



## Feedstock Production and Utilization

Monday, December 9, 2013 |  
8:30am – 10:00am

### Opportunities for Alternative Feedstock Crops

*US Department of Energy and related Investments in Camelina - Creating a new industrial oilseed platform for high value renewable feedstock production*

Moderator: Jack Grushcow, Linnaeus Plant Sciences Inc.

Jonathan Burbaum, U.S. Department of Energy

*A sustainable biorefinery of Agave atrovirens in Central Mexico. From traditional pulque, to novel bioproducts.*

SergioTrejo-Estrada, Instituto Politécnico Nacional

*Evaluation of second generation biofuels production from native halophytes by chemical-characterization of Salicornia sinus-persica*

Ayah Alassali, Masdar Institute of Science and Technology

*Guayule, An Established Industrial Feedstock For Biorubber and Biorefineries*

Jeff Martin, Yulex

### Abstracts

Jack Grushcow

Jonathan Burbaum

With steadily increasing demands for renewable, scalable, non-food sources of high quality feedstocks many Corporations, Researcher and Government groups believe that Camelina can be THE industrial oilseed platform – delivering a truly game changing opportunity. The US DOE has invested over \$30 million in camelina research projects, in Europe the Framework 7 program has invested 10 million euro in developing a camelina supply chain and in Canada several initiatives over the last five years have delivered over \$15 million in Camelina crop development and research. Camelina is a drought tolerant, non-food oilseed crop that has the potential to deliver increased revenues to the farmer while at the same time reducing global CO2 emissions. Camelina can be grown on a larger area than canola since it has 10 days shorting growing requirement, uses 30% less nitrogen and half the water. It is seeded and harvested with the same equipment that growers are accustomed to use in canola production. Because of this, the crop has the potential to be produced on millions of acres in Canada and the northern US states. It can deliver renewable, bio-degradable feedstocks that can substitute for petroleum in a variety of applications including bio-lubricants, hydraulic fluids, greases, rigid foams and polymers; each having value well beyond bio-fuel. The panel will review the latest applied research in this rapidly evolving field.

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Jonathan Burbaum, Program Director of ARPA-E for advanced biotechnology applications for biofuels and the production of biologically-based chemical feedstocks is responsible for roughly \$30 million in camelina projects. He will discuss the ARPA-E program goals and offer an overview of key camelina projects.

### Sergio Trejo-Estrada

Agave atrovirens or maguey pulquero is cultivated in the highlands of central Mexico. Since prehispanic times, its rich sap, aguamiel, has been transformed into pulque, a mixed lactic and yeast fermented beverage of proven functionality. After decades of abandonment, a new initiative of rural producers, government, academic institutions and agribusiness entrepreneurs, is building a pilot scale plant for the sustainable transformation of Agave juices and biomass. The dry cool season provides for highly concentrated sap, useful for its transformation in lactic probiotic beverages and mild fruity wines, through fermentation. During the dry-warm and the rainy seasons, the complete biorefining of agave biomass is achieved. Bioproducts such as fructose sweetening syrup; prebiotic fructooligosaccharides and inulin, are obtained from the agave juices, whereas the agave lignocellulose is transformed into bioethanol and fertilizer vinasses. Due to the ability of Agave atrovirens to grow in eroded marginal lands, under harsh climate conditions, its cultivation is enforced for soil and agro-ecological restoration. Several bioproducts are

now ready for commercial development. Lignocellulose transformation in bioethanol is limited by the enzyme conversion cost, and by the unusual concentration of fermentation inhibitors from hydrolysis and saccharification of agave biomass by classical thermochemical methods. The value added and the unique characteristics of the new bioproducts, make it economically feasible to scale up a balanced process of Agave biorefinery, which is contrasting to other agave-based processing facilities of tequila and mezcal. A projection of agro-ecological restoration of marginal lands, through sustainable bioprocessing of Agave atrovirens is presented. Further biotechnological and benchmarking efforts are needed for the achievement of the full potential of this new biorefining integrated technology.

### Ayah Alassali, co-author Thomsen, M. H.

Introduction: Abu Dhabi exemplifies a coastal desert, where seawater could be used for salt-tolerant crops (halophytes) cultivation. The produced halophyte biomass could be utilized in feed, food and/or energy production, depending on its chemical composition. In this study the UAE native halophyte *Salicornia sinus-persica* was studied for its potential to be used as a feedstock for bioethanol production. Fresh *Salicornia sinus-persica* contains more than 65% of water. For such green biomass direct fractionation and fermentation can be advantageous. This allows for water preservation and the ability to run at

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lower dry matter in the fermentation step. Chemical characterization and ethanol potential of the juice and fibers of the fractionated *Salicornia sinus-persica* was examined in this study.

**Methodology:** Two batches of *Salicornia sinus-persica* (washed and unwashed) were juiced, where two main fractions were obtained (juice and fibers). Washing of the fresh biomass aims to reduce or remove the nonstructural ash (salt deposits). Both fractions were tested for their total dry matter and ash content and sugar monomer composition (applying acid hydrolysis) as described in (Sluiter et al., 2008a). The extent of glucan-to-glucose convertibility was tested for the fiber fraction, where it was subjected to Simultaneous Saccharification and Fermentation using 15 FPU/g DM of cellulases (Novozymes), with a pre-hydrolysis time of 2 hours at 50 °C with intensive shaking (120 rpm) prior to addition of *S. cerevisiae* at 32°C for 7 days. Final ethanol yields, sugars and other metabolites were analyzed by High Performance Liquid Chromatography as described in (Sluiter et al., 2008b).

**Results:** The juice fractions were found to represent 67.78% of the unwashed batch and 74.09% of the washed batch. The wet fiber fraction of the fresh-unwashed salicornia biomass was found to contain 38.88% DM, of which 19.18% is ash. Dry matter content of the juices were found to be 13.53% (unwashed) and

11.58% (washed) of which 61.12% (unwashed) and 57.37% (washed) was ash. Sugar analysis revealed relatively low concentration of glucose, xylose, and arabinose in the juice fractions (7.15 g/L glu, 4.62 g/L xyl, and 2.488 g/L ara in juice of unwashed biomass and 6.62 g/L glu 3.87 g/L xyl, and 1.86 g/L ara in juice of washed biomass) and not much difference was observed between the washed and unwashed biomass. The fiber fractions contained 9.77 g/100 g DM glu, 6.97 g/100 g DM xyl, and 8.44 g/100 g DM ara for the unwashed biomass and 10.25 g/100 g DM glu 7.79 g/100 g DM xyl, and 8.24 g/100 g DM ara for the washed biomass. This is comparable to the lignocellulose content of the mature (dry) plant (Cybulska et al., 2013). We tested *S. cerevisiae* on pulp fraction from wet separation of *Salicornia sinus-persica* pretreated at low severity (121°C for 30 and 60 minutes). Both pretreatment conditions showed ethanol yields between 70-80%, and no significant inhibitory effect was observed in pretreated hydrolysates, probably due to the low pre-treatment severity. These results show that the green pulp of *Salicornia sinus-persica* can be pretreated at low severity and the glucan fraction fermented to ethanol by *S. cerevisiae*.

