



## **Track 1: Advanced Biofuels and Biorefinery Platforms**

**Sponsored by: Ensyn**

### **Session 1: Process in the Aviation Biofuels Industry**

**Tuesday, May 13 8:30am-10:00am**

Moderator: *TBD*

Jeff Trewella, PetroTech Consultants LLC,  
*The Alternative Jet Fuel Qualification Process*

If a load of jet fuel does not meet rigid quality and performance standards there is potential for catastrophic losses. This is the reason that aviation turbine fuels like Jet-A are required to meet more stringent specifications than any other fuel product. Therefore compared to gasoline and diesel fuels, this creates a more daunting entry barrier for jet fuels derived from nonconventional sources such as sugars, crop oils, algae, and biomass. Fortunately, a protocol for qualifying new fuels has been developed and is still evolving. This presentation will discuss four major topics. First, the market and political factors driving the use of alternative jet fuels will be discussed. Second, key jet fuel technical requirements will be reviewed. These will include the range of allowable fuel compositions, key specification properties, and fit for purpose tests. Thirdly we will review the overall qualification process from the perspective of a fuel supplier seeking to qualify a new aviation fuel. (Note that the authors have successfully qualified the first alternative jet fuel, Synthetic Paraffinic Kerosene, worked with DARPA on the second, Hydrotreated Esters and Fatty Acids and are currently leading two other jet fuels through the qualification process.) Resource requirements (including fuel volume,

time, and budget) will be discussed for fuels that already have been qualified as well as those currently navigating the qualification process. As a final topic we will examine pressures and constraints on the existing process and discuss how they might impact the qualification of future jet fuels.

Steve Csonka, Commercial Aviation Alternative Fuels Initiative (CAAFI)

*Update from CAAFI on recent progress towards commercialization of sustainable alternative jet fuel for the aviation enterprise.*

This session will explore challenges and opportunities associated with the development and commercialization of sustainable alternative jet fuels. Much work lies ahead for this new industry to reach commercial scale but significant milestones have been already achieved. The speaker will provide perspectives on how government, industry, academia, and multi-lateral organizations are joining forces and collaborating to facilitate the deployment of alternative jet fuels.

### **Session 2: Renewable Hydro-Carbons Biofuels Tuesday, May 13 10:30am-12:00pm**

Moderator: *TBD*

Perry Toms, Steeper Energy,

*"Cost Competitive Biomass-to-Liquids":*

*Lignocellulosics and Low-grade Coals to Liquid Hydrocarbons via Hydrothermal Upgrading*

Steeper Energy's proprietary Hydrofaction™ technology produces sustainable and renewable energy that can also play an important complementary role in existing petroleum infrastructure. Based on proven science and a commercially emerging technology, Steeper



Energy aims to become a renewable adjunct and bridge to the oil industry not only through the production of synthetic crude oil, but also the production of bio-chemicals and liquid CO<sub>2</sub> captured from the Hydrofaction™ process. Hydrofaction™ uses a catalytic hydrothermal conversion using super critical chemistry. Super criticality is a state of matter achieved when a fluid, like water, is heated well beyond its boiling point, while being contained and pressurized. Any moisture inherent in the feedstock subjected to supercritical conditions becomes an aggressive chemical and physical force that, in presence of low-cost catalysts, aids in the transformation into synthetic crude oils. The process conditions allow for the selective removal of oxygen from the organic feedstock molecular structure thus increasing the carbon-hydrogen ratio of the resulting fuel oil. Steeper Energy's Hydrofaction™ technology is currently being commercialized to convert biomass (wood and agricultural waste or energy crops) and/or peat or lignite coal into liquid hydrocarbons (middle distillates). Steeper has built and installed a continuous-bench-scale (CBS) unit at the University of Aalborg in Denmark. This 15 kg/hr institutional scale Hydrofaction™ reactor and balance of plant was commissioned and is producing excellent results. After over a year of demonstration testing in partnership with Aalborg University, Steeper is moving forward with the development of commercial-scale pilot facilities in Canada. Steeper Energy has signed a letter of intent with the Port of Frederikshavn (Northern Jutland, Denmark) to design, build and operate a bio-refinery estimated to produce 1,000 barrels of low-sulfur marine propulsion fuel per day by 2018. The new Steeper marine bio-fuel is a fully miscible, low-sulfur, petroleum-like fuel,

and can be safely mixed into residual fuels that may be in ships' tanks already. There is a considerable supply of available biomass in North America that can be used to help meet new the 2015 low-sulfur marine regulations by adopting and expanding Hydrofaction™ technology.

### **Session 3: DOE Bioenergy Research Centers Achievements**

**Tuesday, May 13 2:30pm-4:00pm**

Moderator: Brian Davison, Oak Ridge National Company

Timothy Donohue, Great Lakes Bioenergy

Paul Gilna, Bioenergy Science Center (BESC), Oak Ridge National Laboratory

Blake Simmons, Joint BioEnergy Institute (JBEI)

#### US DOE Bioenergy Research Centers: Achievements, Capabilities, and Technologies: a Science Café

The US DOE established three Bioenergy Research Centers (BRCs) to focus the most advanced biotechnology-based resources on the biological challenges of biofuel production. In the past 6 years, each center has pursued basic research underlying a range of high-risk, high-return biological solutions for bioenergy applications. Advances resulting from the BRCs are providing the knowledge needed to develop new biobased products, methods, and tools that the emerging biofuel industry can use. New strategies and findings emanating from the centers' fundamental research ultimately will benefit all biological investigations and will create the knowledge underlying three grand challenges at the frontiers of biology: Development of next-generation bioenergy crops, of enzymes and microbes with novel



biomass-degrading capabilities, and of transformational microbe-mediated strategies for biofuel production. This session will cover major accomplishments including IP and key capabilities at the three centers. Great Lakes Bioenergy Research Center: producing sustainable cellulosic biofuels GLBRC ([www.glbrc.org](http://www.glbrc.org)) conducts genome-enabled, systems-level research to provide the underpinnings for production of ethanol and advanced biofuels from the cellulosic fraction of plant biomass. GLBRC's research portfolio is built on four integrated Research Areas. The goals of each research area are Sustainability: to identify factors affecting the ecological and economic sustainability of biofuel production, Plants: to improve the traits and productivity of bioenergy crops, Deconstruction: to improve chemical and enzymatic methods for releasing monomers or short oligomers from lignocellulosic biomass, and Conversion: to improve microbial and chemical systems for converting materials derived from lignocellulosic biomass to biofuels. The BioEnergy Science Center (BESC): overcoming recalcitrance The primary goal of BESC ([www.bioenergycenter.org](http://www.bioenergycenter.org)) is to enable the emergence of a sustainable cellulosic biofuels industry by leading advances in science resulting in the removal of recalcitrance of biomass as an economic barrier. This overview will discuss advances in our two focused approaches to overcoming recalcitrance: the development of microbial strains capable of consolidated bioprocessing, and the development of improved feedstocks for biofuels. This has led to identification of key genes for low recalcitrance plants and for improved bioconversion. Advanced Biofuels R&D the Joint BioEnergy Institute (JBEI) The

DOE-funded JBEI is focused on the production of infrastructure compatible biofuels derived from lignocellulosic biomass. Biomass is a renewable resource that is potentially carbon-neutral. The development of cost-effective and energy-efficient processes to transform cellulose and hemicellulose in biomass into fuels is hampered by significant roadblocks, including the lack of dedicated energy crops, the difficulty in separating biomass components, low activity of enzymes used to hydrolyze polysaccharides, and the inhibitory effect of fuels and processing byproducts on the organisms responsible for producing fuels from monomeric sugars. This presentation will highlight research underway at JBEI to overcome these obstacles, with a focus on the development of ionic liquid pretreatments for the efficient production of monomeric sugars from biomass. Roundtable

#### **Session 4: Next Wave of Commercial Cellulosic Projects**

**Wednesday, May 14 2:30pm-4:00pm**

Moderator: *TBD*

[Patrick Foody, Iogen Corporation](#)

[Commercial ethanol production from bagasse in Brazil using Iogen's process](#)

There is a large opportunity to produce ethanol from sugar cane bagasse in Brazil. Raizen is building a plant in Piracicaba, Brazil to carry this out. The process includes: pretreatment with dilute sulfuric acid; enzymatic hydrolysis of the cellulose; fermentation of the sugars to ethanol by *Saccharomyces* yeast; and, distillation of the broth to recover cellulosic ethanol. The plant is a scale-up of Iogen's Demonstration plant in Ottawa and is scheduled to be operational by



the end of 2014. This presentation will describe how the design of the commercial plant builds upon the experience of operating the Demonstration plant and the synergies created by integrating with an existing sugarcane mill.

Craig Stuart-Paul, Fiberight LLC

Novozymes and Fiberight Deploy World Class Cellulosic Ethanol Technology from Municipal Solid Waste to Market

World cities generate about 1.3 billion tonnes of solid waste per year. This volume is expected to increase to 2.2 billion tonnes by 2025. Waste generation will more than double over the next twenty years in lower income countries and cost about USD 375.5 billion on a global scale. The global impacts of MSW are growing fast. Solid waste is a large source of methane, a powerful GHG that is particularly impactful in the short-term. This is made particular challenging as landfills remains the most widely used disposal option globally. By 2050, as many people will live in cities as the population of the whole world in 2000, with urban residents producing about twice as much waste as their rural counterparts. By using the same technological solutions that have allowed advanced biofuels to reach commercial scale production, we now have a viable alternative to converting municipal solid waste (MSW) into a range of value-added products, such as biofuel, biogas and not least biochemicals. Combining biotechnology advances with more efficient sorting processes should not be seen as a substitute to existing waste management practices, but rather as an opportunity to make more efficient use of MSW as a growing resource and in co-existence with current infrastructure and waste incineration facilities. Municipal solids waste (MSW) can be converted

into sugars, biochemicals, or biofuels. The total generation of MSW in the US has increased steadily from 88.1 to 249.9 million tons in the last 50 years. The partnership between Fiberight, one of the first U.S. based companies to successfully produce biofuel from waste on an industrial scale, and Novozymes, the world leader on biosolutions and bioinnovation, are using biotechnology to produce bioethanol from MSW under economically viable conditions. Optimized enzyme cocktails developed in partnership with Fiberight have significantly improved the enzymatic digestibility and reduced the enzyme use cost per gallon of ethanol. Fiberight is currently operating a fully integrated demonstration scale plant in Lawrenceville, VA providing data and process information that will be used to transfer technology to the commercial scale plant in Blainstown, IA. The organic biopulp recovered from MSW fractionation is the key material for the sugar platform and biofuel production. The process creates a homogenous lignocellulosic biopulp substrate from a heterogenous MSW stream. The biopulp fraction is about 45% of the MSW, and is used today to produce cellulosic ethanol. The MSW biopulp can be treated at mild conditions and hydrolyzed with high performing enzyme cocktails for optimized enzymatic hydrolysis into sugars. The sugars can then be used for production of chemicals, cellulosic ethanol or other biofuels. • The ability to rethink MSW as a valuable resource could become a strong competitive advantage for governments in the face of growing urbanization • Preliminary cost analysis suggest a significant reduction in project economics by focusing on co-existence with current waste management infrastructure and facilities • More efficient sorting processes



eliminate the need for presorting by households while still capturing most value of every waste fraction • A clearly defined regulatory framework is critical to provide the necessary economic incentives to allow for the production of value-added products

Martin Mitchell, Clariant

*Cellulosic Ethanol: Using New Biomass Sources for Commercial Scale Production of Advanced Biofuels*

The energy industry is currently undergoing a paradigm shift. More and more economies are looking at possibilities to substitute fossil resources by renewable ones, both to secure energy supply and to increase sustainability. The transport sector in particular will continue to be largely dependent on liquid energy sources and advanced biofuels are the preferred way to efficiently reach renewable substitution goals in the short to medium term. The key technical hurdle in the past has been how to access the sugars bound in the lignocellulosic material. In recent years technological breakthrough has been achieved through a variety of technologies, in particular enzymatic conversion. The matter now is to validate production processes and optimize the efficiency for large scale competitive production, in particular through high process yields combined with low OPEX and CAPEX. Clariant's sunliquid® technology overcomes these challenges through a thorough and entirely integrated process design and innovative technology features offering a one-stop shop solution flexible to be used to convert different feedstock and adopt to various plant concepts. The production cost can compete with those of first-generation bioethanol and the greenhouse gas savings of the sunliquid®

ethanol are 95% compared to fossil fuels. Realisation on an industrial scale is no longer merely a dream. Since July 2012 Clariant has successfully been operating a demonstration plant in Straubing, Germany, with an annual capacity of 330,000 gallons (1.25 million litres, 1,000 tons), converting approximately 4,500 tons of lignocellulosic feedstock per year. After focussing on wheat straw for the first months of operation, in May 2013, first runs with corn stover and sugarcane residues also showed good results and validated the technology further. This was a new milestone reached by the project, confirming that the technology can be implemented worldwide. The results obtained in the demonstration plant are being incorporated into plans and design for the first commercial production plant. Agricultural residues are the most important feedstock globally available for the efficient production of advanced biofuels, such as cellulosic ethanol. These by-products from agricultural production have only limited use today and are broadly available in large volumes. According to estimates by the US Department of Energy the volumes of corn stover and cereal straw available in a sustainable way accumulate to 190-290 million metric tons annually in the US. These substantial amounts highlight the economic value of a cellulosic ethanol as a high quality product made from this so far largely unused renewable feedstock.

Bill Brady, Mascoma

Mascoma will discuss commercial progress with its proprietary and innovative consolidated bio processing (CBP) technology. In addition to the commercial penetration into first generation ethanol markets, the company will review



approaches to deploy CBP across a variety of biomass to petroleum replacement applications.

#### **Session 5: New Uses for CO<sub>2</sub>**

**Thursday, May 15 8:30am-10:00am**

Moderator: Laurel Harmon, LanzaTech

*Global Diversity: The Key to Commercialization.*

Increasingly there is a trend in being able to supply products such as fuels and chemicals that are not only economic, but that address key sustainability criteria. Today there is significant research into improving the overall carbon and energy efficiency of biorefineries to overcome challenges such as high energy demands, high capital costs, poor carbon utilization, or a combination of these factors. There is a potential to overcome these challenges when using wastes and residues to produce fuels and chemicals but commercializing such technologies involves strategic global thinking.

Wastes and residues are produced from a wide range of industries including gaseous waste carbon from industrial processes (steel mills, refineries, ferro-alloy plants and PVC plants) or from thermochemical processes such as gasification of biomass/MSW or reformed natural gas. These are conventionally used as a fuel source or flared before being release as CO<sub>2</sub>.

LanzaTech offers an alternative solution.

The CO, H<sub>2</sub>, and/or CO<sub>2</sub> contained in such feed gas can be fermented via a biocatalyst to fuels/chemicals such as ethanol, acetic acid or butadiene which also serve as building blocks to longer chained hydrocarbon drop-in fuels.

LanzaTech's bio process uses a broad spectrum of gases from a variety of sources and offers superior carbon conversion, energy efficiency,

and greenhouse gas emission performance compared to conventional and emerging routes to the same products.

The pathway to commercialization will be outlined showing how diversity of input gas streams enables the technology to be deployed across a variety of geographies at both a regional and national level, depending on which feedstocks are available.

A diverse product portfolio that includes chemical precursors for the production of plastics, nylon and 'drop-in' jet fuel will also be described. This has led to successful partnerships across a variety of sectors including steel, aviation and chemicals. These commercial and research partners across multiple industries and disciplines globally has helped maintain a level of innovation to continue to deliver an approach that makes intuitive sense the world over – why waste carbon when you can put it to good use?

Jonathan Carley, CO<sub>2</sub> Solutions, Inc.

*Deployment of Enzymatic Technology for Low-Cost Carbon Mitigation In Emissions-Intensive Industries*

CO<sub>2</sub> Solutions Inc. of Québec, Canada is deploying a patented biotechnological approach for the cost-advantaged, operationally superior capture, sequestration and beneficial reuse of carbon dioxide (CO<sub>2</sub>) emissions from various industries. Using genetically optimized variants of the enzyme carbonic anhydrase, simple aqueous salt solutions can be enabled within conventional gas scrubbing equipment to replace costly and toxic chemical solvents as an elegant biomimetic alternative for CO<sub>2</sub> capture from combustion effluent gases. The presentation will discuss the basis of the technology and its commercial validation in the



oil production and power generation industries.

Sissi Liu, Joule

*CO<sub>2</sub>-to-Fuels: Producing above-ground fuel reserves without upstream exploration risk or downstream refining cost.*

Nearly \$700 billion is being invested in pursuit of diminishing petroleum, including newer and even riskier practices to obtain “unconventional oil” from oil sands and oil shale. A CO<sub>2</sub>-to-fuels platform can offer a lower-risk and higher-reward alternative to oil E&P – both traditional and unconventional – by creating above-ground fuel reserves in real time; eliminating both upstream exploration and downstream refining. This platform, now operating at demonstration scale, requires only sunlight, waste CO<sub>2</sub> and non-potable water. Product-specific catalysts directly convert the CO<sub>2</sub> to targeted molecules, including ethanol or hydrocarbons that comprise diesel, jet fuel and gasoline. With abundant inputs, single-step production and a process that avoids depletion of precious natural resources, this platform represents the height of sustainability. This presentation will cover the progress, challenges and future prospects for global deployment of a solar CO<sub>2</sub>-to-fuels platform. What makes this approach unique? Conversion Technology Breakthroughs: Product-specific catalysts are self-replicating before a “carbon switch” diverts nearly 100% of the carbon from cell growth to fuel production. Combined advances in catalyst engineering, solar capture and process engineering result in high CO<sub>2</sub>-to-product conversion efficiency. Abundant Inputs, Valuable Outputs: With readily available low-cost feedstocks, the platform can be tailored to produce specific products simply by exchanging the catalyst. The end products require no “crude” intermediates

or refining. Modular, Scalable System: A novel, modular system enables direct conversion of CO<sub>2</sub> to fuels in a single step. The system is designed for ease of installation and expansion in modular increments, making it readily scalable and customizable to targeted output levels based on solar insolation, land and waste CO<sub>2</sub> availability. Highly Efficient Production: The platform produces in minutes what conventional oil takes millennia, avoiding the extreme complexity, volatile resources and escalating risks of fossil fuel production. At full-scale commercialization in ideal locations, a production facility could produce up to 25,000 gallons of ethanol or 15,000 gallons of diesel per acre annually, at costs as low as \$1.28/gallon and \$50-60/barrel, respectively. Localized Production: Without feedstock or land constraints, the platform is suitable for deployment in many regions of the world, creating the opportunity to localize fuel production and reduce the burden of oil imports. CO<sub>2</sub> Mitigation: The technology offers an attractive opportunity to utilize waste CO<sub>2</sub> emissions rather than implement costly CCS solutions.

Kef Kasdin, Proterro

*Making Sugar from CO<sub>2</sub>*

A critical barrier to scale for the production of biofuels and biobased chemicals is access to reliable and economical feedstock. Feedstock is the largest component of end-product costs and is subject to the variables and challenges of various agricultural product markets, including sugarcane, corn and biomass. Current approaches to the cultivation, harvest, storage, transport and processing of crop-based products add substantial cost - when all that is required is simple sugar. Proterro is the only



biofeedstock company that makes sucrose instead of extracting it from crops or deconstructing cellulosic materials. Using CO<sub>2</sub>, sunlight and water, Proterro lowers the cost of sugar production to around five cents per pound, unleashing the economic value of biofuels and biobased chemicals for industry partners. The company has developed a novel, scalable biosynthetic process that integrates a patented, highly productive microorganism with a robust, modular photobioreactor made from off-the-shelf materials. This process yields a fermentation-ready sucrose stream, rather than a mixture of sugars, allowing simple, low-cost downstream processing. Proterro will present updates on its key development milestones. The company has scaled up its novel photobioreactor design and conducted independent functionality tests that confirmed the unit's robustness: the photobioreactor is able to withstand category 1 hurricane winds. Because of the innovative photobioreactor design and materials used, Proterro has also been able to validate low fabrication costs. Proterro is in the process of commissioning a pilot plant in Florida and has completed a preliminary design, layout and associated cost estimate for a demonstration-scale plant.

**Session 6: TBD**

**Thursday, May 15 10:30am-12:00pm**

Moderator: *TBD*

## **Track 2: Algae, Specialty Crops, and Biomass Supply**

**Session 1: The Future of Algae Production Systems**

**Tuesday, May 13 8:10am-10:00am**

Moderator: *TBD*

John McGowen, Arizona State University, AzCATI

Algae Testbed Public Private Partnership (ATP3): Multi-Region, Long-Term Algae Biomass Cultivation Trials

ATP3 is made possible by a \$15 million U.S. Department of Energy competitive grant from its Bioenergy Technologies Office. Our vision is to establish a sustainable network of regional testbeds that empowers knowledge creation and dissemination within the algal research community, accelerates innovation, and supports growth of the nascent algal fuels industry. Our goal is to create a network of operating testbeds, bringing together world-class scientists, engineers and business executives to lead the effort to increase stakeholder access to high quality facilities by making available an unparalleled array of outdoor cultivation, downstream equipment, and laboratory facilities tightly managed by a multi-institutional and transdisciplinary team. ATP3 is utilizing that same powerful combination of facilities, technical expertise, and management structure to support DOE's TEA, LCA and resource modeling and analysis activities, helping to close critical knowledge gaps and inform robust analyses of the state of technology for algal biofuels. Anchored by the existing 300,000L, open testbed facilities at ASU and augmented by university and commercial facilities in Hawaii (Cellana), California (Cal Poly San Luis Obispo), Ohio (Touchstone Research Laboratories), Georgia (Georgia Institute of Technology), and Florida (Florida Algae). ATP3 provides comprehensive cultivation and harvesting facilities along with the required experience of operating an open testbed



system. A primary objective has been to utilize our expertise and world-class facilities to perform long term cultivation trials with process and statistical rigor, producing robust, meaningful datasets across different regional, seasonal environmental and operational conditions. These data are critically important to support TEA and LCA activities that will guide research and development towards the transformative goal of cost-competitive algal biofuels by 2022. To achieve our goals, ATP3 has implemented an experimental framework named “Unified Field Studies” (UFS). Through our UFS framework, we are generating data for the assessment of the current and future state of technology informing modeling efforts to establish economic and sustainability metrics and project future targets for algal biofuel production. We will provide an overview of our multi-year experimental plan and summarize on our current progress to date and results from our first UFS run across six distinct geographic regions using our mini-raceway pond systems that have been installed and are operational at all six of our testbed sites. We will also discuss our data management system and preview our strategy for making the data we generate easily and readily accessible to the research community.

