediated Fenton reaction

M. Kent, A. Rodriguez, B. Simmons, K. Sale and S. Singer, Joint BioEnergy Institute, Emeryville, CA, USA; D. Martinez, M. Juarros, E. Martinez and T. Alam, Sandia National Labs, Albuquerque, NM, USA*

Generating value from lignin through depolymerization and biological conversion to valuable fuels, chemicals, or intermediates has great promise but is limited by several factors including lack of cost-effective depolymerization methods, toxicity within the breakdown products, and low bioconversion of the breakdown products. High yield depolymerization of natural lignins requires cleaving carbon-carbon bonds. We report that a chelator-mediated Fenton reaction can efficiently cleave C-C bonds at or near room temperature in sulfonated polymers and that repolymerization can be minimized through control of the reaction conditions. This method was used to depolymerize lignosulfonate from Mw = 28,000 g/mol to Mw = 800 g/mol. The breakdown products were characterized by FTIR, NMR, and GC-MS and evaluated for bioavailability. The breakdown products are rich in acid, aldehyde, ether, and alcohol functionality but largely devoid of aromaticity. A panel of monocultures were tested for growth on the breakdown products. Growth at a low level was observed for several monocultures on the depolymerized LS in absence of glucose. Much stronger growth was observed in the presence of 0.2% glucose. These results suggest that this method may be promising for biological conversion of lignin into higher value chemicals or intermediates.

Revealing Grass-specific Xylan-cellulose Interactions in Native Sorghum Secondary Cell Wall via Solid-state NMR

Y. Gao, Lawrence Berkeley National Laboratory, Berkeley, CA, USA*

In order to fulfil the increasing global demand for fuels, chemicals and materials, but also to reduce emissions from using fossil sources, lignocellulosic biomass is a sustainable alternative feedstock. However, plant cell walls, which constitute most of the lignocellulosic biomass, are highly recalcitrant and their architecture is poorly understood. As a result, the efficient deconstruction of lignocellulosic biomass is challenging, although the conversion of all components into biobased products is economically necessary. Grasping the detailed structural information of the plant cell wall architecture and understanding the arrangement of plant cell wall components will provide not only invaluable insights to predictively design and refine dedicated engineered biomass crops using synthetic biology, but will also improve strategies to deconstruct the cell wall. Here, we present our recent findings where we reveal the native architecture of wild type sorghum secondary cell walls, which is one of the U.S. Department of Energy key bioenergy crops, by using multi-dimensional solid-state NMR. With 2D INADEQUATE experiments, we demonstrate that, unlike dicot and softwood plant cell walls, most grass cell wall xylan is in the three-fold screw conformation. Also, we use PDSD experiments to show that three-fold screw xylan is responsible for the most of the cellulose-xylan interactions via amorphous cellulose. Additionally, we determine that sorghum secondary walls have approximately three times more amorphous cellulose

compared to Arabidopsis, a model dicot plant. We propose a model of grass secondary cell wall with a new configuration of xylan-cellulose interaction, which will provide insights for future sorghum engineering strategies.

Impacts of biologically induced heating on surface energy, wettability & cohesion of corn stover

J. Leal, T. Rouse, L. Cheng, E. Meierdierks, C. Moore and T. Semelsberger, Los Alamos National Laboratory, Los Alamos, NM, USA; A. Sutton, Oak Ridge National Laboratory, Oak Ridge, TN, USA; A. Hoover and A. Ray, Idaho National Laboratory, Idaho Falls, ID, USA; B. Donohoe, National Renewable Energy Laboratory, Golden, CO, USA*

The impacts of thermal degradation of bulk and fractionated (leaf, stalk, and cob) corn stover as a result of microbial action on surface area, surface energy, wettability, and cohesion of corn stover samples were examined in this study. Surface areas of corn stover were measured ~1 m^2/g, using a gas adsorption method; a dramatic increase in leaf surface area was observed (~0.5-1.1 m^2/g). The degraded corn stover was found to undergo significant changes in surface chemistry evidenced via inverse gas chromatography analysis. Surface energy increased monotonically in dispersive and specific components of the bulk samples, and in the leaf fraction. The cob was the fraction least affected by degradation. Hydrophilicity was calculated using surface energy data, predicting increases in wettability with increased degradation, mainly through the rise in specific surface energy. The work of cohesion was found to increase as a function of biological heating, which may produce bulk solids and handling problems such as rat holing, arching and discontinuous flow patterns from increased agglomeration and segregation.

Conversion of Food Waste to Levulinic Acid Using A Catalytic Membrane Reactor

Z. Zhu, X. Qian and R. Wickramasinghe, University of Arkansas, Fayetteville, AR, USA*

One-pot conversion of biomass to Levulinic acid (LA), a promising platform chemical of bio-refinery, is an important technology in the biomass conversion process. If a biomass feedstock competes with food supply, it becomes a significant concern due to worldwide food shortage thereby limiting the application of the conversion technology. Multiple biomass waste materials such as paper waste, agriculture residues, forest residues and food waste have been considered as promising feedstocks for bioprocessing and bio-refinery. Here, cellulose as well as food wastes from vegetables and other starch-based food materials have been investigated for their conversion to Levulinic acid using a unique solid acid catalyst immobilized on a membrane substrate. Our reusable membrane catalyst is superior to the corrosive homogeneous acid or toxic metal-based catalyst. In addition, the membrane substrate enables the immediate separation of the Levulinic acid from the rest of the feed stream driving the reaction to completion and improving the yield. Finally, hot water extraction as a pre-processing step was found to be effective in removing some of the proteins and other soluble carbohydrate. A Levulinic acid yield of over 80% has been achieved for the model cellulose compound. For starch-based food waste such as rice and noodles, over 90% of Levulinic acid yield was obtained. For the more recalcitrant cellulosic vegetables, 50% Levulinic acid yield has also been achieved indicating the promising potential of our technology for food waste utilization.