#### Jeff Martin

Guayule is a desert shrub native to the southwestern US and northern Mexico that now serves as one of the best feedstock options available for biorefineries after more than 15 years

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of crop science, plant breeding, and improvements in cultivation as well as agronomic practices by Yulex. Some of the key milestones achieved to date with regards to Yulex agricultural program with guayule include 1) consistency in phylogenetic traits optimized for rubber production with yields to achieve profitability, 2) establishment of the most advanced crop breeding program in more than 100 years, 3) crop establishment via direct seeding with high germination rates to replace previous costly approach of transplanting seedlings grown in greenhouses, and 4) second generation harvesting technology that dramatically increases yields per acre and hauling efficiency to achieve maximum highway weight limits per truckload.

While Yulex built its business around guayule for rubber production, this feedstock offers the greatest potential for biorefineries to offer a wide array of products that come from mutually complimentary processing technologies. In addition to its core guayule rubber processing technology platform, Yulex has been developing a wide array of supporting and complimentary guayule processing technologies that yield resins, waxes, oils, cork, energy (electricity and biofuels), green building materials, and specialty chemicals.

**Monday, December 9, 2013 |  
10:30am – 12:00pm**

**Advances in Reeds and Grasses**

*Customized by Chromatin, Renewable by Nature*

Moderator: John Fulcher, Chromatin Inc.

*Giant reed (Arundo donax L.) as a sustainable energy crop for 2nd generation ethanol in relation to its refinery "wastes"*

Enrica Bargiacchi, Consortium INSTM

*Development of an integrated system from cultivation of the cellulosic energy crop Napier grass to production of ethanol for biofuel*  
Masahiro Samejima, The University of Tokyo

*Turning the Technology Flywheel: Moving Up the Yield Curve*  
Steve Bobzin, Ceres, Inc.

### **Abstracts**

John Fulcher

Chromatin is a vertically integrated sorghum feedstock provider with experience in biotechnology, seed, and feedstock growth, harvest, and delivery. Sorghum has seen resurgence in the US as a feedstock for advanced bioethanol with the added benefit of using the sorghum stover and/or high biomass sorghum for the production of biogas. Sweet sorghum is an attractive rotational crop with sugar cane in Brazil. In addition, high biomass sorghum has great potential for cellulosic in Brazil and the US. The growing need for global non-food feedstocks over the next 20 years will be met by a

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combination of three primary sources: energy crops; agricultural residues; and woody biomass. By 2030 it is estimated that 70 million acres of agriculture will be needed to supply the demand for non-food biomass. By way of comparison: there are 50 million acres of sugarcane grown globally; 20 million acres of canola in Canada; and 70 million acres of rice in China today. By 2020 the projected demand for biomass feedstock will be approximately 600 million tons going to cellulosic ethanol, US heat and power, EU power, chemicals and animal feed. Today's markets for purpose-grown biomass for cellulosic and the U.S. power industry are in the \$50 - \$70 per ton range while the EU power market is \$150 - \$200 per ton. Global markets for purpose-grown biomass are emerging. In order to meet that demand, rapid increases in feedstock production will require: proven genetics; established cropping systems (agronomics); yield quality (cellulose, sugar, starch, etc.); global cultivation; existing infrastructure; and economic viability (profit). One of the key economic drivers for the selection of biomass feedstocks will be water. Agriculture accounts for greater than 90% of fresh water consumption. Sorghum is well suited as a purpose-grown biomass given its water use is 85% less than sugarcane and 50% less than corn. Sorghum is also sustainable and scalable. Sorghum can also be grown on marginal land with high yields and has a reduced environmental impact due to fewer chemical inputs (for example herbicide and pesticide use is 40-80% less than

corn). Sorghum has a four-month growing season and in Brazil, sweet sorghum is being co-cultivated with sugarcane to extend sugar harvests. Customers are looking for sustainable customized feedstock solutions to meet their needs in power, transportation fuel, and chemical markets. Biomass production must be economically produced, harvested, stored, and transported. A combination of purpose-grown energy crops along with crop residues may be required to provide adequate biomass supplies year round while minimizing transportation and storage costs. Through strategic alliances Chromatin can harvest, store, and transport a wide variety of biomass to provide customers with the right feedstock solution at the right time.

Enrica Bargiacchi, co-authors Sergio Miele & Antonio Pompeiano

Giant reed (Adx) is the leading candidate among potential ligno-cellulosic feedstocks for 2nd generation ethanol, under warm temperate climates, for its high yield of ethanol-per-hectare and low ecological demands. This perennial C3 grass is diffuse in natural landscapes in Southern Europe and the Mediterranean areas, pioneering a wide range of soils, including saline soils and metal contaminated soils. It can produce 20-30 t ha<sup>-1</sup> DM in Southern Europe, usually with no irrigation and Nitrogen fertilization, due to its high photosynthetic rates and little photoinhibition. It is able to reach early after establishment, and consistently maintain for +10 years,

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high yields with favorable logistics (extended harvest period, and good bulk density). Undesired escaped plants are easily controlled by glyphosate. The research activity in collaboration with Chemtex and Chemtex Agro, has aimed at investigating: i) the plant ability to sustain environmental and soil stress, to better exploit marginal soils, and avoid competition with food crops; ii) the possibility of recycling Adx "wastes" from ethanol refinery to return to the soil most of the nutrients removed by the plant. In comparison with other potential feedstock sources, such as wheat straw, potassium concentration in Adx lignin and ashes from ethanol refinery makes these materials very interesting for soil fertilization and correction, with increased sustainability of this crop.

Masahiro Samejima, co-authors  
Shigenori Morita, Shyu-ichi Mihashi &  
Ei-taro Morita

To develop an integrated system from cultivation of cellulosic energy crop to production of ethanol in Japan and Southeast Asian countries, Research Association of Innovative Bioethanol Technology (RAIB) has been established in cooperation with the University of Tokyo since 2009. The member companies of this association are JX Nippon Oil & Energy Corp., Mitsubishi Heavy Industries, Ltd., Toyota Motor Corp., Kajima Corp., Sapporo Engineering Ltd., and Toray Industries, Inc. The project has now been advancing to an integrated bench-scale test.

In this presentation, recent achievements on the project will be summarized. Particularly, to reduce the production cost of ethanol from cellulosic biomass, year-round operation of the factory by continuous supply of the feedstock is a key point. For this purpose, the multi-cultivating and harvesting system of Napier grass with a low input has been elaborated in Indonesia as a model site of cultivation.

In pretreatment of our project, two-step ammonia treatment system has been developed to attain high efficiency of enzymatic saccharification for both xylan and cellulose. As well known, ammonia treatment can cleave not only the ester linkages on xylan and lignin, but also convert the crystalline structure of cellulose I to cellulose III. After that, optimal selection and combination of enzymes for saccharification of the pretreated feedstock has been done. In addition, recycle use of enzymes has been considered to reduce the total enzyme cost. To produce ethanol from xylose, a new fermentation process has been developed by utilizing a novel yeast strain selected from nature and then improved by non-GM mutation. Finally, by integration of all these technologies, the research association aims to establish the total system to produce ethanol from cellulosic energy crop with low production cost.

Steve Bobzin, Ceres, Inc.