Peter Lindblad, Uppsala University  
*Design, Engineering, and Construction of Photosynthetic Microbial Cell Factories for Renewable Solar Fuel Production*

There is an urgent need to develop sustainable solutions to convert solar energy into energy carriers used in the society. In addition to solar cells generating electricity, there are several options to generate solar fuels. Native and engineered cyanobacteria have been as model

systems to examine, demonstrate, and develop photobiological H<sub>2</sub> production. More recently, the production of carbon-containing solar fuels like ethanol, butanol, and isoprene have also been demonstrated [1]. We have initiated a development of a standardized genetic toolbox, using a synthetic biology approach, to custom-design, engineer and construct cyanobacteria to produce a desired product [2]. One bottleneck is a controlled transcription of introduced genetic constructs. I will present and discuss recent progress in the design, construction and use of (i) cyanobacteria for renewable solar fuel production, and (ii) artificial genetic elements for the regulation of transcription in cyanobacteria. Specific focus will be on the native system present in cyanobacteria to generate hydrogen, metabolic modulations [3] and genetic engineering ([4, 5] for enhanced hydrogen production, as well as the introduction of custom-designed, non-native hydrogenases. In addition, I will report on the development of engineered TetR-regulated promoters with a wide dynamic range [6]. By altering only few bases of the promoter in the narrow region between the -10 element and transcription start site significant changes in the promoter strengths, and consequently in the range of regulations, were observed. Finally, I will give an up-date summary of the present status on the direct production of solar fuels using photosynthetic microbial cell factories (algae and cyanobacteria). [1] Lindblad, Lindberg, Oliveira, Stensjö, Heidorn. 2012. Design, Engineering, and Construction of Photosynthetic Microbial Cell Factories for Renewable Solar Fuel Production. *Ambio* 41 (Suppl 2): 163-168. [2] Heidorn, Camsund, Huang, Lindberg, Oliveira, Stensjö, Lindblad. 2011. Synthetic Biology in Cyanobacteria:



Engineering and Analyzing Novel Functions. Methods in Enzymology 497: 540-579. [3] Khetkorn, Baebprasert, Lindblad Incharoensakdi, A. 2012. Redirecting the electron flow towards the nitrogenase and bidirectional Hox-hydrogenase by using specific inhibitors results in enhanced H<sub>2</sub> production in the cyanobacterium *Anabaena siamensis* TISTR 8012. Bioresource Technology 118: 265–271. [4] Baebprasert, Jantaro, Khetkorn, Lindblad, Incharoensakdi. 2011. Increased H<sub>2</sub> production in the cyanobacterium *Synechocystis* sp. Strain PCC 6803 by redirecting the electron supply via genetic engineering of the nitrate assimilation pathway. Metabolic Engineering 13: 610-616. [5] Khetkorn, Lindblad, Incharoensakdi, A. 2012. Inactivation of uptake hydrogenase leads to enhanced and sustained hydrogen production with high nitrogenase activity under high light exposure in the cyanobacterium *Anabaena siamensis* TISTR 8012. Journal of Biological Engineering 6:19. [6] Huang, Lindblad. 2013. Wide-dynamic-range promoters engineered for cyanobacteria. Journal of Biological Engineering 7:10

Esteban Hincapie Gomez, Colorado State University

*Development of a Continuous Flow Ultrasonic Microalgae Harvesting System*

Microalgae have vast potential as a sustainable and scalable source of biofuels and bioproducts. If implemented sustainably into the global energy and food portfolio, microalgal products can reduce greenhouse gas emissions while simultaneously displacing a substantial percentage of our declining fossil fuel resources. However, algae dewatering is a critical challenge that must be addressed. Because algae are typically cultivated at highly

diluted concentrations (approx. 99.9% water), algae dewatering represents the most significant energy sink in the entire microalgae to biofuels value chain. The use of ultrasonic standing waves has been reported in the literature as an approach to manipulate particles in a fluid. This phenomenon of particle migration with the sound pressure is known as acoustophoresis. These waves exert an acoustic force (F) that agglomerate the cells and is directly proportional to the cell acoustic contrast factor (k). Ultrasonic microalgae harvesting is a promising low cost and energy approach. An acoustic separator does not have moving parts and do not require flocculants, providing very low operational expenses for cell separation. This research proposes a high efficiency continuous flow separation for microalgae and yeast by combining the concepts of acoustic settling with inclined plates that are acoustically transparent. By this novel approach, *Nannochloropsis oculata* and *Saccharomyces cerevisiae* cells were successfully harvested with efficiencies of 75%±5% and 95%±2% respectively at a flow rate of 25 mL/min or more while using a specific power input of 3.25 kWh/m<sup>3</sup>. When compared under the same conditions with other existing apparatus reported in the literature, the proposed device exceeds by 2 to 7 fold in terms of filtration efficiency and biomass recovery. It is shown that the separation efficiency is affected strongly by flow rate, power input, and settling angle. Computational Fluid Dynamics data is presented to explain this behavior and the results are in good agreement with the model.

**Session 2: Feedstock Development for the Next Generation of Biofuels**



**Tuesday, May 13 10:30am-12:00pm**

Moderator: *TBD*

Kevin Caffrey, North Carolina State University  
*A techno-economic evaluation of camelina*  
*(Camelina sativa) oilseed production scenarios*  
*for value added farm processing*

Camelina (*Camelina sativa*) is a promising oilseed with a high omega-3 fatty acid concentration, low agronomic requirements, and short maturity window. The beneficial characteristics of camelina have been proposed for use in various markets including: health food, animal feed, biofuels (e.g. aviation fuel, biodiesel), industrial products, and various niche markets (e.g. pulp & paper). A techno-economic evaluation will be conducted to compare processing scenarios varying in the extent of operations taking place between the farm and oil extraction refinery. Twelve total scenarios will be modeled including transportation between facilities. The two major products to be investigated over the production systems are cold pressed oil and biodiesel (also included are forage and grazing scenarios) with a number of co-products including: seed trash, presscake, and glycerin. The co-products will be considered for different markets corresponding to their production location: compost amendment, animal feed, local heat, and industrial purposes. The products and co-products defined for a given scenario will affect the potential uses of the material and the overall economic benefit. A shift towards more on-farm processing can provide benefits related to: increased rural development, reduced capital investment at manufacturing facilities, reduction in transportation requirements, and increased farm buy-in. This work will provide insight into

the economic benefits of several different configurations of camelina production and processing systems, as it relates to location of operations, transportation and diversification of useful product streams. The manner in which product markets, farmer involvement, and specific characteristics of the production system are integrated may show a greater proportion of on-farm processing to be beneficial.

### **Session 3: Securing Feedstocks: From Logistics to Conversion**

**Tuesday, May 13 2:30pm-4:00pm**

Moderator: Sarah Hickingbottom, LMC  
International

*Second Generation Fermentable Sugar*  
*Feedstocks - How Viable Are They?*

Producers of bio-based chemicals and biofuels are seeking to move away from first generation, food-based feedstocks and towards second generation raw materials. They are driven by supply chain, policy and sustainability concerns, such as food vs fuel and land use. For fermentation processes, this means a switch from using starch glucose, derived from crops, to cellulosic sugars generated from biomass sources. However, with the bio-based chemicals sector still in its infancy, the critical question is: will there be sufficient cellulosic sugars, in reliable commercial quantities and at competitive prices, to supply both the fuel and chemical markets? This paper will highlight the most economic dynamics underpinning both starch glucose and a potential, new second generation sugar supply derived from cellulosic (biomass) raw materials – cellulose and hemicellulose – and, as such, include C6 and C5 sugars. Will such a second generation market evolve into a merchant market? Or will



economic and physical supply chain constraints ensure vertical integration along the value chain? How will the physical supply chain need to develop before large, commercial-scale operations can source sufficient, reliable supply at a price that is competitive with first generation fermentable sugars? These and other key factors will be discussed. Please note: LMC works in a huge range of relevant topics and so can adjust this submission accordingly to fit your agenda needs. Please do not hesitate to contact me with any suggestions or requests in terms of a subject matter that you may be seeking more than the potential for second generation fermentable sugar market development.

Chenlin Li, Lawrence Berkley National Lab  
Scale-up of Ionic Liquid Based High Solid Biomass Deconstruction for Biofuel Production

Ionic liquid (IL) pretreatment is receiving significant attention as a potential process that enables fractionation of lignocellulose and produces high yields of fermentable sugars suitable for the production of renewable fuels. However, successful optimization and scale-up of IL pretreatment required for further commercialization involves challenges such as high solids loading, biomass handling and transfer, washing of pretreated solids, formation of inhibitors, and IL recycling, which have not been effectively resolved during the development stages at the bench scale. As a first in the research community, the Joint BioEnergy Institute (JBEI), in collaboration with the Advanced Biofuels Process Development Unit (ABPDU), a DOE-funded facility that supports academic and industrial entities in scaling their novel biomass deconstruction and biofuel/biochemical production technologies,

performed benchmark studies to address key challenges associated with IL based high solid biomass deconstruction critical for commercialization. Using switchgrass, eucalyptus and mixture of both as feedstocks, we have successfully executed 600-fold, relative to the bench scale (6L vs 0.01L), scale-up of IL pretreatment and 60-fold (1.5L vs 0.025L) scale-up of subsequent enzymatic saccharification at 15% (w/w) biomass loading. The results generated are consistent with those from the small-scale experiments that have been conducted at JBEI and elsewhere, and indicate there are no fundamental issues in terms of performance associated with the scale-up of the IL-based conversion technology. The further scale-up effort at ABPDU includes JBEI's two novel one-pot technologies, i.e., IL pretreatment/acidolysis, and IL pretreatment/saccharification with IL tolerant enzyme cocktails, followed by sugar extraction and IL recycling. The scale-up development at ABPDU will leverage the opportunity and synergistic efforts towards the development a cost-effective IL based deconstruction technology by drastically reducing/eliminating enzyme/water usage, and simplifying the downstream sugar/lignin recovery and IL recycling. The knowledge gained from these initial scale-up studies is an essential first step in demonstrating the commercial viability of the promising IL deconstruction technology.

Camilo Perez, McGill University  
Small scale biomass processing and handling approach for bioenergy production

In 2050 it is estimated that the world will reach nine billion people, and as a consequence there will be a significant increase in demand for energy. It is imperative to search for energy



sources beyond non-renewable fossil fuels in order to meet future energy requirements. Bioenergy can play a key role for our future energy needs. Biomass is an energy source that is availability worldwide and is renewable. The conversion of biomass to energy has different methods of use including gasification. Gasification is defined as combustion without the presence of oxygen and as a result the biomass is transformed into syngas which is predominantly composed of CO and O<sub>2</sub>. The logistics behind biomass to energy conversion is one of the major challenges that need to be addressed, with a specific focus on biomass transportation. Conveying biomass from farms to bio refinery transformation sites has a direct impact on the logistical cost, due to the low energy density of the feedstock. Additionally in large scale industrial gasification (higher than 500 Kw) logistic cost increases due to the large amount of biomass that needs to be provide in order to run the process. Most bioenergy approaches using biomass consider a central large scale facility and defined the supply chain network in order to optimize biomass transportation and storage, however, this paper proposes and investigates a different approach. Instead of transporting the available biomass from multiple farms to central facilities, this paper evaluated the feasibility of transforming the available biomass to electricity directly at the farm and then conveying the electrical energy through the electric grid. Three different regions in Quebec were selected based on biomass availability. In these regions a large scale centralizes facility and a small scale network were analyzed to determine the approach that has the highest overall performance.

#### **Session 4: Successful Strategies for Commercial Algae Production**

**Wednesday, May 14 2:30pm-4:00pm**

Moderator: *TBD*

Valerie Harmon, Cellana LLC

*Progressing Biofuel Production from Microalgae towards Commercial Success*

Progressing Biofuel Production from Microalgae towards Commercial Success Valerie Harmon, Senior Director, Research and Development, Cellana LLC (valerie.harmon@cellana.com) Cellana is a leading developer of algae-based bio-products, including ReNew Fuel, ReNew Feed and ReNew Omega 3. Cellana operates a demonstration scale production facility in Kona Hawaii that is capable of producing all three product lines simultaneously from the biomass generated, providing the company with a business model that allows cost effective production of biofuels. Over 100 high-performing algal strains for various bio-products have been isolated in Hawaii and are maintained at the facility. With our patented ALDUO™ algal production system, the facility is capable of reliably switching from one strain to another with ease allowing flexibility in product generation. To date, Cellana has screened a large collection of algal strains and produced more than 10 metric tons of biomass from numerous natural marine algal strains for the development of biofuels, feed, and high-value nutraceuticals. Cellana has recently tested the utilization of flue gas from our diesel generators as the primary carbon source for our production system. We have proven that production of quality biomass for biofuels, feeds and nutraceuticals is possible with flue gas as the primary carbon source. Additionally, our facility has been utilized to test multiple production



methodologies to improve yields contributing to the industry's ability to cost effectively commercialize. Our defatted biomass has been shown to be an effective feed ingredient for both livestock and aquatic animal feeds, and our fuel product has been evaluated and accepted as quality biofuel product by a large oil company. All of this research moves the industry forward towards successful commercialization.

Nick Donowitz, Heliae

*Algae as Bio-Fertilizer: Going back to the 'source' to feed 9 billion people*

The overlap of climate change and global food production limitations is beginning to loom large for academics and policymakers who are putting these two trend-lines together. If global population growth tracks as projected, we'll see the nine billionth person born sometime in 2050. Add to that the rapidly increasing purchasing power of poorer nations and you get a world that will soon be demanding 50-70 percent more food (special emphasis on the meat and fish-based protein) than we produce today, according to a United Nations study. Match this data with information leaked from the most recent IPCC report (to be published in March 2014) linking climate change with food supply disruptions. The leaked report points to a widening gap, with agricultural output rising a mere two percent each decade for the rest of the century, while the demand for food is projected to rise at a staggering 14 percent each decade during the same timeframe. In the face of a rising population, a more volatile climate and an increasingly difficult food production paradigm, new tools are needed to adapt to this new status quo. The world is going to need new ways of producing food, feed and

fiber. We need production systems that produce more efficiently (i.e. with more production per resources used) and production systems that are not so susceptible to the unpredictable changes in climate. Algae, the source and supporting base of much of life on earth, present an enormous opportunity here. At Heliae, we've begun to unlock the potential of this family of organisms and are eager present the algae opportunity as it relates to agriculture, plant, and soil science. Algae hold the key to a new wave of sustainable fertilizers that increase plant yields while giving nutrients and structure back to the soil for future harvests. Our production trials show increases in the amount of food we can produce per acre, thereby reducing some of the demand pressure we see on food stocks in the future. We will present our trial results with academics and commercial growers, and our continued product development efforts.

Leslie van der Meulen, BioProcess Algae  
*From Co-location to mass cultivation*

15 minute presentation: From Co-location to mass cultivation BioProcess Algae is a global leader in attached growth algal cultivation. With the second generation of a proven and patented membrane technology, BPA is leading the way in novel algal biomass creation. The platform can be connected to a large variety of co-location opportunities that have virtually no restrictions in geographical locations as long as the key criteria is met: access to CO<sub>2</sub>. The search for novel algal biomass has gained in importance over the past decade and has distinctively moved beyond just being a possible renewable fuel source. With BPA's strain agnostic platform, the large needs for new sources of oils, proteins, carotenoids and



carbohydrates can now be met. In a system that carefully connects containment with scale, BPA is able to provide different grades of biomass. The focus now is on going from promise to products, taking another step in the maturation of the algae market. BPA will provide a look into the commercial opportunities and progress beyond biofuel. In the presentation we will provide a brief overview of the technology history and improvements and provide details on some of the core markets in the lipids & protein market opportunities. We will also highlight some of the prerequisites for displacing current commodities and entering new and existing markets. The presentation will focus on BPA's product development efforts in the algae space.

**Session 5: Potential Yield, Composition, and Supply of Dedicated Energy Crops: Results and Outcomes of the Sun Grant Regional Feedstock Partnership**

**Thursday, May 15 8:30am-10:00am**

Moderator: Vance Owens, North Central Sun Grant Region, South Dakota State University

Chris Daly, PRISM Climate Group, Oregon State University

Gary Gresham, Idaho National Laboratory

Laurence Eaton, Environmental Sciences Division, Oak Ridge National Laboratory

*Potential Yield, Composition, and Supply of Dedicated Energy Crops: Results and Outcomes of the Sun Grant Regional Feedstock Partnership*

The Sun Grant Regional Feedstock Partnership commenced in 2007 with the goal of supporting the realization of the biomass potential envisioned in DOE's 2005 Billion Ton Report. The partnership sought to increase the knowledge of bioenergy through coordinated

feedstock research across the lower 48 states and Hawaii with partners in academia, government, and private industry. The core of the research includes over 130 field trials and regional resource assessment activities focused on agricultural residues and a suite of likely dedicated energy crops. This panel focuses on the synergistic activities across partnering institutions to incorporate field trial outcomes into national bioenergy models and strategies for commercial deployment. This panel builds upon a 2013 BIO Panel focused on herbaceous energy crop production to include woody species and modeling activities that integrate field trial data into feedstock logistics and supply systems. In 2013 a series of meetings was held across the US with each of the crop teams and the resource assessment team, led by the Oregon State University and Oak Ridge National Laboratory, to review, standardize, and verify yield trial data and assimilate their outcomes into a national model of biomass yield suitability. The meetings provided a way to "ground truth" yield estimates in order to accurately capture interactions of climate and soils for dedicated energy crops, including switchgrass, energycane, biomass sorghum, CRP grasses, miscanthus x giganteus, hybrid poplar, willow, and pine. From these sets of funded trials and historical data, yield was estimated across spatial gradients according to soil characteristics and climate history at a 2-week interval. The resulting spatial grids provide critical information for policymakers and planners of the potential productivity of these pre-commercial crops. Production and management data, as well as biomass composition characteristics, provide empirical support of logistic design and feedstock supply systems. The Biomass Library contains the



critical compositional characteristics from these trials and is used to develop efficient and cost-effective designs of logistics systems for conversion to usable industrial products. Finally, feedstock yield, cost, and quality are considered in generating commercial feedstock price and supply projections. The 2014 Feedstock Supply and Price Projections provide a 15-year outlook of the agricultural landscape that reflects the growing connectivity of the agricultural and energy sectors. Moderator- Terry Nipp, Executive Director, Sun Grant Association 1. Field Trials of Herbaceous and Woody Dedicated Energy Crops from the Sun Grant Regional Feedstock Partnership: Vance Owens, North Central Sun Grant Director, South Dakota State University 2. Potential Yield Mapping of Bioenergy Crops: Chris Daly, Director, PRISM Climate Group, Oregon State University 3. Biomass Composition Library and Feedstock Logistics Interface: Gary Gresham, Idaho National Laboratory 4. 2014 Feedstock Supply and Price Projections for Commercial Biotechnology: Laurence Eaton, Oak Ridge National Laboratory

### **Session 6: Meeting Your Customers' Need for Sustainable Sourcing**

**Thursday, May 15 10:30am-12:00pm**

Moderator: Jack Huttner, Huttner Strategies, LLC

Erin Simon, WWF

Matt Rudolf, Roundtable on Sustainable Biomaterials (RSB)

Joel Velasco, Amryis

#### MEETING YOUR CUSTOMER'S NEED FOR SUSTAINABLE SOURCING

This panel will bring companies together who are dealing with the challenge of fulfilling their

customer's need for sustainably sourced agricultural materials, in this case used for industrial bio materials production.

### **Track 3: Renewable Chemical Platforms and Biobased Materials**

**Sponsored by: Bergeson & Campbell**

#### **Session 1: Renewable Commodity Chemical: Butadiene**

**Tuesday, May 13 8:30am-10:00am**

Moderator: James Iademarco, Strategic

Avalanche Consulting

Alexandre Zanghellini, Arzeda

Sean Simpson, Lanzatech

Jean-Baptiste Barbaroux, Global Bioenergies

Gary Smith, INVISTA

#### Bio-Based Butadiene

Recent successes in bio-based chemical commercialization have opened the door for the introduction of new molecules to the market. 1,3-butadiene is a molecule produced solely using petrochemical methods using unsustainable feedstocks. Butadiene is used in synthetic rubber to aid in crosslinking, but its foremost application is the production of HMDA and adipic acid, the two monomers used in Nylon-6,6. This nylon is used throughout the fabric and textiles industries due to its mechanical strength, superior water-handling properties as well as its dye retention and texture. Its market (\$10B), relatively high price, and large number of applications and derived materials have made finding a bio-route for butadiene production extremely attractive for both chemical and biotechnology companies. A bio-based approach to butadiene is desired not only for sustainability reasons, but also to provide an alternative feedstock to stabilize the



market price. In the last couple of years, the decrease in naphta processing and the resulting butadiene production decrease has translated into high volatility in the butadiene trading price. Additionally, a bio-based approach can provide a method to selectively produce butadiene with no contamination by other small olefins- something endemic to the petrochemical cracking process. This panel will bring together some of the major players in bio-butadiene technology development and present recent progress towards industrial production. The current market and end-user perspective will also be discussed.

## **Session 2: Designing Biobased products with Enhanced Performance**

**Tuesday, May 13 10:30am-12:00pm**

Moderator: TBD

Tom van Aken, Avantium

Paul Casswell, Cathay Biotech

David Fenn, PPG

Mike Schultheis, The Coca-Cola Company

Angela Harris, Ford

### *Biobased Products with Enhanced Performance*

Recent product developments have focused both on renewable feedstocks and/or on biobased processing to produce new products with enhanced performance compared to the fossil-based standard. The performance can be the result of functionality inherent to the biobased feedstock, for polymers such as poly(ethylene furanoate) or for new plasticizers designed to replace petrochemical based phthalates, or the additional functionality can result from the specificity available to materials produced using tailored microorganisms or advanced catalysis. These new materials have properties which are superior to the incumbent

materials. Setting up the supply chain for these new materials requires significant development work, both for the technology developer and for the end user, and requires the involvement of intermediate parties in the novel supply chain. In this track speakers will include representatives from both the technology developers and from the end users, describing their route to market and the features which they find most important for the new developments. • What is the relationship between technology developer and end-user? • How do they form working relationships? (one/one, collaborations, ...) • How are development costs shared? • What are the remaining hurdles for full commercialization? (technical, economic, product qualification/acceptance?) • What is the time for market development? (how fast is the development cycle, and what factors are controlling; what is the expected long term growth rate) • How do the performance benefits drive the market acceptance vs other critical factors? (cost/price , functionality, purity, reactivity, stability, carbon footprint, degradability...) • How will the end user promote the new product and introduce it to the market? • How important is life cycle assessment? • Are social and societal factors a consideration? • Are 3rd party certifications or endorsements an important consideration? • What is the future view for feedstock economics and availability? • Does the end-of-life scenario for the product differ compared to the incumbent?

## **Session 3: TBD**

**Tuesday, May 13 2:30pm-4:00pm**

Moderator: TBD



#### **Session 4: Bringing Renewable Elastomers to Market**

**Wednesday, May 14 2:30pm-4:00pm**

Moderator: Bill Hyde, Chemical Market

Associates, Inc. (CMAI)

Damien Perriman, Genomatica

Alexandre Elias, Braskem

Karl Sanford, DuPont

##### *Bringing renewable elastomers to market*

Elastomers are one of the most important and widely-used classes of polymers. Major firms are now investing in development of processes for a range of renewable ingredients for this category, including butadiene, natural rubber and isoprene. This session explores the application opportunity and stage of development for these efforts.