Açai waste valorization for polyphenols production: techno-economic analysis

F. Thimoteo Azevedo Jorge and A. Sant'Ana da Silva, National Institute of Technology, Rio de Janeiro, Brazil; G. Victor Brigagão, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil*

Around 1.2 million tons of *açaí* (*Euterpe oleracea* Mart.) seed – an agricultural waste of *açaí* fruit pulp production – are produced annually in the Brazilian Amazon region. Most of this biomass is currently discarded without recovery of any chemical compound and adequate disposal. However, *açaí* seeds contain polyphenolic compounds with several pharmacological properties. This work evaluates the

techno-economic viability of a novel biorefinery conceived as an alternative for *açaí* seeds monetization. The proposed biorefinery comprises the solvent extraction of phenolic compounds and the combustion of the residual biomass for power generation. Process simulation was performed in Aspen Plus V8.8, which supported equipment sizing, utilities consumption and plant investment evaliation. Two processing capacities were assessed: 2 t/h and 6 t/h of *açaí* seeds. For the lower capacity, fixed capital investment (FCI) was US$26 million, while for the higher capacity the FCI was 1.9 times higher: US$50 million. Considering a polyphenols price of US$10/kg, payback time is 5 years for the lower capacity and 2.3 years for the higher capacity. However, sensitivity analysis was performed to investigate how payback time and final net present value are affected by variations in the price of the polyphenolic extract. The extract break-even prices for project feasibility are US$6.15/kg and US$3.48/kg for the lower and higher capacities, respectively. Therefore, this work indicates the feasibility of a biorefinery of *açaí* seed that would add value to this essential productive chain in Brazil while alleviating an acute environmental problem.

Influence of Moisture and Ash Content on the Flowability of Corn Stover Biomass Feedstocks

L. Cheng, J. Leal and T. Rouse, Los Alamos National Laboratory, Los Alamos, NM, USA; T. Semelsberger, Idaho National Laboratory, Idaho Falls, NM, USA*

One of the key technical challenges in the economic viability of integrated biorefineries (IBRs) is the continuous processing and handling of raw biomass feedstocks. Currently, however, flow stoppages often occur such as arching and ratholing, resulting in equipment down time and hampering the economical feasibility of the IBRs. For biomass particles to move through the handling equipment such as hoppers and silos without blockages, the handling equipment needs to be designed using the flow properties of biomass that needs to be measured. The flow properties of biomass are dependent upon many factors including the particle size, shape, moisture, composition of the biomass material, the applied normal stress, and the wall of the handling equipment. Here, we investigate the effect of moisture and ash content on the flowability of corn stover biomass samples by using an FT4 powder rheometer. The results of shear and dynamics testing, as well as implications on the design of handling equipment, are presented.

L-LACTIC ACID PRODUCTION BY *Bacillus coagulans* DSM2314 USING HYDROLYSATES DERIVED FROM STEAM-EXPLODED SUGARCANE BAGASSE

W. Rodrigues Alves and L. Pereira Ramos, Federal University of Paraná, Curitiba, Brazil*

Lactic acid (LA) is a chemical compound with many industrial applications, led by the production of sustainable biopolymers such as poly(lactic acid). Its production is normally carried out by fermentation of simple sugars, but efforts have been oriented to the use of non-conventional carbohydrate sources such as sugarcane bagasse (SCB), with hexoses and pentoses being converted to LA by bacteria such as *Bacillus coagulans*. Due to its highly recalcitrant nature, SCB has to be pretreated to facilitate the recovery of hemicellulose sugars and enhance the susceptibility of lignocellulose to enzymatic hydrolysis (EH). This work aimed to produce L-lactic acid from steam-exploded SCB. Pretreatment was carried out by auto-hydrolysis in two severities (195 ºC for 7.5 or 15 min), followed by the EH with Cellic CTec3 (Novozymes) for 96 h and co-fermentation of sugar streams obtained in both stages by *Bacillus coagulans* DSM2314. Fermentation inhibitors in pretreatment hydrolysates were characterized and detoxified by physical adsorption on activated carbon. Analyses were carried out using HPLC with a Rezex RHM column at 65 ° C and eluted with H_2SO_4 5 mmol·L^{-1} (0.6 mL·min^{-1}). The fermentation tests were conducted in duplicates using the Multifors 2 bioreactor (Infors HT, capacity of 0.5 L). The detoxified hydrolysates fermented by *B. coagulans* resulted in the production of 11 and 15 g·L^{-1} of LA, with higher yields obtained from the pre-treated materials washed with water. It was possible to obtain 56.7 g·L^{-1} of LA in the SHF process starting from the explosion carried out with greater severity and in the SHCF process 76.6 g·L^{-1}. For samples treated with less severity, it was possible to obtain 64.2 g·L^{-1} in the SHF

process and 73.4 $g \cdot L^{-1}$ in the SHCF process. Finally, both fractions could be used with great efficiency for the production of LA, revealing their potential for biorefinery applications.

Kinetic evaluation of one pot fractionation and saccharification of mixed Sugarcane bagasse varieties using Deep Eutectic Solvents

V. Chourasia and R. Henry, The University of Queensland, St. Lucia, QLD, Australia; K. Pant, Indian Institute of Technology Delhi, New Delhi, India*