This presentation will examine how agricultural technologies are improving the economics and

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availability of feedstock, and their implications for scaling up biofuel production in Brazil, the United States and elsewhere. Ceres is an integrated seed company that combines advanced plant breeding and biotechnology to develop products that can address the limitations of first-generation bioenergy feedstocks, increase biomass productivity, reduce crop inputs and improve cultivation on marginal land. Its development activities include sweet sorghum, high-biomass sorghum, switchgrass and miscanthus. The company's integrated technology platforms include elite, proprietary breeding materials (germplasm), genetic mapping, biotechnology (traits) and agronomy, among others. Its largest immediate opportunity is in Brazil, where the company is currently working with ethanol mills to introduce its sweet sorghum hybrids as a low-cost, season-extending complement to sugarcane.

**Monday, December 9, 2013 |  
2:30pm – 4:00pm**

### **Gases as Feedstock: The New Renewable?**

*High Acreage Energy Yield of Novel Energy Crops Offers Grid-Competitive Novel Advanced Biofuels*

Moderator: Michael Schuppenhauer, Farmatic Inc.

*Feasibility Analysis of Gas and Waste Derived Fuels*

Julia Allen, Lux Research Inc.

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

*WRI'S CAT™ Technology for Carbon Capture and Re-Use*  
Karen Wawrousek, Western Research Institute

### **Abstracts**

Michael Schuppenhauer

Current progress towards meeting renewable advanced and cellulosic biofuel goals are falling short of expectations due to technical and financial hurdles. However, those efforts have focused on a narrow set of lignin containing feedstocks and the quest for liquid biofuels. Reviewing domestic and international large-scale yield data from alternative, novel feedstocks, using a biochemical pathway (anaerobic digestion) to gaseous advanced biofuels would on a broad base offer a vast novel pool of feedstocks and highly cost-competitive production processes with 3-5 times the yield per acres than pursuing liquid biofuel routes, while being GHG negative. In fact the electricity cost would be at 3-15 c/kWh and fuel cost at \$0.40 to \$1.70 per GGE, while offering an IRR of >20% and payback of less than 4 years. We are presenting several cases with economic feasibility how anaerobic digestion of non-food crops such as corn straw, sugar beets, various cellulosic tropical grasses, press cakes and organic wastes can replace liquid fuels in energy and transportation fuel applications at

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much lower cost than current fossil fuels. These applications are particularly suitable for insular and rural environments across the Pacific Rim.

### Julia Allen

First-generation biofuels dominate the alternative fuels market today, but issues like indirect land use exchange and food vs. fuel drive demand for new feedstocks. The glut of cheap natural gas from shale as well as trends toward the use of waste biomass feedstock open up opportunities for new technologies that will alter the alternative fuels landscape. Overlapping technologies between gas-to-liquid (GTL) and biomass-to-liquid (BTL) allow developers some degree of freedom to choose the most attractive feedstock. BTL is inherently more capital-intensive than GTL due to the solids handling step. The future of BTL hinges on advancements in gasification and pyrolysis technology. While BTL requires higher upfront capital, advantages include low cost of certain feedstocks such as MSW as well as the potential for long-term feedstock agreements. At current oil prices, most thermochemical GTL and BTL technologies will fail; we analyze the cost of 21 conversion pathways to show that gas and waste biomass processes can produce liquid fuels at \$80 and \$75 per barrel, respectively. A further analysis on return on investment reveals the cost competitive pathways, as well as the impractical.

### Sol Resnick

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.

### Karen Wawrousek

WRI's patent-pending CAT™ process is a novel biological carbon capture and re-use technology for the recycle of carbon dioxide (CO<sub>2</sub>) from stationary emitters that is compatible for use with both small and large industrial CO<sub>2</sub> sources. Currently R&D efforts have concentrated on the production of biodiesel using the CAT™ process, but CAT™ could also be used to produce other fuels and products. Economical operation of the CAT™ process is achieved through unique biochemical systems utilized in the

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process, systems for biomass and water recycle, and a system for biomass residue conversion to nutrients. The microorganisms used in WRI's CAT™ process show a rapid growth rate, indicating efficient conversion of CO<sub>2</sub> into biomass. However, unlike many biologically-based carbon capture and re-use systems, WRI's CAT™ process is not dependent on light, which affords this process multiple advantages. Not only can the CAT™ technology be directly integrated to an existing stationary CO<sub>2</sub> source, it is able to operate 24 hours a day, year-round. Deployment of this technology with large, cylindrical reaction vessels reduces the necessary land area to less than 2% of that required for equivalent biodiesel production from open pond algae processes. Additionally, this closed system uses significantly less water than that required for algal open ponds algae or renewable crops, since evaporation is not an issue. Economic analysis of the process predicts that the CAT™ process-produced biodiesel will be economically competitive with petroleum-based diesel, and a preliminary limited life cycle analysis of the CAT™ process estimates that CO<sub>2</sub> emissions from industrial sources can be reduced by greater than 80%.

**Tuesday, December 10, 2013 |  
2:00pm – 3:30pm**

### **Sugars: Exploring New Sources**

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

Moderator: Kef Kasdin, Proterro

*Low-Cost Sugar from Energy Beets: A largely Unexplored Opportunity*  
Steve Libsack, Betaseed

*American Process Cellulosic Sugar Technologies "Sugar is the New Crude® in Asia"*  
Theodora Retsina, American Process, Inc.

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

### **Abstracts**

#### Kef Kasdin

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

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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.

Steve Libsack, co-author David Bransby

In North America, about 1.2 million acres of sugarbeets are currently grown for sugar production. These beets supply over 50% of the US total sugar supply. Growing regions of North America could be expanded to include almost every state in the U.S., and energy beets can be grown as a winter crop in most southern regions. The potential use of beets for purposes beyond sugar for human consumption is virtually untapped. Energy Beets are an excellent source

of simple sugars for the production of ethanol and many advanced bioproducts. Energy beets will typically produce twice the ethanol per acre compared to corn, and may even compete with sugarcane. Specifically, production costs are lower than for sugarcane, the pulp has much higher value (as animal feed) than sugarcane bagasse, the region where the crop can be grown is multiple times larger than the region suited to production of sugarcane, and it is possible that energy beets could supply sugar for a longer period each year than sugarcane. Therefore, energy beets have the ability to become a major industrial crop for all types of industrial sugars. In addition, they can be used as high energy, high value livestock feed as whole beets, or beet pulp: in the Southeast, beet pulp retails for over \$700 per ton when sold in 50-lb bags as horse feed. Achieving the 2007 federal mandate (Energy Independence and Security Act) to produce 36 billion gallons of renewable fuels each year by 2022, will depend on crops like energy beets. Being a simple sugar plant, it can easily be utilized to produce industrial sugars without the use of enzymes and the need to first convert starches to sugars. First generation feedstocks (mainly corn) for ethanol currently produce about 14 billion gallons of ethanol each year. To achieve the 36 billion gallon mandate we will need about 21 billion gallons produced from second generation advanced biofuel feedstocks. Energy beets are an advanced biofuel feedstock, and may well qualify as a

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cellulosic feedstock. We have recently conducted energy beet trials in the Southeast with good success. For example, an initial commercial-scale test in Baldwin County, South Alabama, yielded 35 tons of beets per acre over the winter with no irrigation ([http://blog.al.com/live/2013/05/manufacturing\\_plant\\_cited\\_as\\_m.html](http://blog.al.com/live/2013/05/manufacturing_plant_cited_as_m.html)). This translates into approximately 830 gallons of ethanol per acre, and at a price of \$30 per ton for beets, a gross income for the grower of \$1,050 per acre and a sugar cost of 10 - 15 cents per pound for the processor. Work on irrigated beet production for conversion to ethanol is also underway in California (<http://www.ethanolproducer.com/articles/9978/lowering-ethanols-carbon-footprint-with-energy-beets>). Our panel will provide an overview of the potential of energy beets for production of biofuels and bioproducts, including the agronomy of the crop and possible end-uses.