#### **Session 5: Market Opportunity for Renewable Chemicals and Biobased Products**

**Thursday, May 15 8:30am-10:00am**

Moderator: Ron Buckhalt, USDA

Sally Van Wert, Bayer CropScience

Arlan Peters, Novozymes

Kristi Snell, Metabolix

##### *The Bio-based Economy: Fueled by Agricultural Biotechnology*

The emergence of a bio-based economy is being driven by increasing demand for sustainable solutions for housing, clothing, feeding and caring for a growing human population. Increasingly, biotechnology is enabling the use of bio-based materials to replace fossil fuels and petrochemicals for delivering the countless products and consumables used throughout our lives. This emergence is being supported by initiatives like the United States Department of Agriculture's BioPreferred Program, which is driving new innovation and new products

coming to the market. It is important to recognize that the use of bio-based materials in place of petrochemicals will require significant increases in agricultural products to deliver the raw materials needed for satisfying the demands of a growing human population and an expansion of wealthy consumers across the world. This panel will explore the USDA's BioPreferred Program, the use of biotechnology to deliver bio-based products, and the importance of agricultural biotechnology in increased crop production efficiency.

#### **Session 6: Is Renewable Succinic Acid a Commercial Reality?**

**Thursday, May 15 10:30am-12:00pm**

Moderator: Oliver May, DSM

##### *Enabling Bio-based Innovations by means of Bright Biotechnology*

Royal DSM N.V. is a global science-based company active in health, nutrition and materials. By connecting its unique competences in Life Sciences and Materials Sciences DSM is driving economic prosperity, environmental progress and social advances to create sustainable value for all stakeholders. Today's market needs are driven by a number of major global trends and challenges. At DSM we're using our innovative strengths to address some of the most important of these trends and challenges, such as climate change, energy independence. Advanced biofuels such as cellulosic ethanol or advanced biodiesel as well as bio-based platform chemicals (e.g. Biosuccinium<sup>TM</sup>, succinic acid) and bio-, or plant-based materials (e.g. Arnitel<sup>®</sup> Eco, thermoplastic elastomer) offer excellent solutions to these challenges for generations today, as well as for generations to come. DSM has been active in the field of biotechnology for



more than a century which a/o led to landmark developments for vitamin and antibiotics production with a clear focus on reduction of process complexity and driving down manufacturing costs. Today we use biotechnology solutions still to economize production processes, but also to achieve greenhouse gas emission reductions in order to maintain economical as well as ecological impact at the same time. What has changed over that century of biotech innovations is not only the shift to add ecological contributions, but also our understanding of how fundamental science can have a huge impact on the efficiency of our toolbox of competencies. This presentation will highlight events in the transition from “White Biotech” to “Bright Biotech” and cover DSM’s unique combination of technology competencies - how we are using these to improve performance and drive down enzyme costs for saccharification of lignocellulosic feedstocks, create novel yeast operating at low pH for succinic acid production and introduce novel pathways that convert by-products from the conversion of agricultural residues to ethanol into cellulosic bioethanol themselves. We will also show the status of commercialization of these disruptive technologies in our business partnerships which are set up to accelerate and commercialize our innovations.

Alif Saleh, Myriant Corporation

*Renewable Succinic Acid: Applications and Solutions Decoded*

One of the best attended and most heated discussions at the World Congress on International Biotechnology--the Renewable Succinic Acid Panel--is back by popular demand. In previous years, we discussed the challenges

and hurdles to achieve the path towards commercialization of renewable succinic acid with the ultimate goal to substitute petroleum-based succinic acid. Myriant, Reverdia and Succinity GmbH--chemical companies who successfully have broken the barriers toward commercialization--are now focused on the creation of every-day products from bio-based succinic acid as a chemical intermediate. The discussion will be centered on the applications and solutions in development by these companies to allow the full penetration of this renewable chemical and its derivatives into a several billion dollar global market.

Jo Kockelkoren

*A few years to double or triple the market size*

The first large scale plant is running, product is being sold, the commercial reality is good, but ambitions are higher. The next step for Reverdia is a plant of about 50kt, meaning the importance of market growth is increasing. The current petro-based succinic acid market is small, around 30 kt, so if we want to develop a market that keeps multiple large scale plants sweating, it’s clear what our focus should be. Key aspects are application development, managing consumer perception, explaining the credibility of LCA’s, and consistent good quality product. Biosuccinium™ among others offers an alternative to adipic acid in polyurethanes, resins and plasticizers, and this of course does have an impact on performance. Application development work clearly helps to accelerate the market growth and identify the key downstream products and players. The presentation will include examples. Managing consumer perception is another one, take for example food competition discussions. The key to success is to help brand owners develop fact-



based communication explaining why their bio-based product is a good choice, also in terms of non-food competition. LCA is a third key element in the development of a more sustainable bio-based economy. The tricky thing with LCA's is that one can come up with any number, even following ISO guidelines. As long as it is not clear what key assumptions are used to calculate LCA's, the audience cannot judge the value of such an LCA, indicating the importance to open up data substantiating the figures. The need for consistent, good quality product is a no-brainer. Reverdia uses low pH yeast technology already since the start, which delivers best-in-class product in terms of quality, economics and environmental footprint.

**Track 4: Speciality Chemicals, Pharma Intermediates, Food Ingredients**  
**Sponsored by: Biocatalysts**

**Session 1: Novel and Sustainable Fermentation Pathways to Natural Products**  
**Tuesday, May 13 8:30am-10:00am**  
Moderator: *TBD*

Jorgen Hansen, Evolva

*A novel & sustainable production route for vanillin via yeast-based fermentation*

A NOVEL & SUSTAINABLE PRODUCTION ROUTE FOR VANILLIN VIA YEAST-BASED

FERMENTATION Natural vanilla is a complex of several compounds obtained from the seed pods of the vanilla orchid, *vanilla planifolia*. From a commercial standpoint, one of the most important components is phenolic aldehyde vanillin. Together, vanilla and vanillin are among the most important fragrance, colourants, and flavouring products in the world, approaching a

market value of USD 600 million. Because of the high cost, limited supply, and other supply chain constraints involved in acquiring reliable and pure supplies of the natural product, industry long ago turned to synthetic vanillin to meet commercial demand for the mass market products that require vanilla flavour, colour, or fragrance. Today, 99% of the market is dominated by synthetic vanillin; it is, however, derived chiefly from petrochemicals, wood pulp waste, rice hulls, and other plant waste products. Although only 1% of the global demand is supplied with ingredients sourced directly from the vanilla bean, commercial chefs, and high-end confectioners and perfumers continue to turn to the material derived directly from the plant. As a result, demand and prices for the vanilla bean continue to increase year over year. History has demonstrated that the biggest challenges faced by the vanilla farmer are not those posed by competition from synthetic vanillin, but rather from weather, available land, political instability, and the supply of counterfeit vanilla bean extract. Evolva has constructed an environmentally friendly and sustainable yeast-based fermentation route to vanillin which represents a major innovation in a market dominated for decades by petrochemical-derived vanillin. Fermentation-produced vanillin is expected to compete favorably with petrochemical-derived vanillin on taste, cost, supply chain reliability, purity, and sustainability. The production route utilizes the yeast metabolite 3-dehydroshikimic acid to form protocatechuic acid, which after reduction and methylation steps, is turned into vanillin. Because vanillin is toxic to most microorganisms, Evolva uses in vivo enzymatic glycosylation to turn vanillin into non-toxic



glucovanillin (vanillin beta-D-glucoside). The glucovanillin is hydrolysed by enzymes, which liberates vanillin. Several challenges have been overcome during the course of the project such as the unwanted reduction of vanillin to vanillyl alcohol. This product is on target for launch in 2014.

David Demirjian, zuChem, Inc.

*Novel Systems for the Production of Rare Sugars and Oligosaccharides*

Carbohydrates play an important role in human biology because of their involvement in a myriad of biological functions, including nutrition, cellular recognition and signaling, cell targeting, and the development and differentiation of disease states. Despite their importance, the development of novel food, nutritional and pharmaceutical-based carbohydrate products has been held back by the lack of availability of rare and modified sugar building blocks and the process technology to manipulate or attach them to other molecules. In order to address these challenges a platform of novel manufacturing methods which can be applied economically at industrial scale to manufacture these components has been developed. These novel methods include unique bioprocesses and specialized systems to produce these valuable products from inexpensive carbohydrate feedstocks. Using a novel engineered dehydrogenase about a dozen different rare L-sugars have been produced from simple and inexpensive sugar alcohols. For example, L-ribose, a potential intermediate for many antiviral drugs, can be produced at yields over 65 g/L using ribitol as a starting material. In order to make short-chain oligosaccharides which have potential uses as prebiotics, human

milk oligosaccharides and in anti-enteric applications carbohydrate precursors must first be converted to their activated form. Several methods were developed and optimized to make a variety of activated sugars. The first step of this is the production of a sugar-1-phosphate molecule. Using a novel engineered thermostable kinase  $\alpha$ -D-galactose-1-phosphate was produced with yields greater than 150 g/L. This same system was used to demonstrate the production of a variety of additional sugar-1-phosphates. This product was purified to synthesize globotriose, an important oligosaccharide. Globotriose was produced at yields of over 50 g/L in a reaction using lactose and  $\alpha$ -D-galactose-1-phosphate catalyzed by a glycosyltransferase. The production of several additional oligosaccharides was also demonstrated. These new processes are now being expanded and scaled up for industrial use.

Wim Soetaert, Inbiose NV

*Inbiose technology: Efficient synthesis of specialty carbohydrates through industrial biotechnology*

Rare sugars such as L-fucose, L-ribose and sialic acid or human milk oligosaccharides such as fucosyllactose and sialyllactose are very difficult to synthesize. These products are consequently very expensive and not available in sufficient quantities. For the synthesis of such highly complex carbohydrates, organic synthesis is not an efficient production method because of the high chirality and excessive presence of hydroxylic groups in the carbohydrate building blocks. Also extraction of these compounds from natural sources is often hampered by the substrate availability and extraction cost. To solve this problem, a highly efficient production method for specialty carbohydrates has been



developed by the start-up company Inbiose. The method is based on the use of cell factories in which a natural pathway has been expressed for the synthesis of the target specialty carbohydrate. Base strains that are engineered to generate the carbohydrate building blocks are equipped with a functional pathway to produce the target specialty carbohydrate. Using the Inbiose technology, any naturally occurring specialty carbohydrate can be produced. Depending on the target, a biochemical pathway will be designed and expressed in one of the Inbiose base strains. Through pathway engineering and optimisation, the production strains are optimised. The target carbohydrate is then produced by fermentation and is efficiently excreted in the culture medium. After fermentation, the target carbohydrate is recovered from the fermentation broth in high yield and purity (generally > 99 %) using a simple down-stream processing method. The production method is generic and has already been proven on an industrial scale. The process is also perfectly scalable so that the availability of these specialty carbohydrates is no longer an issue. Inbiose has access to pilot and production facilities and is able to produce over 1.000 t/a of specialty carbohydrates. The presentation will explain the features of the Inbiose technology and will highlight a number of concrete examples where a process to produce a specialty carbohydrate has been developed and commercialised in collaboration with an industrial partner.

Mangesh Kulkarni, Praj Matrix

*Enzymatic Conversion of EPA and DHA enriched Ethyl Esters to Triglycerides*

Omega-3 ingredients like alpha linolenic acid

(ALA), docosahexanoic acid (DHA) and Eicosapentaenoic acid (EPA) have emerged as vital functional ingredients delivering significant health benefits, particularly those related to a heart and brain. Omega-3 fatty acids are obtained from a number of vegetarian and marine sources. Fish is a major source Eicosapentaenoic acid (EPA) and Docosahexanoic acid (DHA) whereas flaxseed is a major source of ALA. Omega-3 concentrates are classified based on their chemical form. There are two forms of concentrates available in the market that includes ethyl ester concentrates (EE) and triglyceride concentrates (TG). The preference for TG form is due to the fact that Omega-3 exists in the TG form in nature. Hence bioavailability of EPA and DHA in its triglyceride form is higher as compared to its Ethyl Ester form. Praj Matrix has developed proprietary process to enrich EPA and DHA using Sardine fish oil available abundantly on India's south west coast. Various EPA and DHA enriched product grades such as 30, 50, 60 and 80% in their ethyl esters forms are produced using this process. EPA and DHA enriched ethyl esters are converted to their triglycerides using enzymatic process. Praj Matrix has developed process to convert 50% EPA and DHA containing ethyl esters to their 98% triglycerides using an enzymatic process. Praj's process has several advantages: 1. Less reaction time (12-24 hrs) 2. More selectivity to EPA and DHA 3. EPA and DHA ratio maintained in Triglycerides 4. Minimum mono- and di-glyceride formation 5. Process can be converted to continuous mode using fixed bed reactor

**Session 2: The Bridge to Renewable Realities.....Alliances, Experiences and Learning's from Industry Architects**



**Tuesday, May 13 10:30am-12:00pm**

Moderator: James Iademarco, Strategic

Avalanche

Peter Nieuwenhuizen, Akzo Nobel

Joey Zwilling, Solazyme

Adrienne Pedrosa, Solvay

Joe Regnery, Zechem

*The Bridge to Renewable Realities....Alliances, Experiences and Learning's from Industry Architects.*

While investments in renewable chemicals slowed during the recent financial crisis that did not deter companies who shared a longer term vision. Similar to many industries, success will depend on key alliances spanning multiple technologies and feedstocks, global production sites, and varying deal structures all part of a new integrated value chain. This panel will offer valuable insight into their learning's and experiences on how their partnerships were forged (both with one another as well as with other firms) and highlight the successes and struggles that have bridged the gaps. At BIO Orlando in 2012 Akzo Nobel shared for the first time its biobased strategy, and entered execution mode, approaching and inviting potential partners. In 2013, at BIO Montreal they presented their resulting alliances, and started to actively work with its partners in the second half. But what does it really take to achieve alignment in a diverse, multi-business corporation like Akzo Nobel? Akzo will introduce the companies but also share tools, approaches and tactics they employed to "land" these partnerships into their organization. And reflect on how successful they have been up to this point. We will get insights from Solazyme, one of the early architects already with a decade of experience. Throughout their ten-

year history, they have partnered with large corporations and governmental agencies across the transportation, chemicals, food and personal care industries, and also spanning the value chain from raw materials to distribution of products. Solazyme has developed a world-class industrial biotechnology platform, utilizing microalgae to produce tailored oils and bioproducts. They will share their strategy in developing the most successful, high-impact partnerships to compliment Solazyme's core technical capabilities, as well as some of the key lessons learned as they went from startup to an established global operating company it is today. Solvay collaborates with AkzoNobel through three biobased solutions from a wider portfolio that it offers to the market. Augeo® solvents are biobased and offer performance advantages in coatings and other applications. Biobutanol and -acetone are drop-in solvents used widely in the industry, and which Solvay intends to offer from 2015 onwards in Latin America. Epicerol® is the name of the patented Solvay technology to manufacture bio-Epichlorohydrin from glycerol, a renewable raw material available as a by-product of the oleochemical and biofuel industries. We will hear Solvay's overall strategy regarding biobased chemicals, and its experience as it has been building these businesses. And finally, ZeaChem Inc. a renewable cellulose-based biorefinery technology which offers a commercial platform capable of producing advanced sustainable drop-in organic acids, esters and alcohols. At its start in 2002, ZeaChem has collaborated with its feedstock suppliers, equipment suppliers and customers. Through these strategic relationships, ZeaChem has developed its portfolio targeting locations that present reliable long term feedstocks,



incorporating proven equipment packages, utilizing economic logistics, and satisfying its customers product pricing and quality requirements. All 4 architects, Akzo, Solazyme, Solvay and Zechem have different strategies. But together, they will share a common perspective on they plan to deliver the reality of sustainable products for a profitable bridge to the future.

**Session 3: Renewable Specialty Chemicals:  
Additives, Ingredients, Surface Modifiers  
Tuesday, May 13 2:30pm-4:00pm**

Moderator: Stuart West, Biocatalysts Ltd.

*The use of Bioinformatics for rapid and low cost  
enzyme development*

The world of genetics has changed dramatically over the last 10 years as gene synthesis and gene sequencing costs have fallen dramatically and will continue to fall. This has created a completely new bioscience sector – namely bioinformatics. But producing vast amounts of genetic data would give no benefit if it wasn't for the ever increasing speed of computers to deal with this vast amount of information. This enables enzyme genetic maps to be created and rather than randomly produced mutations or doing directed evolution using expensive robotic equipment a wide range of very different enzyme types can be produced intelligently using more of an in-silico approach.

This approach enables Biocatalysts to produce samples of new enzymes in just a few weeks and not several months and to have commercial quantities of enzyme available in a few months. The presentation will include some case studies demonstrating how fast and low cost novel enzyme development can now be.

Lee Tonkovich, Heliae

*Algae: The next Frontier in Sustainable Personal  
Care*

In an industry that is constantly looking for the next new thing, it is noteworthy that one of the 'newest' ingredient trends that will dramatically affect the personal care space is not new at all. The cosmetics and personal care industry has had a legacy comfort with algae for decades, limited mostly to specific seaweeds (macroalgae) and various microalgae species limited to the genus, Chlorella. What's steadily becoming understood is that the personal care industry's utilization of algae as an active ingredient is meager, as compared to a globally available feedstock that boasts over 100,000 species each with its own product development opportunity. The ingredient actives that algae present span complex proteins, polysaccharides, pigments and color, antioxidants, anti-acne, wound-healing, UV protection, and more. With the massive potential this family of organisms present, the missing piece has been reliable and scaled supply to enable the necessary product development and marketing to launch novel formulations into the market. This is changing. With advanced algae production technology mature and the capability to rapidly screen strains for commercial applications available, Heliae is poised to offer a new category of novel marine-based ingredients to the product development pipelines of major market players. We will discuss Heliae's production technology, our current and projected production capacity, and rapid strain screening capabilities, and our product development success to date.



Michael Goldfeld, Advanced Biocatalytics Corp.  
*Protein-Enhanced Surfactants in Environmental and Related Technologies*

Baker's yeast responds to a non-lethal heat shock releasing some small (<30 kDa) proteins. These stress exo-proteins enhance synthetic surfactants, reducing oil/water interfacial tension and critical micelle concentration, compared to the same surfactants without protein. Protein-surfactant complexes (PSCs) are persistent in a broad range of pH, temperature, and tolerant to certain oxidants, such as hydrogen peroxide, iodine, or bleach. PSCs open opportunities in formulating products for such diverse applications as agriculture, enhanced oil recovery, industrial and environmental cleaning, water and soil bioremediation, waste water processing, skin care and more. PSC-based products combine high efficiency with low toxicity and environmental impact. PSCs activate certain industrial enzymes, such as lipase. PSCs also activate the processing of organic pollutants in water and soil by naturally present microflora. In waste water treatment (WWT), PSC's-enhanced bio-oxidation is associated with a reduced accumulation of solid sludge. Field and bench studies indicate that PSCs uncouple the electron transfer in bacterial cells from bacterial proliferation by inducing proton leak across membrane. Formulations have been developed on these grounds, for cleaning chemical cargo tanks, paraffin extraction from clogged petroleum oil wells, as agricultural adjuvants for better wetting and uptake of bio-actives, agents for herding and dispersing spilled oil, and enhancing remediation of water and soil affected by accidental spills. With PSCs, grease in sewer line is converted into surfactant, thus sewer pipe becoming a pre-processing

bioreactor, reducing malodors and corrosion. Only food-compatible surfactants are used in the ABC's products which received certifications from the US National Sanitary Foundation and International Maritime Organization, licensed by the State of California Dept. of Fish and Wild Life, and listed in the US EPA National Contingency Plan. ABC's IP includes 12 patents issued and over 10 more pending.

Katherine Maloney, Novozymes  
*Use of asparaginase to mitigate acrylamide in food*

In 2002, Swedish researchers discovered the presence of high levels of acrylamide in various baked, fried, and roasted foods, including French fries, potato-based snacks, bakery products, breakfast cereals, and coffee. One method for reducing the level of acrylamide in food is to use the enzyme, asparaginase to convert asparagine, a major precursor for acrylamide, into aspartic acid thereby reducing the formation of acrylamide. In this presentation, methods for applying the enzyme in various processes and factors that affect enzyme efficacy will be discussed. The enzyme must be applied prior to heating and given enough time to sufficiently react with the asparagine present in the raw material. For example, in French fry production, fries are dipped in water, dried, and then fried. Adding the enzyme to the water dip allows sufficient time for the conversion of surface asparagine to aspartic acid before frying. Many factors can affect treatment efficacy. It is important to choose an asparaginase with temperature and pH optimum within the normal operating range of the manufacturing process. For example, a moderate temperature asparaginase may be suitable for dough-based snacks that are



typically mixed around room temperature. A more thermostable asparaginase will be required in cereal production where the raw materials are mixed while being cooked at temperatures around 100°C. Reduction in acrylamide content using asparaginase varies based on product and process. Typical reductions in French fries range from 40% to 65%. Reductions in acrylamide formation of up to 95% have been obtained in potato-based and wheat-based snacks. Typical reductions in breakfast cereal products range from 50% to 75%. Acrylamide formation can be reduced by up to 70% in coffee. Asparaginase is an effective tool for mitigating acrylamide in numerous different food products.

Mike Guggenheimer, RSC Bio Solutions  
*The Commercialization and Adoption of Readily Biodegradable, Biobased Functional Fluids and Cleaners*

The results of readily biodegradable, biobased fluids and cleaners can be seen across a wide range of industries and segments. In solely looking at performance, many readily biodegradable, biobased chemicals have proven time and time again to be high performers in comparison to traditional petroleum-based counterparts. This single paper submission would explore lessons learned and successful commercialization examples in a wide variety of industries (marine to utility fleets, waste segment and offshore oil & gas) seeing the benefits of renewable chemical solutions, such as readily biodegradable, biobased functional fluids and cleaners. These products are developed from ingredients from a number of regular BIO meeting attendees. The single submission would also explore the commercial motivations (performance, value and

sustainability) driving the switch from conventional petrochemicals to readily biodegradable, biobased alternatives.

**Session 4: Specialty Performance Materials from Renewable Sustainable Sources**  
**Wednesday, May 14 2:30pm-4:00pm**

Moderator: Atul Thakrar, Segetis, Inc.