Sugarcane is a principal plant in major tropical countries with an annual global production of approximately 1.6 billion tons. Sugarcane waste, generated in sugar production, essentially in the form of sugarcane bagasse (SB) has been widely examined as a valuable lignocellulosic feedstock for bioenergy applications. However, the major hurdle in making this an economically successful biorefinery concept has been the overall utilization of the biomass, where fractionation plays a vital role. In the present work, a kinetic evaluation of enzymatic saccharification was performed to determine the hydrolysis efficiency of four varieties of pre-treated SB in the presence of Deep Eutectic Solvents (DESs). DESs represent a rapidly emerging group of ionic liquids used for fractionation of biomass providing relatively lower toxicities, ease of preparation and biodegradable properties. Choline chloride: Malonic acid (MA)(1:1), Choline chloride: Glycerol (GLY)(1:2) and Choline chloride: Lactic acid (LA)(1:5) were used for pre-treatment and Cellulase enzyme (20 FPU/g) was used for saccharification of SB at 50°C with constant agitation. SB composition and glucan to glucose conversion of four genotypes were determined to study the effect of substrate loading for one pot pre-treatment and hydrolysis for different pre-treatment solvents. The kinetic modelling and validation depicted a first order kinetics and the rate constants (K_i) of cellulose hydrolysis at different substrate loading (4%-10% w/w) were found to be inversely proportional to the initial solid concentration. Furthermore, the percent glucan conversion varied between genotypes, however no genotype showed significant superiority to others. However, the lactic acid treated SB resulted in maximum delignification (52%) whereas the glycerol treated SB demonstrated the highest hydrolysis efficiency (37%).

Production and characterization of high value prebiotics from biorefinery co-product stream

K. Rajan, D. D'Souza, K. Kim, J.M. Choi, D.J. Carrier and N. Labbé, The University of Tennessee, Knoxville, TN, USA*

Hemicellulose, a structural polysaccharide and often underutilized co-product stream of biorefineries, could be used to produce high-value prebiotic supplements with novel functionality. Since hot water pre-extraction is a cost-effective strategy for integrated biorefineries to partially remove hemicellulose and to improve feedstock quality for downstream operations, this technique was employed at 160 °C, 60 min, to fractionate biorefinery-relevant feedstocks. Previous studies have explored the prebiotic potential of hot water extracts from miscanthus, Norwegian spruce and birchwood biomass, but to our knowledge, this will be the first time that the hemicellulosic oligosaccharides from switchgrass, hybrid poplar and southern yellow pine are investigated for their prebiotic applications.
Chemical characterization showed that the hot water extracts of switchgrass, hybrid poplar and yellow pine contained 89.3±0.8%, 91.3±0.9% and 76.5±0.5%, respectively, of oligosaccharides of xylan, mannan, glucan, galactan and arabinan origin. *In vitro* batch fermentations by single probiotic bacterial cultures were performed using M9 minimal salts media that contained 4 g/L of the freeze-dried hot water extracts; xylose, glucose and mannose were used as controls. The tested probiotic microorganisms, namely *Lactobacillus casei*, *Bifidobacterium bifidum* and *Bacteroides fragilis*, exhibited different specificity to the hot water extracts; switchgrass extracts induced the highest growth count in *L. casei* ($1x10^8$ CFU/mL), yellow pine extracts in *B. fragilis* ($1x10^5$ CFU/mL), and hybrid poplar extracts in *B. bifidum* ($1x10^6$ CFU/mL). The observed differences were attributed to the preferential consumption of mannooligosaccharides (in yellow pine), xylooligosaccharides (in switchgrass and yellow pine) and galactose (in hybrid poplar) by *B. fragilis, L. casei*, and *B. bifidum*, respectively. Thus, this research 1) elucidates the potential of biorefinery-relevant feedstocks for prebiotics production, and 2) demonstrates

how the chemical composition of hemicellulose-derived prebiotic oligosaccharides can regulate the viability and selective proliferation of gut microbiota.

Polish for pulp: A combined esterase treatment enhances pulp quality for spinning of regenerated fibers by removing lipophilic extractives

E. Fitz, PhD, Wood Kplus - Kompetenzzentrum Holz GmbH, Linz, Austria and R.H. Bischof, PhD, Lenzing AG, Lenzing, Austria*

With climate change and rising environmental concerns, the demand for textiles from renewable resources is constantly growing. Regenerated cellulose fibers are produced from dissolving pulp gained from wood and require a high pulp purity for efficient spinning. One major source for contaminations are lipophilic wood extractives (LWE), comprising free fatty (FFA) and resin acids, sterols, steryl esters and triglycerides. While FFA and sterols are washed off during bleaching, unsaturated fatty acids like in triglycerides and sterol esters, are hard to remove in totally chlorine free bleaching sequences. Staying there, these compounds can be troublesome during processing of the pulp and fibers and negatively affect the product quality, e.g. by contributing to a bad smell.
We investigated the effect of three commercial esterases on the LWE in ozone bleached beech wood dissolving pulp. Substance group determination of acetone soluble extractives was measured with GC-FID and showed that triglycerides were almost completely removed by all three enzymes (lipase, cutinase, steryl esterase), when fully bleached. Lipophilic compounds in wash filtrates were extracted with solid phase extraction and analysed by GC-FID and all enzymes showed higher contents of washed off FFAs than a reference. Cutinase and steryl esterase treatments led to higher sterol levels and the amount of solubilized steryl esters rose after lipase and cutinase treatment. Overall, the results suggested a combined treatment with all enzymes for the most effective LWE reduction. Additionally, we compared the content of volatile organic compounds (VOC) emitted by the pulps with a solid phase micro extraction-GC-MS. The mixed enzyme treatment completely abolished hexanal from the VOC profile, which accounted for 20% of total VOCs in a reference sample.

We showed that an environmentally friendly enzyme treatment of pulp can remove LWEs on dissolving pulp and an enzyme stage could further increase product quality by curbing hexanal emissions.