## Theodora Retsina

At American Process, we believe that Sugar is the New Crude®. Our vision is to be the leading biomass pre-treatment technology in the world, with a value proposition that combines engineering capabilities, quality of technical support, assurance of high yields, clean sugars, and minimum total cost of operation. We have developed two proprietary biorefinery technologies for producing low-cost cellulosic sugars from non-food based biomass.

The Green Power+® technology is a patented technology for the production of low-cost cellulosic sugars from the hemicelluloses of biomass.

Our AVAP® technology is a patented technology for the production of low-cost cellulosic sugars from the cellulose and hemicelluloses of any biomass.

The two technologies address different market opportunities for biorefineries in the Asia Pacific region and worldwide. For each technology, any biomass may be utilized, including hardwoods, softwoods, and agricultural residues. The Green Power+ process produces low-cost C5 and C6 sugars from the hemicelluloses of biomass feedstocks. Sugars are extracted from the solids which are then utilized for existing applications, in synergy with biomass-based renewable power, pellets, sugarcane plants, and many other existing sites. Co-location minimizes capital costs for commercial implementation. We see tremendous opportunity for Green Power+ configurations Asian sugar cane ethanol facilities, where our technology can substantially increase the production of ethanol in a very capital efficient manner. Other applications include pellet-making facilities and biomass power plants. Our first large-scale implementation of Green Power+ technology is a biorefinery pilot plant in Alpena, Michigan, capable of converting about 20 tons/day of hemicelluloses to sugars and co-products. The plant

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capacity is up to 2 million gallon/year of ethanol. The Alpena biorefinery started up in Q3/2012. The AVAP process produces low-cost C5 and C6 sugars from both cellulose and hemicellulose of biomass feedstocks. The AVAP process employs a solvent for lignin and sulfur dioxide to cleanly fractionate biomass into cellulose, hemicellulose, and lignin. Optionally, the cellulose may be recovered as a cellulose material or precursor for making cellulose derivatives. The cellulose that is produced by the AVAP process is extremely susceptible to hydrolysis by enzymes to produce glucose, thus greatly reducing enzyme costs. We see AVAP as having tremendous potential in Asia in areas of abundant, competitively priced biomass feedstock. An advantage of this technology is that it can operate with softwood, hardwood or agricultural residue, thus increasing the number of locations where it can be deployed. The cellulose sugars from the AVAP process can be converted into a variety of high-value chemicals, many of which are high demand in Asia either on their own or as a feedstock for plastics manufacture. Our first large-scale implementation of AVAP technology is a biorefinery pilot plant located in Thomaston, Georgia. The Thomaston biorefinery is capable of converting about 10 tons/day of biomass to sugars, ethanol, and co-products. The plant capacity is up to 300,000 gallon/year of ethanol or other products, such as butanol, jet fuel, or biochemicals. The Thomaston

biorefinery started up in the first half of 2013.

### Karl Doenges

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 the resulting synergies, and 3) characterization of the vegetable oil to enable optimum downstream processing

**Tuesday, December 10, 2013 |  
4:00pm – 5:30pm**

### **Renewable Oil Feedstocks for the Pacific Rim**

Moderator: Sanjay Wagle, AlphaJet

Naveen Sikka, TerViva

Kirk Haney, SGB

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Mike Cey, Ag-West Bio Inc

Chris Tindal, Office of the Deputy Assistant Secretary of the Navy for Energy

### **Abstract**

Speakers will discuss how they have begun to commercially establish their feedstocks, with specific emphasis on: (1) Business models -- contemplated structures, process for validation, and ultimate winning models with customers (2) Prioritization -- how the feedstock company prioritized landowner customer segments, geographies, and downstream market opportunities (3) Focus on Asia Pac -- evaluation of feedstock appropriateness for Asia Pacific countries.

**Wednesday, December 11, 2013 | 8:30am – 10:00am**

### **Oilseed Derived Feedstocks - Can they compete with Petroleum?**

Moderator: Jack Grushcow, Linnaeus Plant Sciences Inc.

Jonathan Burbaum, U.S. Department of Energy

Andy Shafer, Elevance Renewable Sciences, Inc.

David C. Bressler, Biorefining Conversions Network

### **Abstract**

Oilseed Derived Feedstocks - Can they compete with Petroleum?

The conversation related to oilseed derived feedstocks has been traditionally dominated by bio-fuel applications. As the reality of the disparity between production costs and the market price of oilseed sourced bio-fuels sets in, attention is shifting to higher value oilseed applications such as polymers, lubricants, surfactants, cosmetics and speciality chemicals. Addressing these markets requires a combination of advanced oilseed profiles and processing methods working together to develop next generation bio-refineries. The assembled panel will discuss four unique approaches to delivering high value renewable products from oilseed feedstocks with value beyond bio-fuels.

## **Renewable Chemical Platforms**

**Monday, December 9, 2013 | 8:30am – 10:00am**

### **Differentiation for Bio-based Chemicals**

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## on Industrial Biotechnology and Bioenergy

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Moderator: Damien Perriman,  
Genomatica

Jeffrey Hsu, Far Eastern New Century

*Commercialization - Elevance's World Scale Biorefinery has commenced production and New Products have entered the Market - An update on progress and what's coming*

Andy Shafer, Elevance Renewable Sciences, Inc.

Rodolfo Manzone, Yulex

### **Abstracts**

Damien Perriman

Jeffrey Hsu

In addition to all the other challenges of commercializing bio-based chemicals, Asian markets are evaluating the potential impact of 'low-cost' petrochemical plants now being built in China. This discussion will examine how multiple companies are looking to highlight the value of their bio-based offerings, to have successful market entry and growth despite the low-cost challenge.

Andy Shafer

Elevance Renewable Sciences and Wilmar International Limited have begun commercial shipment of specialty chemicals from their World-Scale biochemical refinery in Asia. The biorefinery, which has a capacity of 180 KTPY (400m lbs.) and is expandable to 360 ktpy, is Elevance's first biorefinery using its proprietary metathesis technology. With commercial capacity now available,

Elevance is now working with its partners such as US-based surfactants producer Stepan to meet product demand and accelerate deployment and commercialization of their high-performance chemicals in end user applications. This paper will discuss the status and progress of the commercialization activity taking place and the benefits the bio-based technology is bringing to the downstream markets.

Rodolfo Manzone

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 "natural"

chemical factory which requires low inputs without competing with fiber and food crops.

The company is developing fully integrated and sustainable biorefineries designed to 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 tailored 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 spans from guayule seed technology,

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agronomics, breeding, harvesting, bioprocessing to 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.