*Emerging Market Opportunities for Levulinic Acid and its Derivatives*

Levulinic acid caught the attention of researchers around the world with its inclusion in the DOE's list of top 12 renewable chemicals in 2004, but its high cost and limited capacity tempered market development efforts. Segetis' new technology to produce levulinic acid efficiently and cost-effectively, first announced at the 10th Annual World Congress on Industrial Biotechnology, removed those concerns and has cleared the path for market expansion of Levulinic Acid and its derivatives. With its dual functionality, this ketone-acid can be converted into a myriad of products ranging from chemical intermediates to specialty chemical products. Some derivatives, like the levulinic ketals based on Segetis' JAVELINTM Technology, are currently being commercialized, while others, like specialty monomers for superabsorbents and polyamides, are just entering the development phase. As the levulinic acid production scale increases and the costs decrease, these new markets will expand. This talk will discuss the growing market portfolio for levulinic acid and its derivatives, both short-term and long-term.

Melanie Venter, Yulex Corporation  
*Guayule BioRubber*

Yulex applies crop science, bioprocessing and materials science for the development of non-



GMO, plant-based biomaterials from guayule, a sustainable and industrial crop. Guayule is a highly productive chemical factory that does not compete against food or fiber crops and requires low inputs. The company is developing fully integrated and sustainable biorefineries that can utilize virtually 100% of guayule to provide a renewable source of rubber emulsion, solid rubber, resins and biomass on a global basis. Yulex's biomaterials are designed to replace traditional tropical Hevea or petroleum based rubber for consumer, industrial and medical markets, with the residual agricultural materials utilized as a feedstock for bioenergy. Yulex's technological innovation includes guayule seed technology, agronomics, breeding, harvesting, bioprocessing, and materials science. Yulex develops natural rubber both in liquid and solid form. Resin can be produced both as a crude extract as well as a fractionated refined resin component. Leftover biomass can either be used for biomass energy to produce electricity in its raw form, or it can be processed to produce both solid and liquid biofuels. Significant progress has been made with regards to specialty chemicals as well. Yulex is based in the U.S. Southwest and has a growing global footprint.

David Sudolsky, Anellotech, Inc.

*Benzene (with p-xylene) from Biomass - Anellotech's Answer*

A decade of volatile and rising oil prices has enormously stressed the global refining and petrochemical industries. Profitability of these industries has decayed as producers struggle to pass through higher raw material prices in sluggish markets. Forecasts of future crude oil prices frequently disagree on the detail but few forecast a downward trend. Refined products

such as aromatics share these trends. Biomass offers the solution. Anellotech is commercializing a unique Catalytic Fast Pyrolysis (CFP™) process, disengaged from high and rising crude oil prices. It converts nonfood biomass to drop in aromatics: benzene, toluene and mixed xylenes (BTX). The process offers substantial benefits over competing processes for biomass to drop in petrochemicals: simplicity, few steps, and conventional unit operations. The company has an exclusive global license from the University of Massachusetts, Amherst, where the original research work was carried out by Professor George Huber, to develop and implement the process. The heart of the process is a tailored catalyst converting ground biomass to gaseous products in a fluid bed reactor. Biomass preparation and BTX separation employ conventional unit operations. Byproduct coke and char provide process heat. Soundly based capital and operating cost estimates show that the process can produce BTX at 60-70% of conventional costs. The catalyst can be tailored to swing mixed xylenes production to predominantly para-xylene. Established in 2008, Anellotech has a well-funded laboratory, pilot plant and headquarters in Pearl River, NY, currently with 24 staff. The primary focus of the development work is on scale up with proven catalyst systems. We have provided liter samples of BTX to our partners and plan to offer even larger samples for evaluation in aromatics and selected derivative markets. Commercially, the cost effective production of bio based para-xylene is a widely publicized industry target. In addition to this product, Anellotech offers bio based benzene and toluene. Benzene especially has numerous market entry points. The major ones include styrene for polystyrene and other



styrenics, caprolactam for nylon, and phenol for polycarbonate. This presentation will provide an update of Anellotech's development work, and a discussion of the competitive advantages of the CFP process.

Joop Groen, TNO

*Bio Aromatics: Breakthroughs in our Shared Research Center Subtitle: Powered by VITO and TNO @ The Green Chemistry Campus, part of the Bio Based Delta*

Shared Research Center Bio Aromatics for Performance materials, Coatings and chemicals. At the Green Chemistry Campus (GCC), part of the Bio Based Delta initiative, TNO and VITO Have started a new Shared Research Center. The GCC is located in Bergen op Zoom, The Netherlands, at the heart of industries between Antwerp and Rotterdam. The initial focus of the Center will be on Biobased Aromatics for performance materials, chemicals and coatings. Setting up a sustainable and economically successful commercial scale business, is a major challenge in this field. A significant research effort is needed, more than one single organisation can handle. Therefore a Shared Research mode is adopted, bringing together collective intelligence of various industries, companies and knowledge organisations. The multi disciplined technological need, as well as the long term roadmap make this effort suitable for shared research, sharing investments, risks and workload. Partners, Industry Driven. The Center will be operated as a Shared Research Program, based on the Open Innovation methodology. We aim is to include the global leaders in the fields of feedstock, conversion, equipment and end-products as partners in the Program. The participating organisations are determining the roadmap and the deliverables

of the Center. The Center is being operated by VITO of Belgium and TNO of The Netherlands, two Research and Technology organisations with a strong starting position in Biobased Technology. There will be a special structure for SME participation. The Center will work together closely with a select group of academic organisations around the world.

### **Session 5: Enzyme Development, Partners in Sustainability Advancements**

**Thursday, May 15 8:30am-10:00am**

Moderator: Tony Pavel, Morgan, Lewis and Blockius LLP

James La Marta, DSM

Vince Sewalt, Du Pont

Roxanna Halich, Verenum

Jessica Pascal, Novozymes

#### *Enzyme development, a partner in sustainability advancements*

Sustainability impacts society, the environment and the economy. Sustainable practices transform industrial processes to reduce waste, energy consumption, and other environmentally unfavorable impacts. A panel of Enzyme Technical Association members will provide insight into enzyme development and deployment that enhance the sustainability of consumer and industrial products or processes. Panel members will highlight the current drivers of enzyme development in the featured industry segments and provide examples of how enzymes are economical, environmentally friendly tools with a long history of safe use. The speakers will address such topics as the use of enzymes in consumer detergents and industrial textiles, biofuel production, shale and deep well petroleum recovery, feed nutrient



utilization by production animals and acrylamide reduction in food processing.

**Session 6: Expediting Bioproduct Commercialization Via Public Private Partnerships**

**Thursday, May 15 10:30am-12:00pm**

Moderator: Dennis Hall, OBIC, The Ohio State

University

Greg Blake, Biosynthetic Technologies

Ray Miller, Verdezyne

Doug Haughn, Team Gemini

Kate Lewis, USDA BioPreferred Program

*Expediting Bioproduct Commercialization via a Public Private Cluster Development Approach*

In 2012, OBIC launched a Bioproduct Network to link public and private stakeholders across the "Cell to Sell"® Innovation Pipeline. This panel will include representative across this network and will discuss strategic issues associated with bioproduct commercialization. Participants include panelists at various segments across a bioproduct supply chain, namely feedstock provider, chemical manufacturer, product developer, and market support. Doug Haughn, President of Team Gemini, will discuss his announced project to utilize biomass from municipal solid waste sources as a cost effective means to obtain large quantities of feedstock. Ray Miller of Verdezyne will address opportunities to produce specialty chemicals via yeast-based biotechnology platform. Greg Blake with Biosynthetic Technologies, has developed a biobased engine oil and can speak to product performance and policy challenges. Kate Lewis, Deputy Program Manager of the USDA BioPreferred Program, will represent the USDA Biobased Certification Program and

opportunities associated with federal procurement. In addition to addressing challenges at each level of the supply chain, the panelist will engage in a conversation about the bioproduct industry, major challenges and strategic opportunities. Dennis Hall, Director of OBIC will facilitate the discussion.

**Track 5: Synthetic Biology and Genomics Research**

**Session 1: New Enzymes and New Chemical Pathways**

**Tuesday, May 13 8:30am-10:00am**

Moderator: TBD

Eric Althoff, Arzeda Corp.

*Enabling Transformative Products, New Enzymes, and New Chemical Pathways using Computational Design*

Arzeda is a biotech company focusing on enabling bio-based chemical pathways as well as industrial enzymes working to commercialize transformative products towards a sustainable future. The recent emergence of industrial and synthetic biotechnology has the potential to radically transform the chemical industry using biomass as a primary feedstock. To this end, we develop novel computational methods and applied them to rationally engineer enzymes with a wide range of activities. To complement our previous techniques, we have recently developed a new algorithm, called Enzyme Identification™, to rapidly engineer enzymes with known catalytic mechanisms for non-native substrates. For a given chemical reaction, our algorithm screens "in silico" large databases of protein structural and sequence information to allow the rapid discovery of existing enzymes that possess both the necessary catalytic



machinery and an appropriate substrate-binding pocket. For those enzymes that possess the necessary catalytic machinery but lack an active site that can accommodate the substrate(s) of interest, Enzyme Identification automatically redesigns and remodels the active site pocket thus enabling catalysis of the desired reaction. Arzeda is applying its technology to develop Designer Cell Factories to produce new bio-based chemicals. The demonstrated success and wide applicability of our methods open the way for the design of a variety of novel biocatalysts necessary for the efficient development of biosynthetic pathways for the industrial scale synthesis of high value chemicals from biomass. In partnership with INVISTA, the world leader in nylon fibers, Arzeda is working on the development of new metabolic pathways for the production of bio-based butadiene, with its \$10B dollar current market made produced from petroleum. Additionally in a partnership with DuPont Pioneer, Arzeda has successfully applied its enzyme design technology to develop a novel trait for corn and soybeans and potentially other crops that will increase agricultural productivity. The traits are continuing through the development process and will be commercially available in the future.

Ian Fotheringham, Ingenza, Ltd.

*Use of a Novel Combinatorial Genetics Platform to Rapidly Clone, Express and Select Target Biocatalytic Activities for Multiqenic Metabolic Pathway Optimization*

Replacement of petroleum-based products and manufacturing processes with competitive bio-based alternatives is attracting increased attention due to environmental concerns surrounding petroleum sustainability and

supply. Replacement of conventional processes for manufacturing valuable industrial products and the selection of optimal biosynthetic routes requires the construction, and in most cases subsequent context-dependent evaluation, and optimization of multicomponent biosynthetic pathways to generate intermediates and end products. This talk will present the use of Ingenza's proprietary combinatorial genetics platform (inABLE®) to rapidly clone, express, select and optimize target activities for many separate enzymatic reactions, from thousands of independent genes derived from metagenomic and phylogenetic discovery approaches. This includes the characterization of a vast library of hitherto undiscovered genes from rumen microbial flora and the deployment of resulting novel enzyme activities in *Saccharomyces* to adapt and expand feedstock utilization for increased process efficiency. Multiple gene variants comprising of up to ten individual genetic elements are combined in single reactions, generating expression libraries with hundreds or thousands of members in diverse heterologous configurations for HTP interrogation. Obvious synergy exists between this approach and versatile, solid phase screening and selection methods using growth-based, crossfeeding or colorimetric methods to identify colonies of interest. This is illustrated through the rapid identification of critical pathway enzymes, optimal gene coding sequences and enzyme variants from inABLE®-derived high quality variant libraries for applications in bio-based polymers, chemicals and personal care products. We will also describe the success of modeling approaches to gene design that, when coupled with our inABLE® approach towards combinatorial biofabrication, enhance the predictability of



heterologous gene expression in diverse hosts. The technology aims to bring increasing predictability and overcome persistent limitations associated with iterative and empirical processes for microbial strain improvement. The successful realization of optimal target reactions enables rapid pathway definition and progression to process optimization and scale-up.

Jason Blake, Novozymes

*Novozymes and Beta Renewables Deploy World Class Cellulosic Ethanol Technology to Market*

Novozymes is proud to be a participant in the commercial cellulosic ethanol industry and continues to develop and deliver best in breed enzymes that enable the cost effective conversion of a variety of lignocellulosic substrates to simple sugars. Our efforts involve not only the development of new enzymes, but also the integration of key process steps; namely pretreatment, hydrolysis and fermentation. Making ethanol from lignocellulosic substrates is a game of tradeoffs. Novozymes has identified that, in order to realize the lowest production and capital costs, delivering cost effective, potent enzymes continue to bring real value to our customers. This presentation will briefly discuss updates on the deployment of the best in class, combined technology solutions from Beta Renewables and Novozymes. Together these winning technologies bring a very compelling value proposition to producers looking for guarantees and certainty.

Roberto Carrillo, ENMEX

*Mexican Association of Industrial Biotechnology Companies*

Embiomex AC is the Mexican Association of

Industrial Biotechnology Companies and it was originated in 2012 as a result of the National Program for Productive Biotechnology 2006-2012, an initiative from the Mexican Government through the Ministry of Economy. Embiomex represents the Industrial Biotech Companies in Mexico to support its interests and future, guide the policy-makers decisions and foster a thriving, long-term development of the Mexican Industrial Biotechnology.

Embiomex was founded by Mexican Companies in different segments from the Industrial Biotechnology and its growing number of members comprises the most important companies in Biopharmaceuticals, Food Biotechnology, Enzymes, Agrobiotechnology and Consulting. Embiomex also includes in its structure a Scientific Council, with the active participation of researchers with industry-partnership experience of the most respected Academic institutions throughout Mexico, such as UNAM, IPN, CINVESTAV, UANL, CIATEJ, CICY among CIAD among others. As one of the most important achievements of Embiomex and its relationship with the National Council of Sciences, a dedicated fund to sponsor Biotechnological projects was launched in 2011. This fund intends to foster the innovation for the development of new biotech products, processes, services and companies in Mexico. Embiomex current members are: METCO, S.A. DE C.V. (Food Biotech) PROBIOMED, S.A. DE C.V. (Biopharmaceuticals) ENMEX, S.A. DE C.V. (Enzymes and fermentation) AGROENZYMAS, S.A. DE C.V. (Agrobiotech) ANÁLISIS TÉCNICOS, S.A. DE C.V. (Biotech Services) DESARROLLOS AGROPECUARIOS DEL ALTIPLANO, S.P.R. DE R.L. (Agrobiotech) GRUPO NUTEC (Feed) GRUPO DE ASESORES EN BIOTECNOLOGÍA, S.A. DE C.V. (Biotech services) SEMILLAS CERES, S.A. DE C.V.



(Agrobiotech) COMPAÑÍA AZUCARERA DE LOS MOCHIS, S.A. DE C.V. (Food Biotech)  
PRODUCTOS QUÍMICOS DE CHIHUAHUA, S.A. DE C.V. (Food Biotech)  
NEKUTLI, S.A. DE C.V. (Food Biotech)  
NUTRAVIA, S.A. DE C.V. (Fermentations and food biotech)  
SIGMA ALIMENTOS, S.A. DE C.V. (Food biotech)  
SINQUÍMICA, S.A. DE C.V. (Biopharmaceutics)  
AGRO&BIOTECNIA S. DE R.L. M.I. (Fermentations and agrobiotech)  
Embiomex and its members are willing to explore and establish productive partnerships outside of Mexico for productive feedback and innovation projects.

### **Session 2: Beyond Transgenics: Biotechnology Tools to Enhance Value**

**Tuesday, May 13 10:30am-12:00pm**

Moderator: Tom Wedegaertner, Cotton Incorporated

R. Michael Raab, Agrivida

Tom Wedegaertner, Cotton Incorporated

David McElroy, Nova Synthetix

Toni Voelker, Monsanto

#### *Beyond Transgenics: Biotechnology Tools to Enhance Value*

Crop biotechnology has been one of the most rapidly adopted agricultural technologies in human history. Today, biotech crops are delivering economic and environmental benefits to farmers and growers across the world. Tomorrow, biotechnology will increasingly deliver benefits for consumers and growers based on new methods for improving plants. This panel will explore several examples of advanced biotechnology approaches to enhancing the value of crops and delivering unique benefits that were previously not possible. This discussion will cover the use of

RNAi technologies and advanced genome editing to demonstrate the future potential for biotechnology to impact our lives.

### **Session 3: Public Perception for an Emerging Technology**

**Tuesday, May 13 2:30pm-4:00pm**

Moderator: TBD

Neil Goldsmith, Evolva SA

John Halligan, Flavor & Extract Manufacturers Association (FEMA)

Reshma Shetty, Gingko Bioworks

Linda Eatherton, Ketchum

#### *Poisoning The Well: Will scientists, industry and the public once again allow activists to sabotage an emerging biotechnology capable of delivering major benefits to consumers and the planet?*

Food Ferment A growing number of biotechs are leveraging synthetic biology to solve some nagging problems that have frustrated scientists, engineers, and consumers for generations. Namely, produce commercially important small molecules that have been fiendishly hard to extract from nature for the global, billion-dollar health, wellness and nutrition industries—using brewer's yeast and fermentation much in the same way that brewers brew beer or bakers bake bread. The gating items are not just capital, scale-up optimization, cost of goods sold, and good taste. An equally daunting challenge is advancing this innovation in an open and transparent manner, despite the fact that food ingredient industries are famously loathe to publicly disclose how they make their products. All of this is unfolding against the headwinds of "food purity evangelism" and growing fear-mongering stoked by anti-synthetic biology



activists. The same platform that allows companies like Evolva to produce stevia (with Cargill) and vanillin (with IFF) from yeast can potentially also be used to produce myriad other problematic ingredients used in the food, beverage and fragrance industries. Likewise, it can be used to produce oils that are molecularly identical to those extracted from deer, fish, and sea mammals, the extraction of which led to the near extinction of the musk deer and contributed mightily to overfishing. For the average consumer of products like orange juice, yogurt, biscuits, cakes, and bottled drinks, fermentation-derived ingredients translate into more and better tasting products formulated with sustainable ingredients at a price point that they can afford. This kind of "closed-loop" process could be a boon for the food industry because it is reliable, resource efficient, flexible and less susceptible to climate change impact in the future. Despite facts to the contrary, activists assert that synthetic biology will actually harm the environment and that ingredients made from biological processes optimized with synthetic biology are not safe for humans, either. These same activists have mounted similar charges against the makers of fermentation-derived artemisinin, which has helped save the lives of millions suffering from malaria. And this begs the question: Will scientists, industry and the public once again allow a vocal minority of activists and self-appointed sentries to sabotage an emerging biotechnology capable of delivering major benefits to public health, consumers and the planet? There is evidence that suggests that this is exactly what is happening. There are other signs to the contrary. These are still relatively early days for yeast fermentation-based biotech in the food business. One thing is certain, left to

define and dictate the narrative, activists and their enablers (the media, bloggers, food evangelists) will control the images, words, politics, terms of engagement, and moral certainty that could delay, undermine or even possibly destroy one of the most powerful new scientific and industrial production advancements to come along in a generation.

#### **Session 4: Microbial Conversions and Metabolic Engineering to Renewable Chemicals**

**Wednesday, May 14 2:30pm-4:00pm**

Moderator: *TBD*

[Kevin Jarrell, Modular Genetics, Inc.](#)

#### [Synthetic Biology Enables Oil-Free Production of Surfactants](#)

Modular Genetics, Inc. (Modular) is a sustainable chemistry company focused on converting underutilized agricultural material into specialty chemical products. Modular has developed engineered *Bacillus* strains that synthesize particular acyl amino acid surfactants. Traditionally, surfactants are manufactured using synthetic chemistry processes, such as ethoxylation, to link an oil (e.g., palm oil or petroleum) to a charged chemical. Significantly, no oil of any sort is used to produce Modular's surfactants. The microorganism synthesizes the oil (a fatty acid) and the charged chemical (an amino acid) and links them together enzymatically to create the surfactant. Modular has demonstrated that synthetic biology can be used to produce particular surfactants, without the use of oil as a raw material. Commercial opportunities for these green bio-surfactants will be discussed.

[Sean O'Connor, Nucleis Inc.](#)



### Nucelis BIO Conference Abstract

3,457 Nucelis Inc. is a pioneering bio-technology company with a unique, patented technology for optimizing cell structure and function. The Rapid Trait Development System (RTDS™) enables Nucelis scientists to make precise changes in the DNA of virtually any living organism. RTDS takes advantage of the natural gene repair system which occurs in all cells and is typically used to correct naturally-occurring DNA mistakes and mutations. This means that the Nucelis RTDS system allows for predictable results without introducing foreign DNA. RTDS has been certified as a non-GMO technology by the USDA. Nucelis is using RTDS as its core technology to develop specialty chemicals using a variety of microbial platforms. The lead product is the production of squalene using the yeast *Yarrowia lipolytica*. Squalene is a natural oil produced by the *Yarrowia lipolytica* yeast via the terpene biochemical pathway. Squalene is typically obtained today either from the livers of deep-sea sharks, or as a by-product from processed non-virgin olive oil. Squalane (C<sub>30</sub>H<sub>62</sub>) is made via the hydrogenation of squalene (C<sub>30</sub>H<sub>50</sub>). Squalene is naturally occurring, and is found in very small amounts in many plants and animals—including humans. Both of these hydrocarbons are liquid at room temperature. The Nucelis process has several advantages over other sources of squalene. Our yeast is enhanced using RTDS, a non-GMO technology. Other fermentation derived sources of squalene use GMO organisms. Additionally, the Nucelis process utilizes a waste-stream carbon source as its feedstock. This allows the production to be scalable to meet demand, and is more sustainable due to the use of glycerol, which is a by-product of biodiesel production. Nucelis has successfully induced this

microorganism to increase its conversion rate of glycerol into squalene to commercially-relevant quantities. By developing an alternative production process for squalene from a waste carbon source (glycerol), we enable squalene users to choose a more sustainable and more predictable supply of this valuable specialty chemical for the cosmetics, nutritional, and lubricant markets. In addition, the Nucelis process produces the highest purity squalene that is currently available on the market. Squalene is only one of many important products Nucelis is targeting with its R&D efforts. There are over 30,000 terpenes known to be produced by microbes. Most of them can be produced by modifying similar biochemical pathways in microbes or plants. Potential product areas could include, but are not limited to: flavors and fragrances, vitamins, nutraceuticals, pharmaceutical intermediates, pigments, and monomers and polymers. Furthermore, products such as ergosterol, also synthesized from the terpene-sterol pathway, can also be targeted for production. Finally, these microbial platforms are often suitable for the production of complex proteins, such as lipases and proteases. RTDS can also give Nucelis the ability to engineer new high-value enzymes, or to improve current enzyme production for greater stability and efficiency. Future platforms that are targeted for development include programs in fungi, and algae. Nucelis is also actively developing RTDS in human cell lines, with the goal of applying the technology as a method of curing disease. Genetic targets in animals could cure disease or create valuable traits in pets and livestock.

Dave Schisler, Cargill, Inc.