Cassava starch saccharification improvement using sugarcane-bagasse-assisted extrusion pretreatment

D. Oluwagbotemi Fasheun, R. Alves de Oliveira and R. Sposina Sobral Teixeira, universidade federal do rio de janeiro, Rio de Janeiro, Brazil; A. Sant'Ana da Silva and V. Santana Ferreira-Leitão, Instituto Nacional de Tecnologia, Rio de Janeiro, Brazil*

The enzymatic hydrolysis of starch is generally slow due to its semi-crystalline nature. A pretreatment prior to enzymatic hydrolysis would be desirable to speed up the process. In this study, cassava starch (*CS*) saccharification was improved using sugarcane-bagasse-(*SB*)-assisted extrusion pretreatment. CS_{only} and *CS:SB* mixtures (1:1, 1:0.5, 1:0.25) were pretreated in a twin-screw extruder at 100rpm and 130°C in 5 cycles before enzymatic hydrolysis. *Extruded-*$CS:SB_{(1:0.25)}$, $CS:SB_{(1:0.5)}$ and $CS:SB_{(1:1)}$ resulted in 71.5%, 65.1% and 53.7% glucose yield after 3h of enzymatic hydrolysis, respectively. Hydrolysis of *extruded-*CS_{only} gave a low 3h glucose yield (22.0%) similar to *untreated CS* (17.2%), which may be due to the fine particle nature of *CS* and its free flow within the extruder barrel, which generated a low extrusion torque (~3Nm). *Extruded-*$CS:SB_{(1:0.25)}$ was hydrolyzed faster within the first 3h compared to *extruded-*CS_{only}, with 260% increase in glucose productivity. This may be due to the coarse nature of *SB*, which traps the *CS* within the barrel and increases the torque (~40Nm) and residence time for better mechanical and thermal pretreatments. After successfully increasing the torque to ~40Nm during the extrusion of CS_{only} through increased feeding rate, its 3h glucose yield after hydrolysis increased to 57.3%, but was still lower than that of *extruded-*$CS:SB_{(1:0.25)}$. Furthermore, upon hydrolysis, the glucose yield at 24h from *extruded-*CS_{only} (higher torque) (65.9%) was less than the 3h yield from *extruded-*$CS:SB_{(1:0.25)}$. The pretreatment conditions were optimized for $CS:SB_{(1:0.25)}$ using a *central composite rotatable design* 2^2. Regression analysis showed that linear and quadratic terms of temperature and

screw speed had significant effects ($p<0.05$) on the 3h glucose yield. The optimum speed and temperature were 132rpm and 129°C respectively ($R^2= 0.91$), with a predicted optimum 3h glucose yield of 74.1%. Thus, the presence of *SB* facilitates the extrusion pretreatment of *CS*, thereby reducing the time required for enzymatic hydrolysis.

Multimodal characterization and rational engineering of cellulose-binding domains enables lowering non-productive enzyme binding to cellulose

B. Nemmaru[*] and S. Chundawat, Rutgers University, Piscataway, NJ, USA; J. Yarbrough, National Renewable Energy Laboratory, Golden, CO, USA; M. Johnson and M. Lang, Vanderbilt University, Nashville, TN, USA

Dissociation of non-productively bound cellulolytic enzymes from cellulose is hypothesized to be a key rate-limiting factor impeding cost-effective cellulosic biomass conversion to fermentable sugars. However, the molecular mechanisms of cellulase binding and particularly role of carbohydrate-binding modules (CBMs) in enabling non-productive enzyme binding is not well understood. To firstly understand the molecular mechanism of non-productive binding, we monitored the single-molecule processive motility of full-length exocellulases and developed a single-molecule CBM-cellulose rupture assay employing optical tweezers to characterize the binding of a well-studied Type-A CBM and its mutant to cellulose allomorphs. Next, we examined the subtle interplay of CBM binding and cellulose hydrolysis activity for three model Type-A CBMs (families 1, 3a, and 64) tethered to a cellulase catalytic domain (CD) on two distinct cellulose allomorphs (i.e., cellulose I and III). We finally generated a small-library of mutant CBMs with varying cellulose affinity, as determined by equilibrium binding assays, followed by monitoring cellulose hydrolysis activity of multiple CD-CBM fusion constructs to identify novel mutants with enhanced activity towards cellulose I. Kinetic binding assays using quartz crystal microbalance with dissipation (QCM-D) were then employed to measure mutant CBM adsorption and desorption rate constants towards nanocrystalline cellulose derived from both allomorphs. Overall, this presentation will highlight our ongoing efforts to characterize the role of CBMs in degradation of cellulose using a suite of novel analytical techniques and highlight potential strategies for engineering more efficient cellulolytic enzymes for cellulosic biomass conversion to sugars.

Production of 5-hydroxymethylfurfural (HMF) from organosolv pretreated beechwood by combining enzymatic hydrolysis and isomerization with homogeneous catalysis

G. Dedes[*], A. Karnaouri and P. Champesi, Industrial Biotechnology & Biocatalysis Group, School of Chemical Engineering,National Technical University of Athens, Athens, Greece; A.A. Marianou, K.G. Kalogiannis and C.M. Michailof, Chemical Process and Energy Resources Institute, Center for Research and Technology Hellas, Thessaloniki, Greece; E. Topakas, Industrial Biotechnology & Biocatalysis Group, School of Chemical Engineering, National Technical University of Athens, Athens, Greece

Lignocellulosic biomass can be utilized as a raw material for numerous processes within the biorefinery spectrum to substitute the continually depleting fossil fuel reserves toward the production of value added chemicals. One such route includes the conversion of biomass sugars to furan compounds that serve as monomers for the production of biobased polymers. These furan compounds include 5-hydroxymethylfurfural (HMF) and furfural (FA), produced by hexose and pentose streams respectively, both of which are obtained by the chemical dehydration of sugars. While heterogeneous catalysis upon the use of metal catalysts is required for the conversion of glucose to HMF, its isomer fructose can be directly transformed with the use of diluted Lewis acids. Hence, establishing an environmentally friendly process necessitates the introduction of an isomerization step in which glucose is transformed to fructose. In this work, we have developed a chemo-enzymatic route for the conversion of beechwood biomass to HMF, starting with an mild oxidative organosolv pretreatment to remove lignin, leaving behind a sugar-rich solid fraction for the production of furan derivatives. By integrating a high-gravity enzymatic saccharification at 20% w/w solids loading and an efficient enzymatic isomerization, a concentrated fructose syrup is obtained (104.5 g/L fructose and 2.3 g/L xylulose) which is subsequently used for HMF

production. The results show that over 50% of the initial glucose content is transformed to fructose, reaching 64 g fructose/ 100 g cellulose. For the subsequent step, different homogeneous catalysts are evaluated as potential candidates for the efficient dehydration of sugars to furans, targeting the maximum glucose conversion to HMF. We conclude that formic acid was the best catalyst, reaching an HMF yield of 44.6% with 55.8% selectivity. The HMF produced can function as a substrate for 2,5-furandicarboxylic acid (FDCA) synthesis or its oxidative intermediates, therefore suggesting an alternative eco-friendly pathway towards polymer synthesis.