**Monday, December 9, 2013 | 10:30am – 12:00pm**

**Expanding the Oleochemical Value Chain to Bio-based Consumer Products**

Moderator: Tom Beardslee, Verdezyne

Hirzun Mohd Yusof, Sime Darby

Sam Bhargava, Jarden Applied Materials

*Biobased Synthetic Chemistry*  
Allen Barbieri, Biosynthetic Technologies

**Abstracts**

Tom Beardslee

Hirzun Mohd Yusof

Sam Bhargava

The conventional processing of crude vegetable oils produces an array of

molecules in the current oleochemical value chain such as fatty acids, fatty alcohols, and dimer acids that are used in the manufacture of consumer products ranging from surfactants and lubricants to polymers and cosmetics. These oleochemicals provide a renewable alternative to petrochemical-based products. New technologies are being developed that broaden the reach of oleochemicals into new products and are providing bio-based alternatives for consumers. This panel assembles speakers representing multiple points along the value chain of converting oleochemical inputs into bio-based consumer products. From plantation and vegetable oil refining to renewable chemical conversion technology to polymerization and product applications, the presentations show the current value chain as well as the future potential value chain of the oleochemical industry.

Allen Barbieri

**OVERVIEW**

Biosynthetic Technologies (BT) holds exclusive patented technology that synthesizes plant and animal oils into high performance synthetic oils used in lubricants, industrial chemicals and personal care. These “biosynthetic” base oils meet or exceed the performance characteristics of existing petroleum based synthetic oils. Having tested BTs biosynthetic oils extensively, several of the world’s largest manufacturers of automotive and industrial lubricants are certifying finished products they will bring to market under their brand names. The

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chief formulator of a leading global motor oil company recently stated that these are “the most exciting products to enter the lubricants industry since the introduction of PAOs [synthetic lubricants] 50 years ago.” First-mover advantage, lack of viable competition and patented technology make BT a market leader in the sustainable chemicals sector.

**Business model:** BT is a wholesale manufacturer/distributor of biobased synthetic oils that can be used by finished motor oil, lubricant and chemicals products manufacturers. BT is currently working with dozens of leading global lubricant and additive manufacturers as well as automotive and equipment manufacturers who are each testing, formulating and preparing to launch motor oils and lubricant products based on BT’s biosynthetic oils.

**Manufacturing:** BT contracted with Albemarle to build a demo manufacturing plant which is currently operating in Baton Rouge. In late fall, 2013, BT will begin construction of a full scale continuous flow production plant.

## ENVIRONMENTAL BENEFITS

**Sustainable:** BTs oils are made from fatty acids found in plant oils including canola, soy, palm, and coconut, as well as refined animal fat (all 100% renewable carbon).

**Biodegradable:** Using the most stringent testing protocols, BTs oils all

exceed the industry standard of 60% biodegradation within 28 days.

**Non-Toxic:** BTs oils tested non-toxic, even at contamination levels 100 times higher than the level needed to claim non-toxicity.

**Not bioaccumulative:** BT’s products do not bio-accumulate in living organisms.

**Greenhouse gas reduction:** A Life Cycle Analysis of Greenhouse Gas Emissions (GHG) from BT’s products is 83% lower than the GHG emissions associated with poly-alpha olefin (PAO), a product of similar function and use.

**Machine life / fuel efficiency:** Numerous tests have shown that BTs biosynthetic oils keep metal surfaces cleaner and facilitate lower friction and scarring on bearing surfaces than the highest quality synthetic oils. All of this keeps leads to longer machine life, lower maintenance costs and increased fuel efficiency.

**Water pollution:** BTs biodegradable oils could significantly reduce the environmental impact of improperly dumped motor oil and oil runoff from roads, which accounts for 40% of water pollution in the U.S. (EPA). In fact the amount of used motor oil and lubricants that enter the world’s oceans is the equivalent of one Exxon Valdez spills every week.

**Monday, December 9, 2013 |  
2:30pm– 4:00pm**

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## Renewable Chemicals and Consumer Products

*Renewable Chemicals: The Path to Commercialization*

Moderator: Max Senechal, Metabolix

*DEINOL: The Deino Way to Biofuels.*

Emmanuel Petiot, Deinove SA

*Leveraging a Consumer Brand for B2B Pull - The Nexa Project*

Jeff Uhrig, Bioformix

*Chemo-catalytic conversion of cellulose into para-xylene*

John Bissell, Micromidas

### Abstracts

#### Max Senechal

Metabolix, Inc. is an advanced biomaterials company that is well positioned to address growing market demand for sustainable solutions in the plastics, chemicals and energy industries. The Metabolix scientific foundation and core science is the metabolic pathways for the production of a class of microbial biopolymers – polyhydroxyalkanoates (PHAs) – from renewable resources. PHAs can be tailored through metabolic pathway engineering and have a wide range of applications in industry, including use in polymer form as biobased plastics or in monomer form as chemical intermediates. The first commercial product using the fermentation route are biopolymers marketed under the Mirel and Mvera brands.

Metabolix is also focused on developing biobased four-carbon (C4) and three-carbon (C3) chemicals. The C4 program is currently at the semiworks scale (80,000L) and Metabolix has shipped samples of biobased gamma butyrolactone (GBL) to prospective customers for testing, and GBL has been successfully converted to BDO via hydrogenation. Metabolix is making steady progress with its C3 program as well – the Company is currently running fermentation at the 20L scale and recently successfully recovered acrylic acid from biomass using its proprietary FAST process. In 2013, Metabolix is continuing fermentation scale up, engineering of microbial strains, and development and optimization of its FAST recovery technology to produce biobased C4 and C3 chemicals to match chemical industry specifications for quality and purity.

In this presentation, Metabolix's vice president, biobased chemicals, Max Senechal, will outline the Company's strategy for bringing these renewable chemicals to market and review various strategic options available to biobased chemical producers in commercializing their innovative products. Key question addressed: Is the "drop-in" chemicals strategy currently pursued by several participants in the space the only approach worth considering for successful market entry?

Emmanuel Petiot

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DEINOVE (Alternext Paris: ALDEI) is a cleantech company that designs, develops and markets novel industrial processes using biotransforming platforms based on the untapped extraordinary and advantageous properties of the Deinococci. Originally founded in France, and principally located in Montpellier, DEINOVE is now offering its technology platforms globally, with commercial representation in both the EU and United States ([www.deinove.com](http://www.deinove.com)).

Taking advantage of the unique genetic properties and unusual robustness of this relatively unknown bacterial phylum, DEINOVE optimizes natural fermentation and metabolic capabilities of these bacterial "micro-factories" to produce renewable green chemicals. The company is structured in two groups: while DEINOL is focused on producing renewable advanced biofuels, DEINOCHEM is focused on green chemicals or intermediates. Of notice, two subsidiaries operate independently, yet falling within the DEINOCHEM group: new antibiotics research (DEINOBIOTICS SAS) and plastics bioremediation (THANAPLASTTM).

DEINOL is focused on producing bioalcohols and alkenes of interest for their use as high octane (RON) drop-in components of transportation fuels. The industrial production of biofuels, through a biochemical route, necessitates the hydrolysis of a feedstock, and its fermentation to generate the end product of interest. The main costs of production are

generally concentrated on feedstock, enzymes and capital, with feedstock and biotransformation accounting generally at least 50% of the total cost. However, most processes are constrained to operate at the optimal temperatures of yeast or commonly used bacteria (~35C), not maximizing on biotransformation kinetics, favoring infections and preventing from using enzymes at their optimum activity levels.