Cargill Partnership Opportunities for



### Commercial Fermentations

Advances in molecular biology have created opportunities to produce food, fuel, and industrial chemicals through fermentation. Cargill, Inc. has developed a proprietary platform microorganism that has proven to be robust and versatile for commercial scale fermentations. Company biotechnologists have successfully engineered this microorganism to produce products of interest both internally, and under license to third parties. Specific examples are highlighted. Fermentation scientists at three locations in North America and Europe are skilled in media development and in optimizing fermentor operating conditions. Strain prototypes are tested in benchtop fermenters, and then scaled up to pilot vessels including 600L and 6,000L bioreactors. Cargill also employs highly skilled chemists and engineers to develop analytical methods, QA protocols, and downstream separation and purification processes. Cargill capabilities and how we have worked successfully with third party companies are demonstrated. Cargill invites industrial partners to develop and produce fermentation products that compete well against similar materials from traditional sources. There are also opportunities to co-locate commercial plants in Cargill industrial bio refineries. The partnership includes leveraging Cargill scientific resources from strain engineering through fermentation development and scale-up to commercial volumes.

Ramesh Nair, Chromatin Inc.

### Engineering Sesquiterpene Based Biofuels in Plants

Chromatin is engineering sorghum to accumulate the fuel precursor farnesene, a

molecule that can be readily converted to biodiesel. Sorghum is naturally drought tolerant, requires low agricultural inputs, has broad geographic adaptability and established agricultural practices. We are applying a combination of enzyme engineering and metabolic pathway engineering to increase sesquiterpene production in sorghum. We have engineered sorghum with key mevalonate (MVA) and non-mevalonate pathway genes (MEP) as well engineer the entire MVA pathway in sorghum. A summary of the approaches, progress to date and the challenges in engineering metabolic pathways in plants will be presented.

### **Session 5: Fermentation Process Design and Separation Techniques**

**Thursday, May 15 8:30am-10:00am**

Moderator: Mark Burk, Genomica

### Engineering high performance strains for commercial production

Our industry is getting better at designing and optimizing organisms to deliver commercially-relevant performance and economics. This session dives into the overall methodology and specific techniques that have delivered these gains in rate, titer and yield, showing how we move from lab-scale validation through 'almost-there' performance to world-class. We'll also highlight how organism design can impact overall process design – and vice versa. This session can be thought of as focusing on the 'science' behind bio-products, with part two, the talk on 'successfully scaling up industrial fermentation of chemicals and fuels' which focuses on the chemical engineering side.

Joachim Venus, Leibniz Institute Agricultural Engineering (ATB)



*Pilot plant facility for the scale-up of continuous mode lactic acid fermentation*

Renewable feedstocks (e.g. crops, lignocellulosics, green biomass, residues) are being used as raw materials for the production of microbial lactic acid. Lactic acid, its salts and esters have a wide range of potential uses and are extensively used in diverse fields. The goal is to develop a fermentation process based on the substitution of expensive nutrient supplements by cheaper materials from biomass due to their main proportion of the whole process costs. The scale-up to a technical scale of several processing steps have to be developed for transferable solutions of biotechnologies for renewables. For that purpose a multifunctional pilot plant was planned and built at the site of ATB to investigate different raw materials and products. Several results of the continuous lactic acid fermentation in a 450-L-bioreactor will be presented. One of the usual ways to keep the biomass inside of the system for increasing the overall productivity is the cell retention with hollow fibre membranes. In comparison to the process without cell recycle (e.g. chemostat) there is a triple up to four time's higher productivity of lactic acid. Depending on the further processing of the lactic acid the separation of impurities after fermentation is a major process cost too. Therefore an optimization is necessary to find a balance between the substitution of expensive nutrients and the limitation of interfering or undesirable components of natural raw materials respectively. Exploitation of high quality L(+)- and D(-) lactic acid for the production of biopolymers is one of the recent applications. Conventional processes for downstreaming are based on precipitation steps that generate large amounts of chemical effluents.

Consequently the environmental impact of traditional processes can be reduced by using alternative technologies, such as electrodialysis with monopolar and bipolar membranes.

*Donald Glatz, Koch Modular Process Systems, LLC*

*Extraction of Chemical from Fermentation Broths Using a KARR Column*

Fermentation processes have become more important in recent years for the creation of valuable chemical products. Liquid-liquid extraction (LLE) is a logical first step in the recovery process since many of the chemicals produced are organic chemicals with higher boiling point than water. These processes generally require a high number of theoretical stages to achieve sufficient product recovery. As such, agitated LLE columns are necessary instead of static columns, including packed columns and columns with sieve trays. However, most fermentation broths have a high tendency towards emulsification when contacted with solvents for extraction. Agitated columns using rotating internals only intensify the tendency towards emulsification. The KARR<sup>®</sup> Column however, uses a reciprocating plate stack with open area greater than 55% for providing the agitation during counter current flow of the two phases. This type of agitation has proven to work effectively when columns with rotating internals have failed. This result is confirmed in this paper via presentation of a case study where the KARR<sup>®</sup> Column was used to provide the solution for a specific fermentation process.

*Gregory Benz, Benz Technology International, Inc.*

*Economics of Oxygen Enriched Aerobic*



### Fermentation

Mechanically agitated aerobic fermentation has been a mainstay for making such bioproducts as antibiotics, enzymes, vitamins, soluble gums and many other products for more than 70 years. These fermentations require nutrients and oxygen to feed the microorganisms that produce the product. In the vast majority of cases, that oxygen is provided by using air introduced at the bottom of the fermenter, and mechanically dispersing it with an agitator. Air is an attractive gas to use because of low cost. The principle cost of air in most fermentations is the power required to deliver the air. There may also be costs of filtration, both on the inlet and the exhaust of the fermenter. In some cases, it may be attractive to consider the use of supplemental oxygen instead of air only. This paper explores several possible reasons for doing so. Such reasons may include, but are not limited to, reducing the capital cost of agitation and air handling systems by designing for less than peak oxygen transfer rate (OTR) by using supplemental oxygen for a short duration to achieve the peak OTR; reducing the cost of treatment of exhaust gasses by reducing the volume to be treated, and in some cases, allow larger fermenters to be built than are currently practical due to limitations of agitator manufacturing capability. The main technical, safety and economic issues are addressed in this paper.

Niloofer Abdehagh, University of Ottawa

### Adsorptive bio-butanol separation from ABE model solutions

Bio-butanol has attracted attention of scientists and industrialists as a potential promising biofuel showing advantages over other biofuels such as ethanol. Bio-butanol lower volatility and

higher Net Heat of Combustion, closer to gasoline are some of its advantages over other biofuels. However, the production of bio-butanol via Acetone-Butanol-Ethanol (ABE) fermentation is facing significant challenges due to important butanol toxicity to microorganisms, resulting in very low final concentration. It is therefore important to develop an efficient separation technique to recover butanol from fermentation broths to make the production of butanol an economically feasible process. Adsorption, as an energy efficient process, can be used to achieve this objective. Adsorption rate and adsorption capacity are some of the factors that should be considered in selecting a suitable adsorbent. In our previous study, activated carbon (AC) F-400 was selected as the butanol adsorbent amongst different activated carbons and zeolites because it showed the fastest adsorption rate and the highest adsorption capacity amongst different adsorbents tested in adsorbent screening experiments. In the present study, Butanol was produced in fermentation using *Clostridium Acetobutylicum* and after optimizing the operational condition of the fermentation the affinity of AC F-400 toward butanol over the other main components present in Acetone-Butanol-Ethanol fermentation broths was studied. It was observed that it has the highest selectivity to butanol. In addition, the butanol adsorption capacity was not affected by the presence of ethanol, glucose and xylose while the presence of acetone led to a slight decrease in adsorption capacity at low butanol concentrations. On the other hand, the presence of acids (acetic acid and butyric acid) showed a significant effect on the butanol adsorption capacity over a wide range of butanol concentration and this effect was more



significant for butyric acid. Although a wide range of materials has already been tested as butanol adsorbent, less effort has been devoted to desorption of adsorbed butanol as the final product. In this study, thermal desorption with a purge of carbon dioxide was performed and the operational conditions were optimized to reach the best results in butanol recovery. It was also observed that after six cycles of adsorption-desorption the adsorption capacity and butanol recovery remained unchanged so it was concluded that this method could be considered as an efficient butanol desorption method.

#### **Session 6: Genome Engineering for Industrial Applications**

**Thursday, May 15 10:30am-12:00pm**

Moderator: *TBD*

Eric Kmiec, Delaware State University  
*Re-engineering genomes using single-stranded oligonucleotides and TAL-effector Nucleases (TALENs) for industrial technology*

The re-engineering of genomes in bacteria, yeast, plants, and mammalian cells can be directed by single-stranded oligonucleotides (ssODNs) and TAL-effector nucleases (TALENs). We have used these tools in a variety of combinations to create polymorphisms, repair genetic mutations and disable functioning genes; in effect, restructuring the genome. While TALENs can act to knock-out particular genes with remarkable precision, ssODNs modify their behavior by reducing the off-site mutation rates and increasing their capacity to correct a missense mutation. By elucidating the mechanism of action of this genome engineering approach, we have been able to improve the efficiency of the gene editing

reaction while identifying barriers to success and technological limitations. Overcoming these barriers will be the major challenge of genome editing as a useful strategy for re-engineering bacteria, yeast or other useful organisms for industrial biotechnology. We will present the keystone features of genome re-engineering that will enable successful implementation of this revolutionary technology using original data from a variety of model systems, pertinent to industrial biotechnology. By defining these limits we will enable members of the biotechnology community to develop realistic and attainable goals when designing their genomic, industrial applications.

Nokuthula McHunu, Durban University of Technology

*Carbon utilization profile of a thermophilic fungus, *Thermomyces lanuginosus*, reveals a potential of lignocellulose degradation*

Replacement of petroleum-based products and manufacturing processes with competitive bio-based alternatives is attracting increased attention due to environmental concerns surrounding petroleum sustainability and supply. Replacement of conventional processes for manufacturing valuable industrial products and the selection of optimal biosynthetic routes requires the construction, and in most cases subsequent context-dependent evaluation, and optimization of multicomponent biosynthetic pathways to generate intermediates and end products. This talk will present the use of Ingenza's proprietary combinatorial genetics platform (inABLE®) to rapidly clone, express, select and optimize target activities for many separate enzymatic reactions, from thousands of independent genes derived from metagenomic and phylogenetic discovery



approaches. This includes the characterization of a vast library of hitherto undiscovered genes from rumen microbial flora and the deployment of resulting novel enzyme activities in *Saccharomyces* to adapt and expand feedstock utilization for increased process efficiency. Multiple gene variants comprising of up to ten individual genetic elements are combined in single reactions, generating expression libraries with hundreds or thousands of members in diverse heterologous configurations for HTP interrogation. Obvious synergy exists between this approach and versatile, solid phase screening and selection methods using growth-based, crossfeeding or colorimetric methods to identify colonies of interest. This is illustrated through the rapid identification of critical pathway enzymes, optimal gene coding sequences and enzyme variants from inABLE<sup>®</sup>-derived high quality variant libraries for applications in bio-based polymers, chemicals and personal care products. We will also describe the success of modeling approaches to gene design that, when coupled with our inABLE<sup>®</sup> approach towards combinatorial biofabrication, enhance the predictability of heterologous gene expression in diverse hosts. The technology aims to bring increasing predictability and overcome persistent limitations associated with iterative and empirical processes for microbial strain improvement. The successful realization of optimal target reactions enables rapid pathway definition and progression to process optimization and scale-up.

Gajanan Naik, Gulbarga University

*Studies in Conversion of Sugarcane Bagasse into Ethanol – A Genomic Approach*

Sugarcane Bagasse and pigeon pea agro waste

is available in plenty in North Karnataka, India. There is a demand from farmers to develop new technologies for their conversion in to value added products. Low cost technologies based on SSF of *Bacillus* sp. JB 99 has helped in standardizing protocols for cost effective production of crude xylanase. The enzyme has 4856 U/g DBB of activity with optimum pH 10.0 and temperature 70°C. The preliminary experiments carried on conversion of Sugarcane Bagasse to sugars have shown promising results. The Experiments are also carried to improve the efficiency of the conversion of Sugarcane bagasse into sugars by media modification, addition of additives, moistening agents and various physiochemical and physico mechanical pretreatments. The possibility of using cell free system and immobilization technique for repeated use of microbe /enzyme has also been investigated. Cloning and expression of xylanase gene from *Bacillus* sp. JB 99 into suitable host and its Scale up studies for conversion of bagasse and pigeon pea waste in to sugar and ethanol for commercial exploitation, is under progress. A highly thermostable alkaline cellulose – free xylanase was also purified to homogeneity with a molecular size of 20 kDa and N-terminal amino acid was determined to be NH<sub>2</sub>-Asn-Thr-Tyr-Trp-Gln-Tyr-Trp=Asp-Gly. The xylanase was biochemically characterized from *Bacillu* sp. JB 99 and found to be exhibiting endo-xylanase activity on different xylan substrate and no activity on cellulose substrate, which confirm it to be true xylanase. The xylanase showed maximum activity at 70oC and pH 8.0. The gene encoding cellulose-free xylanase (BJBxyn11) was also successfully PCR amplified, cloned and sequenced from *Bacillus* so. JB 99. After Bioinformatics analysis of gene sequence, the



gene BJBxyn was found to be 1278 bp long containing ORF of 633 bp consisting of 210 amino acid and conserved Shine-Dalgarno sequence (5' AAGGAGG 3') at 11 bases upstream to initiation codon Met. In addition, a putative promoter -35 signal (TTGCAA) and -10 signal (TATAAT) sequence at 153 and 130 bases upstream of initiation codon was identified and has the typical consensus sequence in Bacillus subtilis promoter. The gene sequence showed BLAST homology search with 97% identity with thermostable alkaline xylanase from Bacillus halodurans and Bacillus firmus respectively, 76% with Paenibacillus sp. HY8 and 75% with Bacillus stearothermophilus.. The deduced amino acid sequence comprises of 210 amino acid residue with a calculated mass of 23 kDa consist of first 28 amino acid as signal peptide and followed by 182 amino acid residues of mature xylanase with calculated mass of 20.4 kDa and pI 8.84. The molecular basis of thermo and alkali stability was predicted after determination of amino acid composition of BJBxyn11. After comparison with other thermostable and alkaline xylanase, BJBxyn11 showed an increased frequency of Arginine (Arg) (3.8-5.0%) as compared to other mesophilic and thermostable xylanase (1.7-3.7%). This factor may contribute in enhanced thermo stability of xylanase as reported earlier (Mrabet et al., 1992; Turunen et al., 2002). The gene BJBxyn11 was functionally expressed under the control of strong promoter using pET-28(+) expression vector in heterologous system E.coli BL21 DE3. The recombinant BJBxyn11 expression was also confirmed by purification and SDS-PAGE analysis with a molecular size of 20 kDa.

Nigel Mouncey, Dow AgroSciences

#### Industrial Biotechnology at Dow AgroSciences

Dow AgroSciences is one of the world's leading Agriculture companies specializing in not only agricultural chemicals such as pesticides, but also seeds and biotechnology solutions. Dow AgroSciences has had a long history of development and use of Natural Products for agricultural solutions. To support the development of new, and continued optimization of existing products, Dow AgroSciences has established an integrated bioprocessing capability that provides the technologies for strain discovery, strain optimization, process development and optimization, product recovery and scale-up. In this talk I will provide an overview of this Industrial Biotechnology capability, highlight some of the innovative methods we are employing and share examples of where we are using this capability to support product development and optimization for products for both Dow AgroSciences and The Dow Chemical Company.

### **Track 6: Growing Global Markets**

#### **Sponsored by: DuPont**

#### **Session 1: BiotechCorp: Facilitating Malaysia to a Developed Economy through Industrial Biotechnology**

**Tuesday, May 13 8:30am-10:00am**

Moderator: TBD

Ybhg Dato' Dr. Mohd Nazlee Kamal,  
BiotechCorp

#### BiotechCorp: Facilitating The Nation's To A Developed Economy Through Industrial Biotechnology

Biobased chemical produced via a biotechnology/fermentation platform must



meet environmental, cost, and performance criteria in order to become viable candidates for industrial adoption. Large majority of biobased products are biodegradable and/or ecologically friendly. While many factors may affect the production cost of a particular biochemical, a major contributing factor has been the starting material or feedstock based on several cost analysis models. Agricultural byproducts, which are also renewable, could provide relief in this case. Naturally produced biobased products may not necessarily possess physical or chemical properties needed to render acceptable performance. Structural modification via chemical reactions to change their properties is a powerful route to achieve performance requirement. In this panel presentation, speakers will discuss development of bioprocess and chemical routes to produce industrially important biochemicals from agricultural renewables.

**Session 2: The Brazilian Developing Biobased Economy: Investment Opportunities**  
**Tuesday, May 13 10:30am-12:00pm**

Moderator: Mariana Doria Abiquim  
Carlos Eduardo Cavalcanti, BNDES  
Felipe Pereira, BNDES  
Jose Vitor Bomtempo, Flavia  
Chaves Alves, UFRJ

*The Brazilian Developing Biobased Economy: Investment Opportunities*

The objective of this panel is to present investment opportunities for the biobased industry in Brazil. First, an outlook of the Brazilian chemical industry will be shown together with Governmental initiatives to promote innovation. Secondly, an update of the innovation support plan for biofuels and

biobased chemicals (PAISS), implemented by BNDES and the Brazilian Innovation Agency (FINEP) since 2011, will be presented. Thirdly, business opportunities highlighted by the study “Brazilian chemical industry diversification opportunities” will be understood and, finally, the last presentation regards relevant technological routes with capacity for development in Brazil.

**Session 3: Global Energy Trends: The Development of Localized Feedstocks to Supply the Global Cellulosic Industry**  
**Tuesday, May 13 2:30pm-4:00pm**

Moderator: Jim Lane, Biofeuls Digest  
Stuart Thomas, Dupont  
Pablo Gutierrez, Abengoa  
Steve Hartig, POET/DSM  
Vonnie Estes, GranBio

*Global Energy Trends: The Development of Localized Feedstocks to Supply the Global Cellulosic Industry*

Cellulosic Ethanol technology is being commercialized globally, where regional renewable fuel needs are being met with local, abundant and low cost biomass. Leading companies will give us an overview on the latest trends in feedstock supply from around the world and how cellulosic technologies are being successfully implemented.

**Session 4: A Year in Review – President Rouseff’s First Term Policies to Benefit the Bio-economy**

**Wednesday, May 14 2:30pm-4:30pm**  
Moderator: Nelson Akio Fujimoto, Brazilian Ministry of Development, Industry and Foreign Trade  
Carlos Eduardo Cavalcanti, Biofuels Department, Brazilian Development Bank



(BNDES)

Manuel Teixeira Souza Júnior, Agroenergy

Nacional Research Center (EMBRAPA

Agroenergia)

Ricardo de Gusmão Dornelles, Brazilian Ministry  
of Mines and Energy

*A year in review-President Rousseff's First Term  
Policies to Benefit the Bio-economy*

**Session 5: Regional Bioeconomy Development  
– Models and Success Factors**

**Thursday, May 15 8:30am-10:00am**

Moderator: Manfred Kircher, CLIB2021

Manfred Kircher, CLIB2021

Stan Blade, Alberta Innovates Bio Solutions

Viondette Lopez, USDA, National Institute of  
Food and Agriculture (NIFA)

Bruce Babcock, Cargill Chair of Energy  
Economics & Director of the Biobased Industry  
Center, Iowa State University

*Regional Bioeconomy Development – Models  
and Success Factors*

Biorefineries address all three grand societal challenges i)energy and resource scarcity, ii)climate protection and iii)employment. Bio-based feedstock and resource efficiency as well as new transformation processes to materials, chemicals and energy carriers are its crucial building blocks and draw most attention in Research and Development. However, turning the modern bioeconomy into reality needs more than technical solutions. It needs awareness of the business potential by SME, big industries, and investors, of product benefits by consumers, of infrastructure adaptation by public administration, future labor requirements by professional training institutions and a supportive legal framework by governments. National biorefinery strategies

have been published and translated into regional programmes since about 5 years . Generally the starting point for spreading the biorefinery value chain has been an industry the region is already strong in – for example agriculture in Iowa and Alberta or chemical industries in Germany. This panel will present regional models with a special focus on success factors. As it needs normalized data about a region's biorefinery qualification key parameters for preparing and evaluating suitable initiatives will be discussed.

**Session 6: Metabolic and Process Engineering:  
A Dual Approach to Address the Industrial  
Scaling up of Biobased Products**

**Thursday, May 15 10:30am-12:00pm**

Moderator: Yvon Le Hanaff, ARD

Johan De Coninck, Cluster IAR

Brian Davison, Oak Ridge National Laboratory

Pierre Monsan, Toulouse White Biotechnology

Pierre Calleja, FermentaIg

*Metabolic and Process Engineering, an essential  
complementarity to optimize the added Value of  
complex processes*

Metabolic engineering is used to optimize or create new metabolic pathways within cells to increase the production of a desired product. Genetic engineering techniques can be used to modify the network in order to relieve these constraints. Metabolic Engineering specifically seeks to mathematically model the process, calculate the yield of useful products, and pin point parts of the network that constrain the production of these products. Process engineering focuses on the design, operation, control, and optimization of chemical, physical, and biological processes to produce valuable substances on an industrial scale in a cost



effective manner. Metabolic network changes in cells can have drastic effects on its ability to survive. Process engineering can help considerably to overpass hurdles and to limit trade-offs in metabolic engineering. Optimized engineered microorganisms are not necessary the best candidates to reach the best productivities at industrial scale. The integrated approach, make possible to accelerate the development while lowering the risks of failure. This approach allows to return easily at the gene level and to perform genetic improvement operations in a representative, but less stressful, environment and jump back to larger scale to validate newly engineered systems. The use of high throughput techniques for screening or strain engineering operations can also reduce time for strain development, to access robustness of the system and to border the strain limitations in various conditions approaching industrial environment. From lab cellular plants to industrial plants the way is very long, but the synergy between Metabolic and Process engineering can shorter time to market by optimizing economics to produce Biobased polymers and materials. This is why demo is unavoidable. In this panel, practical examples will be described to illustrate this approach: -Although biofuels production has the same key requirements as other existing energy supply chains, biofuels are a unique multi-factorial problem. Consideration must be given to the land used for feedstock production, the feedstocks employed, the process technologies used, and the products. Modern biotechnology has the potential to impact many aspects of the biomass supply chain. For example, technological advances in feedstock production (e.g., genetic modification or cross breeding, etc...) and process technologies (e.g.,

new enzymes or bacteria for the conversion of cellulosic feedstocks) will have a significant impact on the future cost and feasibility of possible biofuel supply chains. Other biotechnological advances may increase the production of fungible biofuels and bioproducts. -Biodiversity of Microalgae makes these microorganisms good candidates to produce high added value molecules. To be successful a rich strain bank and a good identification of candidates is necessary, but not enough. Production process and technologies used on each step from breeding to final extraction of the desired molecules are essential. Fermentalg, a microalgae breeding company has developed a process introducing mixotrophic steps in a predominantly heterotrophic environment that offers high yields of biomass and molecules of interest and uses low-cost industrial by-products as production substrates. -ARD and TWB will describe some process improvement made in the production of: bioethanol, DHA, Eladium and others...