Development of a Biomass Containment System (BCS) for Complex Insoluble Substrates

C. Garcia, E. Monge, PhD, C. Nelson, PhD, N. Beri, M. Levi, J. Forbin, M. Legesse, B. Udo, T. deCarvalho, PhD and J. Gardner, PhD, University of Maryland - Baltimore County, Baltimore, MD, USA*

Studying the microbial degradation of real-world insoluble polysaccharides comes with several challenges. Physiological studies are made difficult as insoluble substrates confound optical density readings of bacterial growth while other modes of measurement may be costly, time intensive, or otherwise incompatible with high-throughput screening. Customizable 3D printed biomass containment devices were designed to separate insoluble materials from microbial cells while facilitating rapid and reproducible growth in large-scale experiments. Development of microplate biomass containment devices was designed to facilitate small-scale and high-throughput reactions comparable with both physiological and enzymatic reactions. Finally, we developed an agar capsule system to study the microbial degradation of insoluble powders and microbial cells. These devices and techniques constitute a complete Biomass Containment System (BCS) that allows for high flexibility when studying insoluble polysaccharide degradation.

Understanding of Bacterial Lignin Depolymerization Mechanisms by *Pseudomonas putida* through Secretomic Analysis

Z. Xu, K. Martin and B. Yang, Washington State University, RICHLAND, WA, USA; J.R. Cort and T. Shi, Pacific Northwest National Laboratory, RICHLAND, WA, USA; Y. Pu and A.J. Ragauskas, Oak Ridge National Laboratory, Oak Ridge, TN, USA*

Efficient utilization of lignin from lignocellulosic biomass is critical for the economic efficiency of a bioconversion process to produce renewable bioproducts. Understanding the mechanisms of bacterial lignin depolymerization will pave the way for further optimization of the lignin bioconversion process. In this study, the lignin degradation capacity of *Pseudomonas putida* secretome was investigated. NMR results revealed that the secretome alone exhibited strong C-C bond cleavage capacity. The addition of H_2O_2 and Mn^{2+} to secretome enhanced lignin degradation by stimulating the β-O-4 bond cleavage. GC-MS confirmed the breakdown products consist of aromatic monomers and oligomers after the enzymatic reaction. Overall, our study provides new insights into the bacterial lignin depolymerization mechanisms that may guide further metabolic engineering design to improve the efficiency of process for lignin bioconversion.

Time and spatially resolved infrared spectroscopy of cellulose undergoing enzymatic hydrolysis in a windowless microfluidic platform

J. Nill and T. Jeoh, University of California, Davis, Davis, CA, USA; W. Zhao, L. Chen, S.R. Narayanasamy and H.Y. Holman, Lawrence Berkeley National Laboratory, Berkeley, CA, USA*

Cellulose has immense potential as a source of renewable fermentable sugar and as renewable nanomaterials. The action of enzymes at the surfaces of insoluble cellulose to cleave bonds and modify surface chemical properties have been a subject of intense study to understand how to control the kinetics and outcome of these interactions. Infrared (IR) imaging can be useful to study spatially-resolved chemical modification of cellulose imparted by enzymes. As a polymer of glucose, cellulose features C-O

bonds at five of the six carbons of the glucose residues that contribute to absorption peaks in the infrared 'fingerprint region'. In a Fourier Transformed IR (FTIR) spectrum, cellulose exhibit absorption peaks most prominently at 1034 cm^{-1}, 1060 cm^{-1}, and 1160 cm^{-1}, corresponding to C-O stretches at the sixth (C6) and third carbon (C3) of the glucose residues, and the glycosidic bond of the polysaccharide, respectively. While IR spectroscopy has been used to study changes imparted by enzyme action on the crystallinity and morphology of cellulose, previous studies have been on post-treated, bulk and dried samples. In this work, we applied IR imaging to examine the C-O stretches in the fingerprint region of cellulose during hydrolysis by a cellobiohydrolase enzyme (Cel7A). The hydrolysis reactions were conducted in a novel, windowless microfluidics device designed to control moisture-levels, humidity and temperature. We documented spatially heterogeneous changes in the abundance and molecular ordering of cellulose during enzymatic hydrolysis over 12 hours, seen from a decline in peak absorbance of the 1160 cm^{-1} glycosidic bond peak, and wavenumber shifts in the 1034 cm^{-1}, 1060 cm^{-1}, and 1160 cm^{-1} peaks. We interpret the average 'blue shift' of these three peaks as indication that cellobiohydrolase action generally removes 'less ordered' cellulose. We discuss our results in the context of intra- and intermolecular bond participation of C3 and C6 in cellulose.

3-Hydroxypropionic Acid Synthesis from Methane and Carbon Dioxidevia Acetylene Carboxylic Acid

K. Draths, A. Sirinimal, H. Nayebi Gavgani, S. Sreedhar and J. Geiger, Michigan State University, East Lansing, MI, USA*

3-Hydroxypropionic acid (3-HP) is a critical building block for expansion of the bioeconomy. As the precursor to multiple value-added chemicals including 1,3-propanediol, acrylic acid, and acrylonitrile, 3-HP provides access to a plethora of adhesives, resins, polymers, and fibers. Current technology for 3-HP synthesis relies on conversion of glucose and glycerol. We describe a nearly quantitative, two-step, three-enzyme conversion of acetylene carboxylic acid into 3-HP. Synthesis of 3-HP using this novel route affords product in which all three carbon atoms originate from methane and carbon dioxide.