DEINOL's platform leverage thermophilic organisms (45-55C) that are not subject to these constraints or limitations, and release their own enzymes in solution during the fermentation/hydrolysis, a rare process advantage named Consolidated Bioprocessing (CBP). Having the ability of produce end-products and expressing the enzymes necessary to break down feedstock for metabolism, all at higher temperatures, gives DEINOL's solutions a unique and significant cost advantage in techno-economic analyses.

Of particular notice, DEINOL's organisms co-assimilate C5 and C6 sugars as well as glycerol and acetic acid, and are particularly resistant to process inhibitors, thereby maximizing both conversion rates and feedstock utilization. When considering their incredible advantages including feedstock flexibility or their efficient metabolism of all organics, Deinove's strains truly are the ideal hosts for biotransforming applications by allowing for maximized process

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efficiencies and reduced raw material and variable costs.

In summary, the exceptional properties (robustness, biodiversity and metabolic properties) of DEINOL's platforms, and DEINOL's developmental research results, demonstrate the huge potential for these microorganism's to become true bacterial micro-factories for the production of biofuels in a cost-effective, environmentally friendly way.

### Jeff Uhrig

While B2B sales channels are attractive to resource limited start-ups, the consumer channel presents a nearer term commercial opportunity, allowing companies to build brand equity, demonstrate market adoption and de-risk larger scale B2B applications. This presentation will address how Bioformix created a consumer brand - Nexabond 2500 - and a respective in internet and consumer distribution channel in 12 months, which has in turn created stronger pull from global manufacturing partners.

### John Bissell

Micromidas has developed a chemocatalytic route to para-xylene from cellulosic biomass and ethylene. The process is expected to be cost-advantaged over naphtha-derived para-xylene, and will enable the production of 100% bio-based PET. The highly selective synthesis allows for high yields of para-xylene without the presence of typical isomers or

other aromatic species. The presentation will discuss (1) the specific advantages of the chemical pathway, (2) which feedstocks have been tested and will be scaled first, (3) the comparative projected economics of the process, and (4) the performance of our recently constructed pilot plant.

**Tuesday, December 10, 2013 | 2:00pm– 3:30pm**

### **Processes Leading to Commercialization**

*Making it Real: Levulinic Acid as a Biobased Building Block of a Renewable Chemical Industry*  
Moderator: Atul Thakrar, Segetis

*Strategic Commercialization of Bio-based Chemicals*  
Sue Hager, Myriant Corporation

*Developing new platform molecules: a case study*  
Marcel Lubben, DSM

*Bio-butadiene: Techno-economics of bioprocessing route via 2,3 butanediol (BDO)*

Jayant Sawant, Praj Matrix – The Innovation Center

### **Abstracts**

#### Atul Thakrar

Segetis creates high performance, sustainable materials, reducing the world's dependence on fossil fuel based petrochemicals. Our Levulinic acid based technology platform,

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including the ketal-based JAVELINTM Technology, is positioned for significant global adoption with renewable alternatives that cut greenhouse gas emissions and address toxicity issues in everyday items. Segetis has successfully developed and commercialized specialty plasticizers for flexible PVC used in everyday objects including flooring, shoes and packaging, as well as formulation aids used in consumer and industrial markets such as detergents and hard surface cleaners.

Levulinic Acid, the starting point for these innovations, is the decomposition product of sugars and carbohydrates, the most abundant feedstocks on Earth. Levulinic Acid is readily transformed into a wide variety of chemicals and has the potential to become a foundational biobased chemical building block of a renewable chemical industry. Despite high levels of interest, the lack of large-scale, cost effective, clean Levulinic Acid production has hampered the growth of levulinic-based products. Segetis has made a breakthrough in making Levulinic Acid a reality with a disruptive process that enables industrial-scale Levulinic Acid production using a wide array of biomass feedstocks, including fructose, glucose and cellulosic sugars. The combination of our ability to make cost effective Levulinic Acid at scale with the market development for JavelinTM plasticizers and formulation aids positions Segetis to meet the increasing demand for high

performance, healthier, biobased materials.

### Sue Hager

The chemicals industry is experiencing a fundamental shift as cost-competitive bio-based chemicals become a commercial reality. Leading this transformation is the market availability of bio-succinic acid, a platform molecule used as a direct replacement for petroleum-based succinic acid and also as a fundamental building block chemical in the production of numerous industrial and consumer applications. Myriant is one of the world leaders commercializing bio-succinic acid with two operating plants in production. Myriant's presentation will discuss the company's pathway for strategic financing of its flagship commercial plant, its commercialization strategy for the production of bio-succinic acid, as well as its plans for accelerated development of its bio-based chemicals pipeline.

### Marcel Lubben

Royal DSM N.V. is a global science-based company active in health, nutrition and materials. DSM is a frontrunner in creating bio-based and environmentally sound solutions, and seeks to demonstrate the commercial viability of renewable technologies in collaboration with strategic partners in the value chain. The development and supply of high value knowledge, ingredients and expertise in the field of bio-conversion technology are critical success factors determining its future. DSM's strategy is to license its

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technology and expertise to bio-based entrepreneurs, enabling them to convert biomass in the most commercial and sustainable ways possible. Today's market needs are driven by a number of major global trends and challenges. At DSM we are using our innovative strengths to address some of the most important of these trends and challenges focused on supporting the transition from fossil to renewable raw materials. The investment wave in the bio-based economy started from the early 00s. After almost one decade, tremendous and impressive progress has been made in technology development for renewable chemicals and materials. Pioneers in the field are making their first commercial steps.

In this speech we shall present a case study to illustrate our vision on what drives success in the commercialization of bio-based chemicals and materials: partnerships & capital, feedstock choice, excellent operations, application know-how and value chain understanding. Building on the experience DSM has assembled over the past years in bio-conversion technologies and the creation of chemical building blocks for (performance) materials from bio-based feedstock, an appeal is made to view the bio-based economy as an opportunity to innovate ourselves out of the global economic crisis. Novel and creative approaches, collaborations and partnerships are needed and will be discussed.

Jayant Sawant

Butadiene (BD) is a top 10 petrochemical primarily produced by heavy crackers. With more US crackers shifting to shale gas based light crackers, a net drop in production of butadiene has created a demand-supply imbalance and sharp price swings in global markets. Alternatively, butadiene can be produced from renewable feedstocks such as sugars using greener routes via 2,3 butanediol-fermentation and subsequent conversion to BD by catalysis. Several research groups, start-ups and few petrochemical majors have shifted focus to bio-butadiene. The focus of our study is to develop deeper insights into the technical and market driven challenges of bio-BD production and shortlist solutions to overcome the challenges. We present a detailed techno-economic analysis of bio-butadiene production starting with sugar based BDO fermentation and purification by solvent extraction followed by catalytic conversion of BDO to BD. Detailed sensitivity analysis and case scenarios with different sugar sources, sugar prices, and process parameters, and break-even analysis for crude versus sugar based BD are presented.