## **Track 7: Research Presentations**

### **Session 1: Biobased Chemicals via Bioprocess and Chemistry from Agricultural Renewables Tuesday, May 13 8:30am-10:00am**

Moderator: Robert L. Fireovid, U.S. Department of Agriculture, Agricultural Research Service  
Nhuan (John) Nghiem, U.S. Department of Agriculture Agricultural Research Service  
David B. Johnston, U.S. Department of Agriculture, Agricultural Research Service  
Jonathan Zerkowski, U.S. Department of Agriculture Agricultural Research Service  
Daniel K.Y. Solaiman, U.S. Department of Agriculture Agricultural Research Service



*Biobased Chemicals via Bioprocess and Chemistry from Agricultural Renewables*

Biobased chemical produced via a biotechnology/fermentation platform must meet environmental, cost, and performance criteria in order to become viable candidates for industrial adoption. Large majority of biobased products are biodegradable and/or ecologically friendly. While many factors may affect the production cost of a particular biochemical, a major contributing factor has been the starting material or feedstock based on several cost analysis models. Agricultural byproducts, which are also renewable, could provide relief in this case. Naturally produced biobased products may not necessarily possess physical or chemical properties needed to render acceptable performance. Structural modification via chemical reactions to change their properties is a powerful route to achieve performance requirement. In this panel presentation, speakers will discuss development of bioprocess and chemical routes to produce industrially important biochemicals from agricultural renewables.

**Session 2: Scaling Up Industrial Fermentation of Chemicals and Fuels**

**Tuesday, May 13 10:30am-12:00pm**

Moderator: Jeff Lievense, Genomatica

Chuck Kraft, Amyris

Marcel Lubben, Reverdia

Mike Hess, Novozymes

John Evans, POET

*Successfully scaling up industrial fermentation of chemicals and fuels*

In this session, we 'go deeper' on a hot BIO topic: the genuine integration of process engineering principles and discipline with

producing organisms that is needed to deploy a commercially-successful industrial biotech process. This session shares key guidance for this integration, including from Genomatica, winner of the 2013 Kirkpatrick Award. This session is also an excellent 'sequel' to the session on 'engineering high performance strains for commercial production'.

**Session 3: Techno- Economic Analysis for Microalgae Growth**

**Tuesday, May 13 2:30pm-4:00pm**

Moderator: *TBD*

*Leah Raffaelli, University of Denver*  
*Wastewater and Flue Gas Effects on Microalgal Growth Sustainability*

Providing nutrients can represent major technical and economic problems at commercial scales for microalgal growth facilities. It is largely assumed that tapping into a wastewater or flue gas source will lower the nutrient costs, and possibly enable a cost effective microalgal growth facility through providing for carbon consumption or wastewater treatment. This study analyzes the validity of these assumptions. Two complex system models reveal the costs of supplying nutrients to algal cultures to be significant, especially for supplements not acknowledged by most studies and which are not supplied by wastewater or flue gas. Other results of the study include the high cost of replacing evaporated water in an open pond growth scenario, even if using brackish water, seawater, or wastewater. Economic sustainability of growing microalgae with the intention of producing biofuels is seriously impacted by the necessity of supplying nutrients, especially for open pond growth



scenario. Integration with wastewater treatment shows potential of producing a cost effective photobioreactor algal growth scenario, especially for solar illuminated photobioreactors. However, integration with power plant flue gas offers little economic incentive and incomplete carbon consumption. Previous studies have not included full impact of nutrient costs on commercial scale microalgal growth for end products. The cost of nutrients reveals the necessity of increasing algal culture density, integration with wastewater treatment, and limiting water evaporation in order for microalgal growth to be considered sustainable.

Jason Quinn, Utah State University  
*Life Cycle Assessment and Techno-Economic Analysis of Wastewater Algae for Electric and Fuel Production*

Global oil consumption is rising at an unprecedented rate primarily due to increasing consumption in developing nations. Oil consumption coupled with volatility in oil prices has caused an interest in renewable energy sources. Traditional terrestrial crops are currently being utilized as a feedstock for biofuels but resource requirements and low yields limit the sustainability and scalability. Comparatively, next generation feedstocks, such as microalgae, have inherent advantages such as higher solar energy efficiencies, large lipid fractions, utilization of waste carbon dioxide, and cultivation on poor quality land. Extensive research is being performed on optimizing the microalgae growth, harvesting, extraction, and conversion techniques to obtain a feasible path to biodiesel production. A novel harvesting and extraction procedure was developed at Utah State University for the microalgae to biodiesel path to increase its

feasibility. The harvesting process is performed through a Rotating Algal Biofilm Reactor (RABR) which is intended to optimize microalgae growth in wastewater while reducing the nitrogen and phosphorous concentrations. The Wet Lipid Extraction Procedure (WLEP) was developed to avoid the energy intensive step of thermal drying. A life-cycle assessment (LCA) and techno-economic analysis (TEA) were performed for electrical and biofuel production through wastewater algae with the integrated RABR and WLEP. LCA results revealed a net energy ratio (NER) of 11.23 and 31.17 for the electrical and biofuel production respectively with a global warming potential (GWP) of 446 and 382 gCO<sub>2</sub>•kWh<sup>-1</sup>. Although the NER is unfavorable for both processes, the GWP for microalgae to electricity ranges lower than the GWP for electricity for coal and natural gas at 1120 and 622 gCO<sub>2</sub>•kWh<sup>-1</sup> respectively. TEA results shows to break even with the annualized cost of a large scale system the electricity output would be sold at \$3.00/kWh and biodiesel would be sold at \$400/gallon. The TEA results show it is not economically viable to produce biofuel or electricity from this process and a higher value product would be needed to make it more economical. A sensitivity analysis was performed to identify the inputs with the greatest effect on the overall system. Incorporating improvements to the most sensitive inputs yielded an average 86% and 80% decrease in the NER and TEA respectively.

Jean-Claude Frigon, National Research Council Canada  
*Anaerobic digestion of microalgal biomass in lab-scale digesters for the production of volatile fatty acids*

Anaerobic digestion (AD) is a well-known,



reliable and proven process for the conversion of a range of organic wastes into methane, a biofuel. It is possible to divert the AD process and focus on the hydrolysis and acidification steps of the targeted organic substrate, to produce a range of low-carbon chemicals, among which volatile fatty acids (acetic, propionic and butyric acids), with a higher commercial value than methane. Recent work with microalgal biomass as a substrate has focused on its conversion into biofuel, mainly biodiesel, and greenhouse gas reduction activities by CO<sub>2</sub> sequestration. However, the conversion of microalgal biomass into carboxylic acids could offer an interesting alternative to be embedded in a biorefinery approach, by producing a value-added product. This study compared the digestion of the microalgal biomass *Scenedesmus* sp.-AMDD in 6L laboratory-scale digesters during 22 weeks, under mesophilic (35°C) and thermophilic (55°C) conditions. The thermophilic conditions were tested to verify if an increased temperature would favourably impact the hydrolytic rate of the bacterial population and increase the final yield of volatile fatty acids (VFA). The digesters were inoculated with bovine manure, which displayed a high hydrolytic activity in preliminary batch assays, and operated under acidic conditions to prevent methane formation. The operating conditions were as follow: pH (4.5), organic loading rate (2.5 gTVS/L.d) and hydraulic retention time (15 days). Both digesters displayed similar hydrolytic activity with  $0.37 \pm 0.02$  and  $0.44 \pm 0.04$  g sCOD / gTVS for the digesters operated at 35 and 55°C, respectively. However, the VFA represented a higher fraction of the sCOD in the mesophilic digester effluent (45%) compared with the thermophilic conditions (22%). The

average VFA yield was 85% higher under mesophilic conditions, at 165 mgVFA / gTVSin, compared with  $89 \pm 10$  mgVFA / gTVSin at 55°C. The lower VFA yield in the thermophilic digester could be explained by a much higher concentration in long chain fatty acids (LCFA), suggesting an incomplete degradation, and higher risks of inhibition of the AD process. In effect, LCFA (> C13) represented 7.7 % of the effluent sCOD of the thermophilic digester (192 mg/L), compared with 4.4 % for the mesophilic one ( $73 \pm 22$  mg/L). Furthermore, there was a significant fermentative hydrogen production at 55°C with a relative hydrogen concentration of 42% in the biogas and a yield of 20 mL H<sub>2</sub> / gTVSin. Hydrogen production was negligible at 35°C. In conclusion, thermophilic conditions did not improve the VFA yield from microalgal biomass over more conventional mesophilic condition. The VFA yield was quite high for this exploratory experiment, although it is presumed that a higher hydrolysis rate and better VFA yield will be required for this process to become more appealing.

#### **Session 4: Uses of Biopolymers in Industrial Applications**

**Wednesday, May 14 2:30pm-4:00pm**

Moderator: *TBD*

Thomas Schultz, Horizon Partners Ventures LLC  
*HydroCoat® Crop Enhancer- A Renewable Resourced, Biodegradable Seed Coating*

The increasing need for better water usage and conservation in agriculture will continue to with the growth in human population and global warming. The demand for food is rising dramatically while the amount of arable land is not. Reducing water requirements for agricultural crops is critical. Seed modifications



have helped lower the amounts of fertilizer and protection materials with more crop yield, but the approaching problem of significant water demand competition between human hygiene and agriculture necessitates lowering water use in food production supply. Genetic modifications and the efficient use of greenhouses will not be enough to meet future water demand. Seed coatings that help germination with lower soil water needs is a solution to this problem. Genetic modifications (unpopular), greenhouse crop production (costly) and petroleum-based, polymeric seed coating (environmental issues) have led to better yielding crops, but each of these technologies fails to effectively address the need to conserve water and utilize this resource more efficiently as overall demand increases. To this end we created HydroCoat©, a renewable resourced, biodegradable super-absorbent-polymer, that reduces seedling water needs. Unlike current technologies, this novel fast-drying aqueous hydrogel increases seed germination yield and provides a solution to the three most critical needs in food crop planting: (1) reduces in irrigation demand during germination, (2) reduces fertilizer and plant protection runoff, and (3) serves a significant societal benefit in balancing global water requirements between human and agricultural needs. Super Absorbent Polymers were originally started under a 1960's USDA program, but these materials performed "too well" in that they failed to release absorb water. HydroCoat©'s desorption of water is modifiable by their gelatin/sugar/clay ratios. HydroCoat© coated vegetable and grass seeds seedlings sprouted more robustly than untreated controls at 25%+ reduced moisture levels, indicating a new means of in-soil water attraction.

Incorporating inorganic and biological additives (fungal or bacterial control materials) HydroCoat© will further increase field capacity with tillage and enable new field usage in areas with poor water supply. HydroCoat© may also be able to substitute partially for seed priming so that pre-germination activities can occur. HydroCoat© hydrogels seed coatings have excellent wet-dry-wet hysteresis and have no negative environmental impact. Unique novel formulations using aqueous gelatins, sulfated saccharides, clays and azeotroping solvents (a new discovery) form fast-drying, non-deliquescent coatings. These seed coatings follow the Gibbs-Donnan equilibrium so compositions tailored for seed species-specific applications can be made for effective water attraction in seedbeds of suboptimal water content (osmotically and matrically) facilitating better germination. Current commercial seed coatings contain few added-value components, whereas our water-based hydrogels provide a means to carry large combinations. The inclusion of water-soluble plant nutrients and beneficial bacteria and fungi in hydrogel seed coatings of both non-primed and primed seeds permits lowering the amount of fertilizer that needs to be broadcast on the field. This in turn reduces the fertilizer runoff that has become a very serious water contamination issue in major areas around the world.

Bernhard Seifried, Ceapro Inc.

*Novel Drying and Impregnation Technology for Water-Soluble Biopolymers*

A novel drying technology for processing water soluble biopolymers, such as polysaccharides, gums and others, into dry micro- and nanofibrils, granules or fine powders is presented. The technology is based on a spray



drying method operating at mild temperatures (40°C) and moderate pressures (10 MPa), which is particularly suitable for high molecular weight water-soluble biopolymers. These biopolymers, such as oat beta-glucan (BG) having molecular weights ranging from 600 to 1,500 kDa, form highly viscous solutions at relatively low weight fractions (1%wt). Drying of biopolymers with high molecular weight forming highly viscous solutions is challenging using conventional spray drying methods, which require high shear rates for atomization and high temperatures having detrimental effects on the biopolymer molecular integrity. The drying medium in this novel drying process is a mixture of food grade, recyclable solvents, generally regarded as safe (GRAS), such as pressurized carbon dioxide (CO<sub>2</sub>) and anhydrous ethanol (EtOH) forming a so-called pressurized gas expanded (PGX) liquid. The unique properties and tune-ability of pressurized gas expanded liquids afford single phase conditions and very low or vanishing interfacial tension in the spray process. This then allows generation of preservative free nano- and micro-scale structures, fibrils and particles, resulting in favorable solubilisation properties and large specific surface area. Furthermore, the dried biopolymer fibers or particles can be impregnated with bioactives directly after formation to further increase their functionality, for example for drug delivery or cosmetic systems, functional food and nutraceutical applications and beyond. The processing conditions facilitate formation of sterile biopolymer matrices. Due to the highly tuneable processing conditions and depending on the molecular structure of the biopolymer a wide range of morphologies and nanoscale features can be generated ranging from cobweb structures, microfibrils, fine and coarse

powders, nano-particles, 3D micro- or nano-structured biopolymer networks. This platform technology has been developed at the University of Alberta and successfully scaled-up from lab-scale to commercial scale by Ceapro Inc., allowing processing of multi-tonnes of biopolymer solutions per day. This scale-able process can be adapted to various spraying conditions and a wide range of aqueous biopolymer solutions. A continuous commercial scale unit is currently being built to generate dry BG in form of fibrous sponge-like material with properties similar to aerogels. In summary, the PGX drying technology may enable for the generation of dry biopolymer matrices loaded with bioactives that can be used in cosmetic, nutraceutical, food as well as in healthcare applications, including slow release drug delivery systems, solid oral formulations or wound healing scaffolds.

Elvis Fosso-Kankeu, North West University  
*Beneficiation of by-products from biofuel plant processes for the production of an eco-friendly polyurethane foam*

Synthetic polymers are very resistant to biological degradation and therefore tend to persist in the environment. The use of natural polymers in replacement of fossil-based polymers for the production of polyurethane foam results in the formation of a bio-based product susceptible to environmental degradation. The reactants involved in the first step of the production of polyurethane foam, namely lignin and glycerol could be obtained as by-products from bioethanol and biodiesel processes, respectively. The objectives of this study are two-folds, firstly to produce a bio-based polyurethane foam from the by-products of biofuel processes and secondly to investigate



the effect of lignin type on the quality of polyol and polyurethane foam. The production of polyols was carried out through liquefaction at 160°C and pH of 8 using sulphuric acid as catalyst, a crude glycerol to lignin ratio of 9:1 (w/w) and a residence time of 90 minutes. Three types of lignin were used for polyol production, i.e. lignin extracted from softwood by the kraft process (KL), lignin extracted from hardwood by the acid sulphite process (SL) and lignin extracted from sugarcane bagasse by an organosolv method (OL). The characterization of the biopolyol produced revealed a hydroxyl number of 360 mg KOH/g, 530 mg KOH/g and 185 mg KOH/g for the three lignin types KL, SL and OL respectively. The amount of biopolyol derived from liquefaction of SL was double that of biopolyol deriving from other lignins. The viscosity of biopolyols varied with the type of lignin used, i.e. 610 mPa.s for KL, 210 mPa.s for SL and 80 mPa.s for OL. The polyurethane foam produced under optimum conditions from the KL biopolyol was of a better quality with characteristics comparable to commercial analogs; it showed a compressive strength around 300 kPa and a density around 78 kg/m<sup>3</sup>. These results suggest that a “No Waste” biofuel production system can be achieved through the beneficiation of by products such as lignin and crude glycerol for effective production of bio-based (green) polyurethane foam susceptible to biodegradation by biotic and abiotic factors in the environment.

Rina Carlini, GreenCentre Canada  
*Novel Catalysts to Produce High-Performance Biodegradable PolyLactic Acid*

Global demand for biodegradable polymers is growing rapidly to address the need for compostable and recyclable plastic products.

Poly(Lactic Acid), or PLA, represents the largest segment of these biodegradable polymers, and annual sales of PLA is expected to surpass \$3 billion by 2016. Several grades of PLA are currently produced with a range of physical and performance properties that enable its use in disposable and biodegradable plastic items such as food serviceware, non-wovens and rigid packaging. However, there is growing demand for high-performance PLA having higher melting temperatures and crystallinity to allow its use in mechanically durable and heat-resistant plastic products. GreenCentre Canada has developed a new technology based on a family of ring-opening polymerization catalysts that produce biodegradable PLA with such high-performance properties. GreenCentre’s novel catalysts produce an isotactic-enriched PLA from D/L-Lactide feedstock monomer mixtures having both high crystallinity (> 60%) and high melt temperatures exceeding 170°C, which are well above the melt temperatures of most other commercial PLA. The catalysts are air-stable, have good processing stability, and perform well under both solution-phase or melt-phase polymerization conditions. The technology is “made in Canada” and offers a new process innovation to produce sustainable, PLA-based biodegradable polymers that are well-suited for high-performance and specialty product applications.

#### **Session 5: Biomass Supply Chain and Pre-treatment Methods**

**Thursday, May 15 8:30am-10:00am**

Moderator: Allen Julian, MBI

*Unlocking the Potential of Biomass: Recent Advances in the Scale Up of AFEX Biomass Pretreatment Technology and its Downstream Applications.*



MBI has recently commissioned a pilot-scale AFEX reactor to pretreat agricultural residues such as corn stover, rice straw, wheat straw, bagasse, Miscanthus or switchgrass. Funded by a \$4.3 MM award from the US Department of Energy, MBI has designed and constructed a 1000-liter packed bed AFEX reactor with a maximum design throughput of 1 ton per day. The MBI technology enables the economic production of pellets which are dense (10-fold higher than raw biomass) stable and shippable. The simplicity and low-capital cost of the process allows it to be practiced in local depots close to the site of agricultural production. MBI is developing multiple applications for AFEX pellets including biorefinery feedstocks and ruminant feed ingredients. This presentation will highlight features of the AFEX reactor design and operation, and present data on the efficacy of the pilot reactor compared to laboratory benchmarks. The presentation will also report salient progress in downstream applications, including saccharification and fermentation trials and related market economics.

Sam Jackson, Genera Energy Inc.

*Building Innovative and Successful Industrial-Scale Biomass Supply Chain Solutions*

An efficient and optimized feedstock solution requires tight integration of all elements of the biomass supply chain. Genera Energy has focused on developing front to back, integrated supply chain solutions to benefit the entire biobased industry. The company works to optimize crop portfolio selection and production as well as downstream operations including harvesting, logistics, storage, and preprocessing. The logistical systems required for cost-effective biomass production are often

overlooked and this oversight can create the potential for supply chain failure. Genera has developed industry leading best practices for feedstock logistics. After a crop has been produced, components of the integrated feedstock supply chain include the timing and management of harvest cycles, the selection of harvesting, collection, and in-field aggregation equipment and the efficient use of that equipment with respect to capital, maintenance, labor, fuel, and scheduling. Managing the labor, equipment and information in relation to harvesting is critical to improving the overall supply chain. Decisions made at harvest and immediately thereafter can have significant impacts on the downstream supply chain. After harvest, the biomass supply chain becomes an inventory management system. This system includes the management of equipment, capital and labor requirements associated with storage; timing and quality management in storage; equipment, capital, labor, and fuel associated with transportation; managing and scheduling pickup, as well as transport and delivery logistics. A well-managed inventory system will prevent many known problems for biomass-based facilities. All feedstocks are subject to physical material losses which inevitably leads to economic losses. Paying close attention to the key contributors of material loss as well as other inefficiencies in the supply chain will make sure that your inventory management does not negatively contribute to the bottom line. Inventory management is followed by feedstock preprocessing. Variable crops from the field must be converted into uniform format feedstocks. Preprocessing is driven by three primary feedstock characteristics: moisture, bulk density, and flowability. Moisture



management is critical to achieve maximum throughput of grinding equipment and to reduce energy consumption. Genera Energy and its partners have developed systems that allow for flexibility in moisture levels during processing. Low bulk density can negatively impact conveyance, processing and storage of biomass and many biomass feedstocks are low in density. Genera has developed tools and systems to overcome density as an issue in preprocessing. Both moisture and density can impact material flowability. Flowability issues include things as simple as reduced rates of flow, but could also include bridging, where biomass binds together and blocks conveyances, and “ratholing,” where storage bins empty in irregular patterns causing potential structural issues. Flowability is also impacted by feedstock type (plant structure), harvesting or grinding methodologies, and other external factors. Addressing these issues is a very critical component to successfully processing biomass. Genera Energy has developed efficient, economical systems to manage decision making as well as cost. This presentation will overview Genera’s lessons learned and best management practices related to biomass supply solutions.

Florian Huber, BASF

*High throughput experimentation for enhanced R&D on biobased chemicals and fuels*

Biobased feedstocks are becoming more and more important as source for renewable fuels and biochemical. Biotechnology in combination with thermochemical routes offer new pathways for selective catalytic transformation to new platform chemicals and further downstream processing to chemicals, polymers and fuels. The integration and intensification of

homogenous and heterogenous catalytic processes for conversion of biogenic feedstocks is crucial for successful commercialization. High throughput experimentation offers a path to accelerate the development of these new catalytic processes. hte GmbH, located in Heidelberg Germany, provides high throughput technology for development and optimization of catalytic processes for biochemicals and renewable fuels. The area of expertise comprises basic screening of catalysts as well as process and catalyst upscaling. This presentation will contain an overview of high throughput technology and case studies with industrial relevance for the biobased industry.

Yinhua Wan, Institute of Process Engineering, Chinese Academy of Sciences (CAS)

*Inhibitory effect of vanillin on cellulase activity in hydrolysis of cellulosic biomass*

Pretreatment of lignocellulosic material produces a wide variety of inhibitory compounds, which strongly inhibit the following enzymatic hydrolysis of cellulosic biomass. Vanillin is a kind of phenolics derived from degradation of lignin. The effect of vanillin on cellulase activity for the hydrolysis of cellulose was investigated in detail. The results clearly showed that vanillin can reversibly and non-competitively inhibit the cellulase activity at appropriate concentrations and the value of IC50 was estimated to be 30 g/L. The inhibition kinetics of cellulase by vanillin was studied using HCH-1 model and inhibition constants were determined. Moreover, investigation of the effect of each group on phenyl ring of vanillin on cellulase activity demonstrated that aldehyde group and phenolic hydroxyl groups of vanillin act inhibitory effect on cellulase. These results provide valuable and detailed



information for understanding the inhibition of lignin derived phenolic on cellulase.