Modeling the air classification of corn stover anatomical fractions for enhanced feedstock quality

D. Cousins, W. Otto and D. Hodge, Montana State University, Bozeman, MT, USA; S. Hernandez, A.H. Rony, J. Lacey and J. Aston, Idaho National Lab, Idaho Falls, ID, USA*

Lignocellulosic biofuel production is inherently challenging due to heterogeneity of feedstock materials. Petro fuel feedstock (crude oil) is also heterogeneous, but its widespread use is backed by centuries of separation technology development. Pneumatic separation (air classification) of lignocellulosic biomass is a facile, inexpensive technology that can enable cost competitive biofuel production at scale. Air classification leverages differences in density and aerodynamic profiles of biomass anatomical tissues to separate a feed into more homogeneous fractions. Biomass fluidization has been extensively studied for gasification and pyrolysis, while air classification has been used to a limited degree for agricultural applications. However, fundamental physics-based models describing the separation of heterogeneous anatomical tissues are lacking. Here, we present a model that describes the pneumatic separation of corn stover based on particle size and shape from image analysis. Particle partition velocities predicted from size and shape parameters accurately describe experimental separations of corn stover tissues in an industrial air classifier.

Effect of pH, temperature, and retention time on the solubilization of lignocellulosic biomass during anaerobic digestion by mixed microbial communities

M. Shreve, PhD, K. Hirl, A. Bharadwaj, PhD, J. Regan, PhD and T. Richard, PhD, The Pennsylvania State University, University Park, PA, USA*

To better design anaerobic digestion systems for conversion of abundant but recalcitrant lignocellulosic feedstocks, an understanding of how the system behaves under different operating conditions is critical. Prior research has looked at process optimization within a fairly narrow range around conditions known to be successful for methanogenesis. Surprisingly little is known of the structure, function, and dominant metabolic products of mixed microbial communities that have adapted under conditions outside of those local optima, and many process conditions have never been explored. This study fills these longstanding gaps in the literature through an extensive investigation of a wide range of process conditions. Triplicate, semi-continuous bench-scale (1-L) anaerobic digesters were used to test 18 combinations of pH (pH 5.5, 7, 8.5), temperature (37 and 55 °C), and retention time (3.3, 5, 10 d) for the anaerobic digestion of senescent switchgrass. All reactors were inoculated using identical composite inoculum collected from diverse environments, flash-frozen, and stored at -20 °C. Process data including carbohydrate solubilization, gas production/composition, and the concentration of various soluble products (volatile fatty acids, alcohols, and sugars), were collected over five retention times (RTs), with a focus on “steady-state” operation (RTs 4 and 5). Biomass samples were collected and preserved for a multi-omics (metagenomics, metatranscriptomics, and proteomics) analysis of the effect of selective pressures on the composition and function of the microbial community (currently underway).

Process data show that conditions cluster into four groups: Low performing (0 ml methane/RT, < 0.5 g VFA/L, conversion of 0.0-7.3% of carbohydrate); Medium performing (0-570 ml methane/RT, 0.5-1.9 g VFA/L, and 7.7-22.9% conversion); High performing methanogenic (1400 ml methane/RT, 0.2 g VFA/L, 27.8% conversion); and High performing acidogenic (350 ml methane/RT, 3.0 g VFA/L, 40.6% conversion).

Enhanced production of cellulase and hemicellulase in DDGS-based media by optimizing nitrogen source and salts

A. Iram and A. Demirci, PhD, Penn State University, State College, PA, USA; D. Cekmecelioglu, PhD, Middle East Technical University, Ankara, Turkey*

The fiber content of Distillers’ Dried Grains with Solubles (DDGS) can be a valuable source for the microbial production of hydrolytic enzymes such as cellulase and hemicellulases. Salt and nitrogen source amendment can further enhance the enzyme production in a DDGS based media. Therefore, this study was undertaken to evaluate the effect of salts (KH_2PO_4, $CaCl_2{\cdot}2H_2O$, $MgSO_4{\cdot}7H_2O$, $FeSO_4{\cdot}7H_2O$, $CoCl_2{\cdot}6H_2O$, and $MnSO_4{\cdot}H_2O$), peptone, yeast extract and ammonium sulfate (as nitrogen source) on enzyme secretion and to optimize the nitrogen source for maximum enzyme productions by various fungal strains. Salts did not have a significant effect on enzyme production ($p>0.05$), while ammonium sulfate was a better nitrogen source supplement compared to peptone and yeast extract. Optimization of different nitrogen sources increased cellulase production from 0.174 to 1.3 U/ml and hemicellulase production from 9.4 to 43.64 U/ml on day 6. In conclusion, the optimization of all three nitrogen sources improved both cellulase and hemicellulase production in DDGS based media.

Alkaline Oxidation of Lignin Using Reversibly-Soluble Bases

J. Kruger, R. Dreiling, D. Wilcox, D. Brandner and G. Beckham, National Renewable Energy Laboratory, Golden, CO, USA*

Production of oxidized aromatic monomers 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. The high hydroxide loading in most scenarios renders the process uneconomical even for high-value products such as vanillin. In this work, we explore alkaline oxidation of a lignin-rich enzymatic hydrolysis residue isolated from corn stover, using alkaline earth metal hydroxides, such as $Sr(OH)_2$ and $Ba(OH)_2$ as base promoters. These materials are soluble at reaction temperature, but mostly

insoluble at room temperature, allowing recovery and reuse by simple filtration. We show that monomer yields and profiles using these bases is similar to that obtained using NaOH as base, and that $Sr(OH)_2$ can be recovered in yields above 90%. Preliminary TEA and LCA suggest that replacing NaOH with $Sr(OH)_2$ can decrease monomer production costs by 40% and decrease global warming potential in lignin depolymerization by 30%, enabling a more economical and sustainable process.