**Tuesday, December 10, 2013 |  
4:00pm – 5:30pm**

### **Fine Chemicals Manufacturing Using Biocatalysts**

*Biocatalytic Preparation of  
Cyclopropanes – A Novel and Cost-  
Effective Approach*

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Moderator: Pedro Coelho, Provivi, Inc

Satish Khanna, Provivi, Inc  
Ajay Parikh, ZCL Chemicals  
Anand Ghosalkar, Praj Corporation

### **Abstracts**

Satish Khanna  
Ajay Parikh  
Anand Ghosalkar

In recent years, biocatalysis has established itself as a key technology for the production of fine chemicals. The fine chemicals industry utilizes this technology to catalyze chemical reactions with unprecedented regio and enantioselectivity.

Implementation of biocatalysis therefore often results in a decreased number of chemical steps required for the synthesis of a given fine chemical, and ultimately reduces the cost of manufacture. In addition, biocatalysis favors mild conditions, resulting in decreased waste streams, decreased energy input, and improved safety. With an ever expanding biocatalysis toolbox and decreasing enzyme engineering timelines, biocatalysis is being embraced by the fine chemicals industry today. This session will discuss the value biocatalysis provides to a number of prominent fine chemical manufacturers, as well as challenges and opportunities.

Pedro Coelho  
Provivi, Inc. is a start-up company aimed at producing high-value chemicals via biocatalytic processes.

Our initial product platform is based on breakthrough technology for cyclopropane biosynthesis developed at and licensed from the California Institute of Technology. There, we developed the most active catalyst ever reported for olefin cyclopropanation, a key reaction in the synthesis of drugs, hormones, and insecticides (Coelho et al., Science 339, 307 (2013); Coelho, et al. Nat. Chem. Biol. 9, 485 (2013)). The combined annual global market size for products that are accessible via Provivi's technology exceeds \$7 billion. Active pharmaceutical ingredients (APIs) that contain a cyclopropane motif have a combined market of over \$5 billion. In addition, pyrethroid insecticides containing a cyclopropane motif have a market size of about \$2 billion. Here we will briefly describe the technology, and then focus on product applications and market opportunities pursued by Provivi.

**Wednesday, December 11, 2013 | 8:30am – 10:00am**

### **Innovation in Renewable Chemical Platforms**

*Bio-based Innovations and New Ventures from the Engineering Research Center for Biorenewable Chemicals (CBIRC)*

Moderator: Peter Keeling, Iowa State University, OmegaChea and SusTerea

Karl Sanford, DuPont/Danisco

Joe Noel, Pareto Biotechnologies

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Jeff Fornero, Glucan Biorenewables

### **Abstract**

CBiRC is developing renewable chemical platforms from bio-based innovations and new ventures utilizing tools, components and materials being explored by the Center. Core knowhow and technologies include bioengineering of fatty acid and polyketide biochemistry in microorganisms, as well as an innovative and complimentary portfolio of developments in chemical catalysis. By combining biocatalysis and chemical catalysis CBiRC creates new knowhow and powerful technologies that have the potential to nurture a sustainable bio-based chemical industry. CBiRC believes the existing petrochemical supply chain can be transformed with key foundational intermediates that deliver an array of drop-in chemistry or similar functionality to existing fossil-carbon-based chemicals. Here we will describe our progress towards creating an advanced manufacturing system with new platform molecules for biobased chemicals.

## **Advanced Biofuels and Biorefinery Platforms**

**Monday, December 9, 2013 |  
8:30am – 10:00am**

### **Cellulosic Biofuels: Unleashing Commercial Production**

Moderator: Brian Conroy, BP

*The Reality of Commercial Scale Cellulosic Ethanol Production*  
Christopher Standlee, Abengoa Bioenergy US Holding, Inc.

*Meeting worldwide demand with cellulose*  
Steve Hartig, POET-DSM Advanced Biofuels

*Delivering Big by Getting Small*  
Wes Bolsen, Cool Planet Biofuels

### **Abstracts**

#### Christopher Standlee

Abengoa is an international leader in the development of Advanced Biofuels. We have pursued a 10 year effort to produce cellulosic ethanol from multiple agricultural residues, culminating in the commissioning and start up of our Hugoton, Kansas commercial scale cellulosic facility in December 2013. Abengoa also started operations at a demonstration scale municipal solid waste to ethanol facility in Spain, and is negotiating a commercial scale MSW facility today. We are also pursuing efforts to produce other advanced biofuels and bioproducts from the same agricultural residue and MSW feedstocks. We currently are sourcing and managing 380,000 tons of ag residues annually for our Kansas facility, and have already conducted 4 harvests. We also are planning to utilize dedicated energy crops at some point in the near future. These efforts, along with start

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up of the Ineos facility and progress in development and construction by other producers of Advanced Biofuels make second generation biofuels a reality.

## Steve Hartig

Cellulosic bio-ethanol is poised to play a crucial role in the energy landscape of the United States, driven by recent process and technology gains as well as a commitment by government leaders to prioritize domestic, sustainable energy production. But worldwide, the potential is even greater. In the U.S., liquid fuels demand has fallen in recent years, and most experts predict this trend will continue. However, worldwide demand, driven largely by growth in Asia, is expected to increase dramatically in coming decades. The potential for expansion of cellulosic bio-ethanol in those areas is enormous. China, as the second-largest corn producer in the world, is uniquely suited to reap the rewards of commercialization efforts such as those by POET-DSM Advanced Biofuels. China has stated that it will not use corn for ethanol production; however, the abundance of crop residue that remains after harvest does provide ample feedstock for biomass-based ethanol production. The Energy Information Administration expects China to lead the world in demand growth for liquid fuel by 2035. India is expected to experience the next-largest demand growth, and the rest of Asia is not far behind. Cellulosic bio-ethanol can help meet that demand. POET-DSM Advanced

Biofuels is constructing a 25 million-gallon-per-year cellulosic bio-ethanol plant in Emmetsburg, Iowa that will use corn cobs, leaves, husk and some stalk as feedstock. Farmers this fall will harvest an estimated 120 thousand tons of biomass to feed this commercial biorefinery. Construction is on schedule for startup in early 2014. POET-DSM is now working to license this technology to other ethanol producers, and we see Asia as an important opportunity for putting this technology to good use. Sound technology, created here in the U.S., is ready to provide solutions for energy demands well into the future.

## Wes Bolsen

Carbon negative fuels is a reality today through the combination of biofuels and biochar. Wes will talk about the company's ability to commercialize Cool Planet's technology through a much smaller, distributed plant model. He will lay out transformative industry models such as mainframe computers becoming personal computers, and the mass production of micro-refinery technology paralleling other industries. He will finish by discussing their first commercial facility and how with the backing of Google, GE, ConocoPhillips, BP, and their other marquee investors, they were able to commercialize.