Mariya Marinova, Polytechnique Montreal  
Renewable Chemicals from the Forest  
Biorefinery

The forest industry in Canada has been engaged for some time in a quest for the penetration of new markets and the development of new sources of revenues by manufacturing products derived from the forest biomass. One class of emerging products are those based on organic wood components, such as cellulose, hemicelluloses and lignin that can be extracted and converted into a broad variety of renewable chemical platforms and biobased materials. This approach is known as the forest biorefinery. The integration of biorefining technologies in pulp and paper (P&P) mills is a privileged option for the following reasons: (i) process streams containing valuable wood components can be easily diverted and used as a feedstock in the biorefinery; (ii) if optimised, the receptor P&P mill can supply most of the energy and material requirement of the biorefinery; (iii) the companies in the forest sector have well-established networks of customers and suppliers. Companies from the chemical sector have demonstrated an increasing interest in biobased products, seeking for drop-in or near to drop-in chemicals. It is expected that the use of those specific products will minimize the cost associated with the potential modification of the production facilities as well as the development and implementation time. The objective of this work is to select biomass-based products that can be generated in the forest biorefinery and used as drop-in products in the chemical industry. A structural approach, taking

into account technical and economic factors has been used. Particular attention was paid to products generated from hemicelluloses, a class of wood components that can be extracted prior to pulping wood chips and converted into a variety of intermediate or finished products. Two organic acids, and a biobased fuel have been selected based on market demand, complexity and constraints of the production process, properties, cost comparison with petroleum derivatives and ability to combine them with petroleum-based products. The process configurations required for the production of the biobased products have been evaluated and the potential interactions with the energy and water systems and the chemical requirements of the receptor P&P mill have been determined.

**Session 6: Production of Bioenergy from Waste**  
**Thursday, May 15 10:30am-12:00pm**

Moderator: Ashvini Shete, Praj Industries Limited

Lignocellulosic biomass to ethanol technology – Simultaneous saccharification and fermentation

Development of second generation ethanol technology is the need of the hour for India to save billions of foreign exchange spent on crude oil. Praj Industries is in pursuit of commercialization of its proprietary cellulosic ethanol technology. Globally, development of novel pretreatment to reduce natural recalcitrance of lignocellulosic feedstocks and recently developed efficient cellulase systems has eluded cellulosic ethanol technology commercialisation. One of the major bottlenecks in cellulosic ethanol technology is efficient deconstruction of cellulose with commercially viable dose of enzyme, especially with tricky feedstocks like bagasse.



Simultaneous saccharification and fermentation (SSF) assists in reducing the product inhibition of cellulases; thereby enhancing the cellulose hydrolysis efficiency with lower enzyme dose and in lesser time as compared to separate hydrolysis and fermentation (SHF).

Thermotolerant yeast strain of *Candida glabrata* is identified as the preferred strain for SSF of Praj pretreated feedstocks like cane bagasse and corn cob. Wild type *C. glabrata* strain isolated from hot water springs in India is capable of fermenting ethanol at 42°C. To develop efficient SSF process with cellulosic feedstocks, strain development as well as process development approaches are necessary. *C. glabrata* strain is modified in order to enhance its thermotolerance and fermentability in presence of ethanol and inhibitors present in lignocellulosic feedstocks. During SSF process, equilibrium between glucose release rate (driven by enzyme) and glucose consumption rate (by yeast) is necessary. With lignocellulosic feedstocks, glucose release rate of cellulases is extremely lower as compared to yeasts glucose consumption rate. As a result, yeast utilizes the only available carbon source that is ethanol and consequently, at a critical time point, ethanol formation rate and ethanol utilization rate becomes same and net ethanol concentration in the fermentation medium remains constant. To mitigate this problem, development of yeast strain which can't utilize ethanol is the way out. Use of mutant of *C. glabrata*, that can minimally utilize ethanol, for SSF lead to enhancement of fermentation efficiency by 25%. Development of the strain which can grow and ferment at 45-50°C (temperature optima of cellulases) is imperative to achieve higher cellulose conversion efficiencies. Thermotolerant strain

of *C. glabrata* has been developed which can ferment ethanol up to 45°C by evolutionary engineering and mutation. The developed mutants can efficiently ferment ethanol with Praj pretreated feedstocks like cane bagasse and corn cob in SSF mode. Role of surfactants in improvement of hydrolysis due to hydrophobic interaction with lignin and subsequent reduction in non-productive binding of cellulase enzymes has been confirmed. An array of surfactants, belonging to different classes, were evaluated against Praj pretreated cane bagasse. An amphoteric surfactant was identified that exhibited 25 to 30% increase in overall cellulose to ethanol conversion efficiency in SSF of Praj pretreated cane bagasse using *C. glabrata* mutant strain. Thus, simultaneous saccharification and fermentation using thermotolerant yeast strain has the potential to increase cellulose conversion efficiency with lower enzyme dosages with lignocellulosic feedstocks.

Chunzhao Liu, Institute of Process Engineering, Chinese Academy of Sciences (CAS)  
*Coproduction of hydrogen and methane via anaerobic fermentation of cornstalk*

Lignocellulosic wastes were considered as one of promising resources for the production of renewable energy. The microbial conversion of these lignocellulosic wastes into hydrogen and methane is attracting increasing interest because hydrogen and methane are excellent clean energy candidates for the future. However, this bioconversion efficiency is still limited because of low biodegradability of these lignocellulosic wastes which are mainly composed of cellulose, hemicellulose and lignin. Therefore, there is a need to develop an efficient technique to disrupt recalcitrant



structure of lignocellulosic wastes and improve hydrogen and methane production. A three-stage anaerobic fermentation process including H<sub>2</sub> fermentation I, H<sub>2</sub> fermentation II, methane fermentation was developed for the coproduction of hydrogen and methane from cornstalks. Hydrogen production from cornstalks using direct microbial conversion by *Clostridium thermocellum* was markedly enhanced in the two-stage thermophilic hydrogen fermentation process integrated with alkaline treatment. The highest total hydrogen yield from cornstalks in the two-stage fermentation process reached 74.4 mL/g-cornstalk. The hydrogen fermentation effluents and alkaline hydrolyzate were further used for methane fermentation by anaerobic granular sludge, and the total methane yield reached 205.8 mL/g-cornstalk. The total energy recovery in the three-stage anaerobic fermentation process integrated with alkaline hydrolysis reached 70.0%. The three-stage fermentation process was successfully scaled up in a 10 L continuous stirred tank reactor integrated with a 10 L up-flow anaerobic sludge bed, and it was proved to be a potential way for rapid and efficient hydrogen and methane production from lignocellulosic wastes.

Edris Madadian, McGill University

*The potential of bioenergy using gasification of poultry manure feedstock*

Chicken manure is one of the major environmental concerns generated at poultry farms. Nutrient content, waste management and odor control of the chicken manure make it necessary to develop new methods to manage it properly. Gasification is a thermochemical technology for handling biomass feedstock such as animal residues and agricultural wastes. In

this study, the potential of producing syngas from chicken manure feedstock using a down draft gasifier has been investigated. The manure was provided from a local farm in Quebec City, Canada (name farm here?). To meet our down draft gasifier specification, we mixed the manure with wood pellet with the ratio of 30% to 70%, respectively. The results of this research showed a great potential of producing syngas with a lower heating value of 1.5 KW/Kg from the chicken manure. The gasification process resulted in an average temperature and pressure of 850 °C and -1.2 KPa, respectively. This technology helps to dispose of the waste appropriately, but also produces a high quality synthetic gas which has the potential to be used for heating and electrical generation .

John Ingersoll, ECOCORP

*Bio-methane Fuel and Bio-Fertilizers Production from the Dry Thermophilic Anaerobic Co-digestion of Poultry Litter and Crop Residues*

The potential of using poultry litter in U.S. as a feedstock for the production of bio-methane fuel and bio-fertilizers is immense. More than 13 million tons of litter is generated annually from broiler and turkey growing operations. This litter (manure) is typically land-applied as a fertilizer for corn and other crops resulting in substantial run-off water pollution to several bodies of water, primarily in the Mid-Atlantic and South-East regions of the country, but also in the upper Mid-West (Minnesota, Wisconsin) and the West (California). ECOCORP is working on the development of a number of projects in Maryland and Minnesota to digest poultry litter anaerobically in order to produce renewable energy and bio-fertilizer and to demonstrate the environmental benefits of digested poultry litter. To this end, ECOCORP, in conjunction



with its European technology partners, has designed a standardized biogas facility unit comprised of a 1,350 m<sup>3</sup> plug-flow digester with integrated biogas storage operating in the thermophilic regime (55oC), under dry conditions (32% solids) and a 16 to 18 days retention time. This biogas facility unit co-digests 8,000 tpy of poultry litter and 3,000 tpy of agricultural crop residues such as wheat straw or corn stover, generating biogas that may either be used for power generation at a rate of 1.2 MWel or be upgraded to 100 million scf/yr of pipeline quality natural gas per year. The avoided emissions of greenhouse gases stand at 8,000 tpy of carbon dioxide equivalent. The digester effluent is post-processed via centrifuging and ultrafiltration into (a) a solid component stabilized via aeration and (b) a liquid component. The solid component is reduced to 350 tpy of phosphate mineral fertilizer via gasification with extraction of additional energy. The liquid component is processed into 3,500 tpy ammonium sulfate by reacting its ammonia content with sulfuric acid and 1,600 tpy of potassium concentrate via reverse osmosis and evaporation. All these bio-fertilizers are of commercial grade quality. The discharged water from the processing of the bio-fertilizers is reused in the anaerobic digestion process to dilute the incoming very dry feedstock materials. ECOCORP plans to study the benefits of the solid and liquid organic fertilizers vs. raw poultry litter and to carry out an educational and public relations campaign across the region. As many as 1,500 identical biogas facility unit. most likely concentrated in about 150 large bio-refineries, can be built across the country within a period of 10 years and up to 1 million acres of energy crops can be also developed to supplement crop residues.

This effort (a) generates renewable methane fuel, ideal for transportation purposes, sufficient to fuel 10 million passenger cars or 150,000 heavy duty vehicles (transit buses, trucks), (b) transforms poultry litter into improved commercial grade bio-fertilizers, sufficient to supply the fertilizer needs of 6 million acres of corn and another 6 million acres of a variety of other crops, (c) relieves groundwater, lakes, rivers and estuaries from a serious water pollution source, (d) reduces greenhouse gas emissions and (e) stimulates economic activity with green jobs in manufacturing, construction, engineering and farming. Keywords: anaerobic digestion, biorefinery, agricultural wastes, energy crops, bio-methane, bio-fertilizers, water run-off.

Serpil Gurna, Rutgers EcoComplex  
*A Decision Support Tool for Evaluating Sustainable Biomass-to-Energy Pathways*

The ever increasing demand for energy, forces us to consider alternatives to existing fossil fuel resources. It is important to recognize that low carbon energy sources, feedstocks and solutions are critically needed to achieve true sustainability and resilience in providing for today's energy needs, while conserving limited resources for future generations. In order to achieve efficient biomass-to-energy and bio-products pathways, industry needs access to tools and resources that can reduce risks such as: strategies and information for securing feedstocks; efficient conversion technologies that are tested and verified; and policies to stimulate market demand for low carbon biofuels and bio-products. The potential environmental benefits of low-carbon energy technologies must also be proven to ensure that the emerging pathways are not only



economically viable, but also environmentally sustainable in mitigating climate change. Displacing fossil fuels with low-carbon, sustainable, biomass feedstocks will not only reduce harmful emissions, but will also increase the potential for success of an emerging biotechnology industry. In order to provide the renewable energy sector in New Jersey with current and comprehensive information on biomass feedstocks, technology yields and efficiencies, and GHG reduction potential, the Rutgers University's New Jersey Agricultural Experiment Station (NJAES) recently released an updated "Assessment of Biomass Energy Potential in New Jersey 2.0". The project team developed a detailed database of New Jersey's sustainable biomass potential which contains data on over forty feedstocks and seven bioenergy technologies, a bioenergy calculator for estimating potential biopower or biofuel yields, and a comprehensive evaluation of bioenergy potential in the state. The study also provides estimated potential greenhouse gas emissions reductions based on several bioenergy technology/feedstock scenarios. The scenarios include waste biomass to advanced gaseous and liquid biofuel pathways and GHG reduction potentials. The presentation will include a summary of the findings of this study, as well as a demonstration of the unique bioenergy calculator.

## **Track 8: Technical Presentations**

### **Session 1: Process Optimization of Sugar and Lignin Production**

**Tuesday, May 13 8:30am-10:00am**

Moderator: *TBD*

Kevin Gray, BetaRenewables

### *PROESA™ TECHNOLOGY: Breakthrough technology for producing advanced biofuels and renewable chemicals from cellulosic biomass*

Since 2006 Biochemtex has invested approximately \$200 million in the development of the PROESA™ technology. The process is designed to provide low-cost, high quality second generation sugars readily convertible into bio-fuels and/or bio-chemicals. PROESA™ integrates an energy efficient, chemical-free biomass pretreatment operation and a novel viscosity reduction, enzymatic hydrolysis step. The unique configuration ensures limited formation of degradation products that could lower yield and inhibit (bio)catalyst performance. One of the features of PROESA™ is the opportunity to process a number of different biomass types ranging from energy crops, agricultural residues, woody biomass, and industrial by-products, without the necessity to change hardware. Biochemtex has engineered and constructed a 1 dry ton/day biomass processing pilot facility in Rivalta, Italy integrating all unit operations required to convert lignocellulosic biomass into fuels and/or chemicals. BetaRenewables, a joint venture between Biochemtex, TPG and Novozymes, was formed in 2011 to license the PROESA™ technology for the production of fuels and chemicals. BetaRenewables has partnerships with leading bioconversion companies, Genomatica, Codexis, and Gevo, to integrate PROESA™ with downstream processes to produce value-added chemicals. The PROESA™ technology is the basis for one of the world's first commercial scale cellulosic ethanol plants located in Crescentino, Italy. This plant is designed to produce approximately 20 MM gallons of ethanol from a combination of



agricultural residues and energy crops.

Construction of a second plant in Brazil is expected to be completed in Q1 2014 while similar scale plants in North Carolina and California are in the design phase. Lastly, M&G Chemicals recently announced plans to build a 1 MM ton/yr PROESA™ biorefinery producing both bioethanol and biobased chemicals in China. This plant will be almost four times the size of the facility in Crescentino.

Michael Lake, Liquid Lignin Company

Low-Cost Process for Recovering Lignin from Papermaking Black Liquor

The popular wife's tale is "You can make anything from lignin but you can't make money" is no longer true. The SLRP Process is a CAPEX and OPEX technology for recovering lignin from papermaking black liquor. Recovery of lignin is a win-win for the papermaker and the potential users of lignin. Many efficient papermakers are near or at their limit in their Recovery Boilers (RB), and removing up to 30% of the lignin allows a proportional increase in RB capacity and eventually paper production. The SLRP Process was developed with SBIR funding from DOE. About 70 RBs are at or near their limit in North America, and each could generate about 50,000 ton/yr of lignin. A demonstration SLRP Process will be built in the near future with 10 ton/day capacity for lignin and will be quickly scalable to 50 ton/day. Competitive lignin recovery processes will be discussed. Near-term applications for lignin will be presented, together with new emerging technologies for lignin, including a high-purity lignin that can be incorporated into polymers.

Fred Moesler, Renmatix

Lignin Development as a Co-Product of Sugar

Production

The growth strategies of today's global chemical and biobased materials producers—a rapidly expanding \$150B global market—hinge on their access to low-cost renewable raw materials like sugar and lignin. Sugar and lignin can replace petroleum-based or first-generation incumbents, providing an alternative to meet increased demand for everyday products ranging from paints and plastics to adhesives and even carbon fiber. Renmatix's Plantrose process uses supercritical water to deconstruct biomass in a matter of seconds resulting in affordable sugars and lignin streams. These streams form the basic building blocks required to produce cleaner chemicals and materials like acrylic acid, isobutanol, polypropylene and phenols. Traditional methods of producing cellulosic sugar that use accelerants like enzymes and acids have been in development over the past few decades. However, these methods incur high costs due to either enzyme consumption or costly acid recovery systems, and lengthy process cycles limit the ability of these technologies to scale. Lignin is an emerging co-product of Renmatix's Plantrose process. Having only been exposed to water, Renmatix's unique lignin product opens up a new set of end-market applications based on both product characteristics and economics. In this session, Renmatix CTO Fred Moesler will provide an overview of lignin development as a co-product of sugar production and will discuss market drivers for increased demand. The economic advantage of producing lignin as a co-product of sugar has opened bio-based markets to meet pent-up customer demand, and as a result, lignin development is shifting from R&D to piloting phases. There are several perspectives relevant to the growing demand



for lignin, including: academic experts like Dr. Martin Feng from the University of British Columbia, industry experts from FP Innovations, end-users from the chemical or forestry industries, like Georgia-Pacific and the established host of cellulosic sugar companies at various points on the value chain.

Stephen Roth, United Catalyst, LLC.

#### Enzymatic Hydrolysis Without Enzymes

#### ENZYMATIC HYDROLYSIS WITHOUT ENZYMES

Cellulose is by far the most abundant biological molecule on earth, and is composed entirely of unbranched,  $\beta$ -linked, glucose monomers.

Although cellulose has been used as an energy source by humans for tens of thousands of years, transforming cellulose to its glucose subunits on a large scale and in a commercially significant fashion remains a complex challenge—a bottleneck in the pursuit of a biobased economy. The critical obstacle is the cost of using biological cellulases, the enzymes that break down cellulose to its glucose subunits. These enzymes must be produced by recombinant methods in expensive bioreactors, are very pH-sensitive, and have short half-lives at even moderate temperatures. United Catalyst is developing CHIPs, Cellulose-Hydrolyzing Imprinted Polymers. CHIPs are enzyme mimics composed of inorganic matrices with covalently attached acid groups that are imprinted with molecular cellulose, thus creating a cellulosic surface texture. The inorganic matrices, after polymerizing, washing, drying, and milling, are entirely free of cellulose and are capable of hydrolyzing a variety of celluloses to glucose when incubated in hot, aqueous solutions. The novel molecular imprints allow for matrix-mediated catalyses that are time- and temperature-dependent.

CHIPs can rapidly and completely transform various cellulose subclasses to glucose. United Catalyst, LLC. is currently optimizing our CHIPs technology by evaluating different polymers, different imprinting molecules, and different reaction conditions. Although preliminary, the data suggest that our inorganic novel imprinted catalysts will far outperform traditional enzymes for converting cellulosic biomass to glucose at commercial scales.

#### **Session 2: Bioprocess Separation Technologies at Commercial Scale**

**Tuesday, May 13 10:30am-12:00pm**

Moderator: Daniel Bar, Ameridia, Division of Eurodia Industrie

#### Optimizing the Downstream Purification Process is Key to Achieve Commercial Success

In manufacturing such as food and beverage, industrial biochemicals, or biofuels, separations can be difficult, costly, and inefficient. The difference between a good and a great separation process can mean the difference between a successful demonstration project and an unsuccessful one. Separation technologies are many and, while the selection and purchase of such components might seem like a straight-forward process, the right components in the right arrangement could mean the difference between the technical and economic success of a new plant or its failure. A case study will be used to illustrate the impact that the development of the right separation process can mean to your organization. In addition, Ameridia, the US Division of Eurodia Industrie, will describe a new affinity-based separation technology that reduces CAPEX and OPEX, eliminates the need for additional treatment steps, and offers reduced contamination risks. Eurodia/Ameridia is



focused on the development of separation processes that are specifically developed for each purification challenge with twenty-five years of experience in industries such as dairy, wine, sugars & sweeteners, specialty chemicals, and bio-based chemicals. In 2013, Eurodia/Ameridia has established CHEMISTRIA, a new division dedicated to the purification challenges of the bio-based chemical industry. World leaders in the design of separation trains that include membrane technologies, chromatography, electro dialysis, and ion exchange, the Eurodia process development team uses proprietary modeling and design techniques to determine the optimal mix, design and delivery of these technologies to provide efficient, cost effective, and reliable separation processes.

Michael Melnick, Microvi Bitotech  
*MicroNiche Engineering: Combining Microbiology and Materials Science to Enhance the Production of Renewable Fuels and Chemicals*

Industrial fermentation has generally focused on exponentially growing cells, based on the assumption that growth and metabolic activity are inextricably linked. Problems with this general approach include, for example, the limitation on yield imposed by carbon being needed to make biomass, genetic drift or competition from adventitious microorganisms, and long fermentation times. Several approaches have been attempted to overcome some of these issues, which involve starting to uncouple growth from bioconversion activity. These include fermentation at very high cell density, or various immobilization approaches including attachment or encapsulation. These have their own drawbacks however, for

example loss of cell viability and mass transfer issues that can limit their application and productivity. Microvi has developed a novel technology to overcome the limitations of cell growth-dependent bioprocesses. Using Microvi's MicroNiche Engineering (MNE) platform technology, Microvi uses materials science to create customized microenvironments associated with superior microbial performance. The resulting polymeric biocatalyst composites contain the desired microorganisms at high density, and are optimized for a particular process. Biocatalysts have been developed for water treatment applications (wastewater, groundwater and water purification) as well as for the production of fuels and chemicals (e.g. ethanol and butanol). In the case of fuels and chemicals the benefits and value of these biocatalysts include enhanced yield due to biomass not being accumulated; enhanced titer due to enhanced tolerance of toxic end-products; enhanced productivity due to extremely high cell density; long lived biocatalyst activity after repeated reuse; reduced risk of genetic drift or competition from contaminants. Examples demonstrating the performance of Microvi's biocatalysts for the production of ethanol and butanol will be discussed.

Edi Eliezer, BioPrizM  
*Key success factors for bioprocess technology selection, scale-up & engineering of new facilities*

Bioprocesses developed for biobased chemicals & biofuels, require six major critical parameters to be considered for successful conceptual through detailed design phases of new facility engineering projects. The first critical parameter is the appropriate selection of the biocatalyst



(in engineering terms) or the culture type (in scientist terms, microbial cell type). The second one is the raw material or substrate selection as a crude or refined one as it impacts processing design and costs. The third one is the process type: batch, fed-batch, continuous types and variations based on integration of upstream and downstream steps. The fourth one is the product type: low or high purity (science), bioburden or contamination tolerance (engineering), low or high-value added (cost sensitivity). The fifth one is the scale of operations (design, engineering) or production capacities (logistics, partially or fully commercial). The sixth one is the economics of processes and products at given production scales (capital & operating costs). The author, a bioindustry expert of many years, including leadership at DuPont, Abbott and Fluor, addressed above subjects in many projects where he developed the most appropriate design solutions. The presentation will illustrate, with real cases, how a good understanding, integration and industrial experience in above parameters will result in the selection of optimal & economical solutions.