High concentration and yield production of mannose from açaí seeds

I. Santos Miguez, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil; A. Ferreira Monteiro, J.P. Rodrigues Barros da Silva and A. Sant'Ana da Silva, National Institute of Technology, Rio de Janeiro, Brazil*

The açaí seed (*Euterpe oleracea* Mart.) accounts for 85% of the mature fruit's weight. Based on the growing pulp production, it is estimated that 1.1 million tons of seeds are generated annually in the Brazilian Amazon region, causing acute environmental and urban problems. To extract the highest value from this residue, this study aimed to evaluate its chemical composition to determine the most suitable industrial applications for this feedstock. The results showed that the açaí seed is mainly composed of carbohydrates (57.2%), of which 82% are mannan. As mannose is a functional ingredient of great interest to the industry, the second step of this work was to develop methods to convert this high mannan content of the seed into mannose. For this, the açaí seeds were hydrolyzed using dilute H_2SO_4 (1.5-4.5% w/w for 30-60 min, 121 °C). However, in the best condition, using 3% acid for 60 min, ~70% of the mannan remained in the seed. To maximize sugar recovery, mannanase-catalyzed hydrolysis was sequentially performed. The acid-treated samples (3.0% acid, 60 min) were hydrolyzed with "BGM Amano 10" using 2-20% solid content. The highest mannose yield, 98.6%, was obtained with 20% solids, resulting in 146.3 g/L of mannose. This sequential hydrolysis method, with acid and mananases, allowed the recovery of 98.8% of the original mannose content of the seeds. Thus, this work contributes to the development of transformation routes for the production of high concentrations and yields of mannose from an agricultural residue, giving value to an abundant and unexplored biomass.

Strategies for improving the production of polyhydroxyalkanoates (PHAs) from industrial wastewater by *Cupriavidus necator* H16

N. Hernández-Herreros and M. Auxiliadora Prieto, Biological Research Center Margarita Salas (CIB-CSIC), Madrid, Spain

Biopolymers such as polyhydroxyalkanoates (PHAs) are considered as an alternative to conventional plastics, but their production can only be economically feasible by waste valorization approaches. The project Afterlife[1], funded by the Bio-Based Industries Joint Undertaking under the European Union's Horizon 2020 research and innovation program, aims a flexible, cost- and resource-efficient process for recovering and valorizing the relevant fractions from wastewater (WW). The AFTERLIFE process will isolate the different components of value using a cascade of membrane filtration units that will separate all the solids in the WW. These will then be treated to obtain highly-pure extracts and metabolites or, alternatively, to be converted into PHA. Volatile fatty acid (VFA) fractions with different compositions were obtained by the acidogenic fermentation of the WW from three food industries. In this study, we present a strategy to revalorize those VFAs for the production of PHA using pure cultures. The model strain *Cupriavidus necator* H16 is a non-pathogenic bacterium that has been the subject of intensive research due to its metabolical versatility and its ability to store up to 90 % of its cell dry weight as polyhydroxybutyrate (PHB). The tolerance studies of H16 strain to VFAs and the determination of key physiological parameters have been applied to optimize the fermentation conditions using three fermented industrial WW on a lab-scale up to 5 liters. The highest PHA concentration (5 g L^{-1}), PHA productivity (0.1 g L^{-1} h^{-1}), and PHA content (80 %) have been achieved by modulating the carbon pulses in a fed-batch system, based on the specific consumption and biomass/product produced.

[1]https://www.bbi.europa.eu/projects/afterlife

VALORIZATION OF 2G BIOREFINERY GENERATED WASTE “TECHNICAL LIGNIN”

R. Agrawal, DBT-IOC Centre for Advanced Bioenergy, Faridabad, India*

The technology advancements in the area of second generation (2G) bioethanol have led to the installation of multiple commercial scale cellulosic ethanol biorefineries globally. However, still the most critical challenge is the commercial viability of the process. Each liter of ethanol produced from biomass generates approx. 2-2.5 Kg of technical lignin which does not have any other value except burning as a low-cost fuel. The chemical composition and physio-chemical properties of the technical lignin is dependent upon the type of lignocellulosic biomass and the pretreatment approach. In this study, the chemical composition and the physio-chemical characterization of the technical lignin generated in a multi-feed demonstration scale plant was investigated. It was observed that the technical lignin which is predominantly composed of lignin, ash and highly recalcitrant residual cellulose is a high value bio-resource available to produce bio-based bulk chemicals, polymers, specialty molecules, and materials. The residual cellulose and lignin present in technical lignin offer unique features for its applications in adhesive, carbon fiber and nanomaterial industries. This study will showcase new avenues in this area of research to valorize the waste technical lignin in a biorefinery concept for improved biofuels sustainability. Furthermore, techno-economic and life cycle aspects of the present approach are discussed to identify the cost drivers, grey areas and process options.

Biocatalysis based on cold-active lipase for silymarin valorization from vegetal waste of cold-pressed milk thistle oil technology

G. Gheorghita and M. Tudorache, University of Bucharest, Bucharest, Romania; C. Purcarea, Institute of Biology Bucharest of the Romanian Academy, Bucharest, Romania*

Silymarin is a natural mixture of flavonolignans extracted from *Silybum marianum* L. Gaertn (milk thistle) seeds [3]. Silybins are one of the most biologically active compounds within the silymarin mixture, with two diastereoisomers (silybin A and silybin B, 1:1) [4]. Their molecular structure is particularly composed of a chromone fragment responsible for the weak acidic properties. Meanwhile, the presence of polyphenol hydroxyls provides the ability to form complexes with transitional metal ions and high antioxidant activity is imprinted to the molecule. Moreover, this active ingredient is tolerated by animals and humans even at very high doses [5].