Elizabeth Schirmer, Sweetwater Energy  
CO<sub>2</sub> or Sugar to Vegetable Oil: Commercial Scale Production of Pure Vegetable Oil Through Plant Cell Cultures

The recent volatility of commodity prices and rapid pace of industry development is stimulating the biorefining and oleochemical industry to diversify its raw material feedstock, using an array of fats and oils. The ability to take various fats and oils is a key risk mitigation strategy; however, reengineering the way oils are made addresses the feedstock problem at a more fundamental and sustainable level. This

presentation will review Sweetwater Energy's deployment of a new CO<sub>2</sub> or sugar to pure vegetable oil technology, whereby a modular, CO<sub>2</sub> source-flexible, commercial scale oil production technology has been optimized for the biodiesel, renewable diesel and oleochemical industries. This talk will highlight: 1) The basic science behind CO<sub>2</sub> and sugars to pure vegetable oils, 2) using this technology as a bolt-on for corn ethanol plants and biochemical refineries and the resulting synergies, and 3) characterization of the vegetable oil to enable optimum downstream processing.

Douglas Machon, NAES Corporation  
Operations & Maintenance of Next Generation Technologies : Lessons from the Real-World

The next generation of biofuels plants using bio-based feedstock faces more than technology validation, feedstock procurement, and challenging capital markets. They face a dynamic commercial operations profile that requires far more expertise than most companies possess. The presentation will outline lessons from the real world of commercial operations (operations & maintenance) for plants comprised of biomass feedstock, and why developers either need to build a suitable infrastructure, or employ a strategic partner, to achieve the commercial production requirements to justify the project development costs. The Importance of Commercial Operations (O&M) • Over the life of a typical project, O&M costs will exceed CAPEX. • Approximately 3 out of 4 projects fail to achieve pro forma financial or production estimates. • Sub-standard operations and maintenance accounts for 3 of the top 5 reasons commercial plants fail. • Plant owners that focus on plant operations as part of their



corporate strategy average significantly higher availability than those that do not. O&M is an Investment, Not a Cost Most of us have heard the old English saying, “penny wise and pound foolish,” and probably have an image of someone who, by fussing over trivial amounts of money to such an extent, misses much more significant opportunities. The commercial operations of a greenfield biomass facility represents a great example. • Does it make more sense to focus on scrutinizing O&M costs to save tens of thousands of dollars, or does it make more sense to invest in an industry-leading operations practice to boost the top- and bottom-lines by hundreds of thousands or even millions? (examples) • How should you structure the scope of O&M? Should you have separate entities responsible for chipping/pelletizing vs. conversion technology/plant systems? (examples) • How important is the safety of plant employees? (examples) • How important is staying compliant with all permits, laws, and regulations? (examples) • How important is the proper implementation of contracts and agreements for feedstock, off-take, and technology? (examples) • How important is your ability to respond quickly and decisively when unforeseen challenges arise with staff, equipment, new regulations, etc. (examples) • How important is having access to bulk purchasing discounts for boiler maintenance, specialty chemicals, consumables, tools, uniforms, etc. (examples) Real World Lessons Following are just a few of the many tangible benefits potentially gained by employing an experienced independent, third-party operator:

- Exceed industry averages for measures such as availability and reliability (a 4% difference in availability can amount to \$2 million annually at

the top-line), • Incorporating the owner’s plant into a much larger portfolio of plants to take advantage of benchmarking, best practices, lessons, learned, and support for problem resolution, • Providing the owner with access to group pricing agreements for purchases of bulk and specialty goods and services, • Providing a career path, and offering and administering a compensation package that attracts top talent, • Leveraging fleet scale for purchases of insurance, workers’ compensation, and benefits which effectively prices them below market, • Deploying a robust home office support infrastructure which supports knowledge access and transfer, performance optimization, and compliance cost avoidance.

**Session 3: Innovation of Agriculture Based Materials from Biobased Building Block Chemicals for the Automotive, Construction and Building Markets**

**Tuesday, May 13 2:30pm-4:00pm**

Moderator: Murray McLaughlin, Bioindustrial Innovation Canada

Peter van Ballegooie, EcoSynthetix

Anne Waddell, BioAmber

Amir Faim, Queens University

*Innovation of Agriculture Based Materials from Bio-Based Building Block Chemicals for the Automotive, Construction and Building Markets*

Global demand for bio-based and sustainable chemistry based products in materials and engineering applications has been forecasted to exceed \$100 billion beyond 2020. Recent developments and new availability of bio-based and sustainable chemistry based building blocks have initiated a surge in development and new models of innovation to address this demand. With support from the Canadian government,



Bioindustrial Innovation Canada has created an open-collaboration model to introduce new products to market quicker while mitigating the financial risks associated with their development. Utilizing a cluster-based, private-public sector model for innovation, Bioindustrial Innovation Centre has launched an agriculture-based science cluster (AgSci Cluster) focused on the development of the agriculture biomass supply chain and the development of agriculture based bioproducts to address the emerging market demand. Examples of the new AgSci Cluster innovations include the development of polyurethanes from succinic acid in a private-public partnership between the Province of Alberta, BioAmber and Woodbridge Foam. BioAmber is a leader in the development of succinic acid and its derivatives. Woodbridge Foam is a top three automotive industry supplier of polyol based products. To address the construction industry, the AgSci Cluster is also supporting the development of new saccharide based binder systems with EcoSynthetix, a global leader in developing starch based alternatives for petroleum based latex products. To provide new engineering solutions to aging civil infrastructure and low cost building needs, the AgSci Cluster is also working with academia and composite research centers to introduce novel applications using agriculture based fibre and resins. This exciting panel will provide examples of successful private-public innovation that addresses a growing demand in the automotive, construction and building sectors.

#### **Session 4: Production of High Value Molecules from Organic Waste**

**Wednesday, May 14 2:30pm-4:00pm**

Moderator: Bill Baum, Genomatica

#### Cesar Granda, Earth Energy Renewables, LLC Production of Valuable Organic Acids from Anaerobic Digestion of Organic Wastes

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



polyols and olefins and drop-in biofuels such as gasoline, diesel and jet fuel. The high value of these acids and the efficiency of its technology allow EER to quickly achieve profitability at small scales, with low investment and risk. This strategy allows EER to proceed with a series of small, but profitable, stair-step increases in scale to decrease engineering risk as the company continues to move to larger facilities in the future.

Danny Haynes, AkzoNobel

*Converting Cellulosic Sludge in Pulp and Paper Mills to Bio-sugars*

Sludge from pulp and paper mills is one of the simplest feedstock for integration of bio-refining technologies. The opportunity to convert pulp and paper sludge, a waste stream costing a mill operation is a good first step into the biorefinery world. Cellulosic sludge has been evaluated at several sites and results are presented. Application of enzyme accelerator technology on differing biomass using enzymatic hydrolysis has shown areas of potential application in these evaluations. The amount of sludge produced favors a pulp and paper just producing bio-sugars or a small distillation plant (demonstration size compared to some of the larger proposed plants) can be shared across several mills located close together. The sludge needs to be characterized with respect to ash content, lignin content, percentage of fines and amount of available carbohydrates. The amount of ash can significantly impact cost (mechanically or chemically) of processing the sludge. An enzyme accelerant increases enzyme binding to the fiber and can result in about a 20% increase in glucose production for the same amount of enzymes. This improvement can be of value

when processing cellulosic sludge.

Timothy Cesarek, Enerkem Inc.

*Green Chemistry: a sustainable and competitive waste diversion solution and a commercial reality – The case of Enerkem and the City of Edmonton*

The United States generated 459 million metric tons of municipal solid waste (“MSW”) in 2011, of which approximately 63% was landfilled, and the remainder was recycled, composted or incinerated (source: Waste Business Journal, Waste Industry Overview, 2012). Approximately 140 million metric tons of this annual landfilled MSW would be suitable for conversion into advanced ethanol and chemicals using Enerkem’s green chemistry technology. Enerkem is considered the first company to have developed a technology capable of breaking down waste materials that are chemically and structurally heterogeneous and convert them into a pure, chemical-grade, stable and homogeneous syngas. This syngas is then converted into renewable fuels and chemicals which when produced using Enerkem’s technology can help reduce carbon dioxide emissions, aid in extended producer responsibility and meet the growing world demand for green chemicals. Enerkem is now commissioning one of the first commercial advanced biofuels facilities in North America as part of a partnership with the City of Edmonton (AB, Canada). This plant, which will begin operations in early 2014, will convert the City’s MSW into methanol and ethanol. The City is seeking to increase its waste diversion rate from 60% to 90%. Following an independent study of over 100 different technologies, Enerkem was chosen to help achieve this goal. Enerkem is also developing a full-scale facility in



Varenes (QC, Canada), where it will convert into methanol and ethanol non recyclable waste from the institutional, commercial and industrial sectors, as well as construction and demolition debris. A third full-scale facility is also planned in Pontotoc (MS, USA). In the past few years, Enerkem was named one of the World's 50 Most Innovative Companies by Fast Company, a GoingGreen Global 200 company by AlwaysOn, a Global Cleantech 100 company by Guardian News, and was part of the Biofuels Digest's rankings of the 50 Hottest Companies in Bioenergy and 30 Hottest Companies in Renewable Energy and Biobased Materials.

Lisa Dyson, Kiverdi, Inc.

#### Bio-based Chemicals from Waste Carbon

Kiverdi is an advanced sustainable oils and chemicals company developing bio-based replacements to oleochemicals and petrochemicals using proprietary bioprocessing technology. Kiverdi's vision is to build a more sustainable world by developing alternative bio-based products that reduce reliance of petroleum and lower CO<sub>2</sub> emissions. Methane is an inexpensive and plentiful feedstock with a high H:C ratio and is highly valued as an energy-rich fuel and raw material for chemicals. However, methods for converting methane to readily transported liquids and other useful chemicals, such as the Fischer-Tropsch (F-T) process, the Mobil Process, and Shell Middle Distillate Synthesis, that are currently available for commercialization are capital intensive or non-selective since. These are indirect methods that convert methane into syngas via production of syngas (H<sub>2</sub>, CO) by steam reforming, which requires high temperatures and pressures and is capital intensive (60% or more of the capital cost of F-T plants is

associated with the reforming of methane). Kiverdi's award winning Carbon Engineering Platform technology uses proprietary bacterial strains that convert low-cost, abundant methane such as biogas, stranded natural gas, landfill gas as well as industrial gases into high-value chemicals at a fraction of the cost of current approaches. Kiverdi's proprietary methanotrophs (methane eating bacteria) with improved efficiency of direct biological methane conversion directly converts methane into higher value hydrocarbons which are used in cosmetic products, biodegradable cleaning soaps and detergents, food flavoring, medicine, and surfactants.

#### **Session 5: Markets for Renewable Chemical Platforms Based on: C3, C4, C5, C6**

**Thursday, May 15 8:30am-10:00am**

Moderator: Joel Stone, Green Biologics Inc.

#### Renewable Butanol: A Foundation for C4 Derivatives

Production of n-butanol via fermentation offers a foundation for a wide range of renewable chemicals built off the C<sub>4</sub> platform. On a standalone basis, n-butanol is a 3.4 million tonne global market growing at 4.4 % per year, with growth in China at over 9% per year. On a volume basis, over 80% of the global n-butanol market is used to manufacture chemical derivatives such as butyl acrylate, as well as specialty solvents such as butyl acetate and butyl glycol ether. The remaining 20% is used in a range of specialty solvent applications in paints, coatings, adhesives, inks, cosmetics, household cleaners, and specialty applications in food ingredients and unique, high performance industrial applications such as heat transfer fluids, high temperature brake fluids, lubrication products, textile swelling

agents, defoamers, and drilling/ore flotation agents. N-Butanol is also used to produce plasticizers, amino resins and amines. More importantly, n-butanol can be catalytically converted to 1-butene, a high value additive in HDPE and LLDPE, polybutene and other high value applications. 1-butene can then be converted via oxidative dehydrogenation to butadiene, and 1-butene can be metathesized to bio-propylene. A co-product to renewable n-butanol is acetone, another unique chemical solvent that can be converted to isopropyl alcohol, which can be catalytically dehydrated to bio-propylene. With tight propylene supplies impacted by shale gas production and lighter cracker feedstocks, the C4 platform offers viable routes to bio-propylene from both n-butanol and acetone.

Josef Modl, Vogelbusch GmbH

*The Use of Crude Glycerol and Lignocellulosic Hydrolysates in the Production of 1,3 Propanediol for Bio-Plastics*

Introduction 1,3 propanediol has been identified as a key- substance chemical in production of bio-plastics. The current industrial biotechnological process is based on glucose as the primary feedstock, thus weakening the commercial competitiveness. A process to convert crude glycerol, directly derived from biodiesel production, to 1,3 propanediol was the primary goal of invention. Methods and Materials A non- pathogen bacterium, *Lactobacillus diolivorans*, was identified as a favorable organism for industrial 1,3-propanediol production. Initial conversion and productivity rates of the wild strain and efficiency in the conversion of crude glycerol were further increased by genetic engineering and optimization of the fermentation protocol.

The application of lignocellulosic hydrolysates as the sole carbohydrate source for biomass formation was evaluated. Results Evaluating crude glycerol samples from various biodiesel productions, 85 g l<sup>-1</sup> of 1,3-propanediol were obtained with a productivity of 0.45 g l<sup>-1</sup> h<sup>-1</sup> in feed-batch cultivation. No inhibitory effects of crude glycerol on growth of the microorganism or production rate could be observed. The use of lignocellulosic hydrolysates as sole carbon source, containing the potential inhibitors furfural and 5-hydroxymethylfurfural at concentrations of 0.7 g l<sup>-1</sup> and 0.3 g l<sup>-1</sup>, did not show a significant decrease of conversion rates and result to 75 g l<sup>-1</sup> 1,3-propanediol and a productivity of 0.36 g l<sup>-1</sup> h<sup>-1</sup> . Conclusions A non- pathogen, genetically modified *Lactobacillus diolivorans* bio-transforms effectively and stable crude glycerol to 1,3 propanediol and lignocellulosic hydrolysates as the sole carbohydrate source for biomass grow as a commercially viable alternative to current production protocols.

Mandy Lin, Evernu Technology, LLC

*A Green & Clean Route for the Production of Methacrolein and Methacrylic Acid from Bio-isobutanol - a 2nd Generation Biofuel*

Human activities in the modern society depend heavily on fossil resources for energy and chemical production, and have thus caused global depletion of crude oil and other fossil resources. The rapid consumption of fossil resources subsequently leads to release and accumulation of greenhouse gases, which has caused global warming and induced ecological imbalance and extraordinary nature disasters in recent years. Fossil fuels are the predominant sources of energy to power the transportation and sources for chemical feedstock. The US



chemical industry is a \$460 billion enterprise, a key element of US economy and about 26% of the global chemical production. Approximately 10% of the U.S. crude oil imports are used to feed the chemical industry. Such dependence exposes US to critical disruptions in crude oil supply and create economic and social uncertainties to the country, businesses and individuals. There is urgency for the US and the rest of the world to explore renewable resources for energy and chemical production to sustain the human activities while protecting the earth. While the need for sustainable energy can be met by utilizing various types of renewable resources, such as solar, wind and geothermal, biomass is the only renewable resource that produces carbon-based fuels and chemicals. The United States has over a billion tons of sustainable biomass resources that can provide fuel, make chemicals and produce power. However, recent data indicate that only about 4% of U.S. chemical sales are attributable from renewable biomass. Transformation from fossil to biomass based chemical production requires the development of enabling new technologies. Methacrylic acid (MAA) and its ester derivative methyl methacrylate (MMA) are monomers used in the production of a wide variety of polymer products for surface coating, construction, appliances and automotive industries. MAA and MMA are among the core components of the US chemical industry. At the present time, the size of MAA/MMA market is about \$2.1 and 8.1 billion in the US and worldwide respectively. Worldwide and in the US, MAA and MMA are predominately produced from highly toxic hydrogen cyanide (HCN) and acetone via an 85-yr old ACH process, which further uses large quantity of concentrated sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) as the

solvent, and generates large quantity of toxic and environmental harmful wastes. Funded by the US Department of Energy under its SBIR Program, EverNu has investigated a clean and green route for the production of methacrolein and MAA from bio-isobutanol, a 2nd generation biofuel in commercial production in the United States since 2012.

Brian Yeh, ZeaChem

*Cost Effective production of C5 and C6 chemicals from Biomass using the ZeaChem process*

The market for 2 carbon (C<sub>2</sub>) and 3 carbon (C<sub>3</sub>) molecules such as acetic acid and propionic acid are worth \$485 and \$595 billion respectively. The key to penetrating these markets is to offer a fungible product that can be produced at costs below those made with petroleum feedstock yet offer the attribute of being renewable. ZeaChem has developed a cellulosic biochemical process that has a 40-50% higher yield than other processes, yet leverages mature technology to enable commercialization with favorable economics. In February, 2013, ZeaChem produced cellulosic ethanol from its demonstration facility in Boardman, Oregon. The same process also produced cellulosic acetic acid as well as cellulosic ethyl acetate.

Julia Allen, Lux Research

*Cultivating Capacity for Bio-Based Materials and Chemicals to 2017—Which sectors grew, where are we going, and what worked?*

Investment and growth in bio-based materials and chemicals continues to increase. The leading technologies are aligning the financing, corporate relationships, and research muscle necessary to succeed in an era of cheap shale, rising feedstock costs, and a push towards non-food biomass inputs. We examined 199



companies' bio-based material and chemical production facilities across the globe (259 sites in total), that were planned, operating, or shuttered between 2005 and 2017 to determine the current state of the market, planned growth, megatrends, and what products are winning the hearts and minds of investors and end-users. We highlighted the growth of key chemicals, such as butanol, succinic acid, and acrylic acid; identified feedstock trends; and determined the fastest growing geographies. The presentation will discuss an assessment of industry growth trends and commercialization successes, with lessons to be learned for rolling out applications with other bio-based products.

**Session 6: Natural Gas to Fuels and Chemicals**  
**Thursday, May 15 10:30am-12:00pm**

Moderator: *TBD*

Michael Schuppenhauer, Farmatic Inc.  
*Beyond Waste: Full Account of US Biogas Potential*

Biogas is per current RFS2 definition and advanced and even cellulosic biofuel for transportation purposes. Historically, however, biogas is in the United States only understood to be coming from organic waste, such as food waste, manure and landfills. This is in contrast to other leading nations that have developed significant agro-energy economies based upon the conversion of crop residues and novel energy crops into biogas, at a much higher yield (3x - 5x) per acre than with liquid fuels (ethanol or diesel). In fact, waste streams account for less than 5% of biogas in Germany, whereas 95% of biogas is made from purpose grown energy crops, crop residues and manure to meet critical fuel needs. In this study we performed a complete bottom-up analysis of

the US biogas potential and present previously unavailable data, including crop residue and energy crop potential for biogas, beyond manure, ag-food processing and residential organic waste streams. The research also details the geographic distribution of biogas sources on a county basis and includes chemical and unused on-shore oil and gas exploration potentials, all of which can be used for biochemicals and distributed energy production. In fact, the aggregated potential of renewable domestic biogas will meet the entire future transportation vehicle fuel demand as supply increases and demand of vehicle fuel gradually sinks. We will present the domestic US biogas potential for all sources, including crop residues and energy crops, the economic impact and investment opportunities as well as return to the national economy in terms of jobs and GHG savings. As those resources are using proven technology and are cost effective, biogas is key to implement the RFS.

Joshua Silverman, Calysta Energy  
*BioGTL platform for the conversion of natural gas to fuels and chemicals.*

Important progress has recently been made toward engineering a number of phototrophic and fermentative microorganisms for biofuels production. Several limitations, most notably the ever-increasing cost and linkage to oil prices of sugar feedstocks, currently prevent the economical production of biofuels from microbial systems. Exploiting methane, an inexpensive, domestically abundant carbon feedstock, represents an attractive strategy towards economically sustainable biofuel production. Calysta Energy™ has developed a genetic engineering platform for host organisms (methanotrophs) capable of metabolizing



methane to a variety of biofuels and biochemicals. The genetic tools, together with innovative fermentation and bioprocess approaches, enable the rapid implementation of well-characterized pathways to utilize natural gas as a biological feedstock instead of sugar.

Richard Troyer, Coskata, Inc.

*The compelling economics of biological conversion of natural gas to fuels and chemicals*

The outlook for natural gas and crude oil prices suggests a sustained opportunity to upgrade a plentiful domestic natural resource into products tied to crude oil. Coskata's biological process, that converts natural gas to fuels and chemicals, will offer the opportunity to diversify away from high cost crude oil. Based on five years of demonstrating our technology, we believe our process offers compelling economics. For example, the production of ethanol from natural gas using Coskata's technology, can achieve significantly lower unsubsidized production costs than any other process in the industry, offering attractive economies for consumers and creating new market opportunities for ethanol. On the route to American energy independence, we now have solutions with the potential to leverage the benefits of both natural gas and ethanol as domestic fuel sources.

Eli Groban, Intrexon Corporation

*Natural gas bioconversion to chemicals, lubricants, and fuels*

The objective of Intrexon's natural gas upgrading program is to develop a microbial cell line for industrial-scale bioconversion of natural gas to chemicals, lubricants and fuels, as opposed to employing standard chemical routes. Intrexon's unique cellular engineering

capabilities will enable the genetic manipulation of a microbe to convert natural gas to higher carbon content compounds at ambient temperatures and pressures, thereby reducing capex and opex requirements compared to standard gas to liquid (GTL) processes. At present, Intrexon's engineered microbial host converts methane into isobutanol in a laboratory scale bioreactor. Natural gas is currently one of the most economical forms of carbon as it is both highly abundant and is the least expensive form of energy other than coal. Moreover, unlike sugar, natural gas is a highly reduced source of carbon, allowing conversion of the entire feedstock to highly reduced products. Methanotrophs have the innate ability to "upgrade" natural gas, by oxidizing methane as the sole carbon source to support cellular metabolism and growth. Unlike many industrial hosts, methanotrophs are challenging to genetically engineer as the requisite tools are generally not available and detailed microbial regulatory and physiological information are lacking. Intrexon's synthetic biology team has developed an advanced suite of tools that enables rapid manipulation of methanotrophic organisms including gene knock in/out, direct transformation/electroporation, and plasmid-based expression systems. Using these tools, we have generated methanotroph strains that are capable of upgrading natural gas to fuels and chemicals. Fermentation of wild type methanotrophs has been performed at commercial scale for conversion of natural gas into cell mass. Intrexon's engineered methanotroph that produces isobutanol, however, presents a unique series of challenges for commercial scale up. Expression of a heterologous pathway for product generation places additional metabolic burdens on the cell,



as carbon is diverted from primary to exogenous metabolic pathways for heterologous product production. We have developed a fermentation bioreactor protocol that supports growth of an isobutanol producing engineered methanotroph at industrially relevant cell densities. The current focus is to scale to a pilot facility. In addition, we are developing a high-throughput platform to systematically test and iteratively optimize media composition and strain performance while also facilitating rapid design and testing of new pathways for improved isobutanol production from engineered strains.