Silybin has low liposolubility, thus consisting its drawback on cellular adsorption in the context of bioavailability strategy. So that, silybin fatty acid esters could be a valuable alternative. We propose a biocatalytic cold-active lipase-mediated system for the acylation of silybin A/B with proper fatty acids or esters. The biocatalyst consists of the protein material extracellularly produced by a novel *Psychrobacter sp.* extracted from perennial ice deposits of Scarisoara Ice Cave (Romania). The lipolytic effect of the protein material is considered for the catalyzed reaction, as many extracellular putative lipases are attributed to species draft genome. The relative enzyme activity was evaluated for both free and immobilized biocatalyst specimens and the optimization of the biocatalytic system has been achieved. All of these experimental aspects will be detailed during the lecture.

Acknowledgements: This work was financially supported by PNCDI III PED project (contract no. 356PED/2020) from UEFISCDI, Romania.

References

1.Lee DY-W, Liu Y (2003) J. Nat. Prod. 66, 1171-1174.

2.Liang Q, Wang C, Li BB, Zhang AH (2015) Pharmacong. Mag. 43, 586-593.

3.Singh RP, Agarwal R (2006) Mol. Carcinogen 45, 436-442.

4.Florczak T, Daroch M, Wilkinson MC, Bialkowska A, Bates AD, Turkiewics M, Iwasnejko LA (2013) Enzyme Microb Technol 53,18-23.

SUSTAINABLE BIOGAS PRODUCTION FROM ANAEROBIC DIGESTION: A PROMISING ALTERNATIVE FOR THE ORGANIC SOLID WASTE MANAGEMENT

J. Lopes[*] and B. Lunelli, Pontifical Catholic University of Campinas, Campinas, Brazil

In search of an environmental, social, and economic balance, it is necessary to adopt measures in urban infrastructure that incorporates integrated management that supplies the increase in energy demand with the subsequent increase in waste generated by human activities. This scenario motivates the investment in technologies that prioritize the quality of clean energy generation added to a set of public policies aimed at sustainable development for the use of natural resources and their waste. Moreover, it may contribute to mitigate climate change by reducing anthropogenic carbon emissions. Biogas is a versatile energy resource that can be used to produce electricity and heat, replacing all the functions of natural gas, including diesel substitution in transport (heavy vehicles) and agricultural (machines) sectors. Its production takes place through the anaerobic digestion process, in which microorganisms naturally degrade organic matter. It is a viable and sustainable alternative for the organic solid waste treatment since it provides an environmentally friendly final disposal and starts to treat the waste as an important substrate, adding value to its use, either in the form of energy or as a by-product that can be used as biofertilizer. As a decentralized component of the overall energy system, the biogas production infrastructure can be used as a center for local consumers within the available organic waste. Based on the above, the present study aimed to perform a SWOT analysis of the biogas production from the anaerobic digestion process and propose a strategic plan for its sustainable development using residual organic waste from local supply centers in Brazil. The results highlight the potential uses of available organic waste in the assessed region for biogas production, as well as the possibility of identifying the main bottlenecks associated with the process.
Keywords: SWOT. Sustainable energy. Waste-to-energy.

Characterization of Carpenter Bee Bacterial Isolates for Biodegradation of Pulp Milling Waste

M. Cuebas-Irizarry[*] and A. Grunden, North Carolina State University, Raleigh, NC, USA; S. Sidhu, University of North Carolina, Chapel Hill, NC, USA

Complex polymers represent a challenge for environmental pollution as well as an opportunity for microbial catalyzed conversion to generate valorized chemicals. As an example, black liquor, a by-product from paper milling contains lignocellulose components but is typically burned for energy. Here, we are investigating biodegradation of the lignin and hemicellulose-derived compounds present in black liquor using bacterial strains isolated from carpenter bees. The isolates were identified to be be *Streptomyces spp.* by 16S rDNA sequencing. Growth was assessed for the isolates cultured in minimal media with the lignocellulose constituents (cellulose, xylan, and lignin) or black liquor (pulping waste) added as the only source of carbon. Filter paper deconstruction, dye decolorization assays, and % lignin reduction assays were used to determine the cellulose, hemicellulose, and lignin degradation potential of the isolates, respectively. Strain 2-6 and 2-10 were able to decolorize Congo Red by 60 and 50%, respectively, Tolouidine Blue by 100 and 70%, Remanzol Brilliant Blue by 30 and 32% and Bromocresol Green by 15 and 18% after one week incubation without the addition of a reaction mediator. Cellulose deconstruction experiments showed degradation of up to 30% of the filter paper within 10 days. Growth on lignin revealed that strain 2-6 could degrade up to 24% of the lignin mass within 30 days. Evaluation of enzymes activities that may participate in lignin deconstruction (e.g. laccases, peroxidases, etc.) have been conducted to provide additional insight into the potential of *Streptomyces spp.* isolate 2-6 and 2-10 for lignin degradation and dye decolorization.

3-hydroxypropionic acid metabolism in *Aspergilli*

K. Pomraning, Z. Dai, N. Munoz, Y.M. Kim, Y. Gao, S. Deng, J. Kim, B. Hofstad, T. Lemmon, M. Swita, K. Burnum-Johnson and J. Magnuson, Pacific Northwest National Lab, Richland, WA, USA*

Biological engineering of microorganisms to produce value-added chemicals is a promising route to sustainable manufacturing. However, overproduction of metabolic intermediates at high titer, rate, and yield from inexpensive substrates is challenging in non-model systems where limited information is available regarding metabolic flux and its control in production conditions. Integrated multi-omic analysis of engineered strains offers an in-depth look at metabolites and proteins directly involved in growth and production of target and non-target bioproducts. Here we applied multi-omic analysis to overproduction of the polymer precursor 3-hydroxypropionic acid (3HP) in the filamentous fungi *Aspergillus pseudoterreus* and *Aspergillus niger*. Metabolic pathways involved in degradation of 3HP were identified, elimination of which improved the yield of 3HP dramatically.