**Monday, December 9 | 10:30am  
– 12:00pm**

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## **Production of Drop-In Hydrocarbon Fuels from Cellulosic Biomass**

Moderator: Thomas Foust, National Renewable Energy Laboratory

Charles Cai, University of California, Riverside

Jesse Q. Bond, Syracuse University

*DME, its advantages and its promise through small-scale production*

Brittany Syz, Oberon Fuels

### **Abstracts**

Thomas Foust

Charles Cai

Jesse Q. Bond

Among sustainable resources, biomass is uniquely suited to production of organic fuels on which our society is so dependent, and only cellulosic biomass offers low enough costs and sufficient abundance to make a large impact on petroleum use. However, although biomass at about \$60/dry ton has an equivalent energy cost to oil at about \$20/barrel, conversion of cellulosic biomass is currently expensive, and new processes are needed that overcome recalcitrance as the primary barrier to competitiveness. In addition, ethanol and other oxygenates made from biomass carbohydrates, currently mostly sugar and starch and latter cellulosic biomass, are not fully compatible with the current fuel infrastructure. And hydrocarbon fuels with higher energy density are

particularly desirable for heavy-duty vehicles and air travel. Furthermore, enzyme costs for biological conversion of lower cost cellulosic biomass to ethanol tend to be high. Catalytic routes are being developed to convert biomass sugars and their breakdown products such as furfural, 5-hydroxymethylfurfural (HMF), and levulinic acid that we call fuel precursors into hydrocarbon fuels and fuel components compatible with the existing fuel infrastructure. A potential advantage of this aqueous processing approach to such "drop-in" fuels is that only thermochemical conversion is needed to make fuel precursors from cellulosic biomass, thereby avoiding use of expensive enzymes. However, yields for traditional approaches to producing such intermediates are too low to be cost-competitive, and the key economic challenge is developing low cost routes that realize high yields of fuel precursors from cellulosic biomass that can be integrated with catalytic conversion to hydrocarbons.

This panel will provide insights and recent performance information for the key operations, leading technologies, and commercial strategies for aqueous processing of cellulosic biomass for catalytic conversion to fuels. Charles Cai from the University of California Riverside will start with an overview of biomass deconstruction to form fuel precursors including sugars, furfural, HMF, and levulinic acid. Various routes to produce these components will be described, yield and operational

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considerations will be discussed, and perspectives will be offered on leading options and challenges.

Next, Jesse Bond from Syracuse University will summarize pathways for catalytic processing of these fuel precursors and identify appropriate catalysts for each fuel option. In addition, theoretical yield limits and current performance will be described for the various fuel options, and opportunities for improvements will be outlined. Tom Foust from the National Renewable Energy Laboratory (NREL) will then provide an overview of the National Advanced Biofuels Consortium (NABC) and describe NABC partnerships to accelerate commercial production of hydrocarbon fuels. Included will be an outline of key metrics that must be met for commercial viability and the stage of development of various processes to meet these goals.

The range of topics covered in this panel will allow the audience to better understand the pathways, progress, and challenges in converting cellulosic biomass into fuels and fuel components that are compatible with the existing aircraft and heavy and light duty vehicle infrastructures.

### Brittany Syz

DME (dimethyl ether) is a clean-burning, non-toxic, diesel alternative. Its high cetane value and quiet combustion, as well as its inexpensive propane-like fueling system, make it an excellent, inexpensive diesel

alternative that will meet strict emissions standards.

DME has been used for decades as an energy source in China, Japan, Korea, Egypt, and Brazil, and it can be produced domestically from a variety of feedstocks, including biogas (plant and food scraps) and natural gas. Ideal uses in North America are in the transportation, agriculture, and construction industries. Because production is not dependent upon the price of crude oil, DME will have stable pricing, which is competitive with that of diesel.

DME has storage and handling advantages over other diesel alternatives, such as CNG and LNG. In current demonstrations using bioDME in Europe, Volvo has shown 95% reduction in CO<sub>2</sub> emissions with companies such as DHL using DME in commercial operations. See <http://www.biodme.eu>

Oberon Fuels has developed an innovative, small-scale production method that produces DME in regional fuel markets, bypassing the initial need for a national infrastructure. In these regional fuel markets, regional feedstocks are used to produce DME to be utilized in regional trucking hauls, creating new regional economies and green jobs. The first such production facility will be online in Imperial Valley, in Brawley, CA in August 2013. The regional model supports not only local job growth and clean air initiatives, it also provides a viable economic structure that allows

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for capital expenditure in sync with market growth – it really hits the triple bottom line concept of sustainability.

The need:

In his climate initiatives speech in July, President Obama identified that we need to address heavy-duty trucking. Why? Because 30% of the particulate matter in our atmosphere is caused by commercial trucking. DME produced using the Oberon Fuels method is actually carbon negative – because it uses feedstocks that would otherwise release methane into the atmosphere and converts them to a clean-burning, zero particulate matter fuel.

Partners entering the field:

In early June, on the steps of the Capitol in Sacramento with representatives of the Governor's office involved, Oberon Fuels, Volvo Trucks North America and Safeway Inc. (the big CA-based food giant) announced a partnership to demonstrate DME in the San Joaquin Valley. They have received a \$500,000 grant from the San Joaquin Valley Air Pollution Control District to help support the project.

At the same event, Volvo Trucks North America announced plans that it would commercialize DME trucks in North America by 2015. Volvo is the largest seller of heavy-duty trucks worldwide. It is committed to DME engines because DME has the best

performance level of any of the diesel alternatives.

On June 20th, Mack Trucks also announced they plan to commercialize DME trucks. In its press release, Mack Trucks noted that DME "offers many environmental and societal benefits, including that it can be made from multiple sustainable feedstocks," including grass clippings, animal waste and other sustainable sources.

See: [http://www.aboutdme.org/aboutdme/files/ccLibraryFiles/Filename/000000002405/Release\\_Mack\\_2013-06-20.pdf](http://www.aboutdme.org/aboutdme/files/ccLibraryFiles/Filename/000000002405/Release_Mack_2013-06-20.pdf)

Conclusion:

DME is the smart choice for an alternative fuel to diesel. Our goal is to educate your audience on the prospects and promise of DME and the small-scale production method that models its success.

**Monday, December 9 | 2:30pm – 4:00pm**

## **Biorefineries: Perspectives on Finance & Construction**

Moderator: Martin Sabarsky, Cellana LLC

*US next-generation biofuel investment: no swing for the fences yet*

Alejandro Zamorano Cadavid,  
Bloomberg New Energy Finance

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# Pacific Rim Summit

## on Industrial Biotechnology and Bioenergy

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*Decision and Risk Analysis in Industrial Biotechnology*  
Alan Propp, Merrick & Company

*Project Financing Considerations*  
Lester H. Krone, Stern Brothers & Co.

*Critical Questions for Bolt-On Biorefineries: Avoiding Unintended Consequences*  
Doug Dudgeon, Harris Group Inc.

### **Abstracts**

Alejandro Zamorano Cadavid  
The Renewable Fuel Standard celebrated its seven-year anniversary on 1 May 2013. Bloomberg New Energy Finance takes a look back at privately and government-funded deals in the US next-generation biofuels industry and appraises conversion technologies based on the amount of funds invested.

Alan Propp  
Making good decisions is critical for companies developing and deploying new industrial biotechnologies. Decision and Risk Analysis (D&RA) is an objective, analytical process designed to help companies navigate through the maze of uncertainty to make sound business and technical decisions. D&RA allows companies to better understand both business and technological risks so they can be proactively managed throughout development. With proper D&RA companies can increase their odds of pursuing the right project with the right technology. D&RA can also be used to develop a compelling, credible

business case to attract needed funding. This presentation will describe the Decision and Risk Analysis process and present an example of how the science was applied to an industrial biotechnology project.

Lester H. Krone  
Project Finance Fundamentals 1. Current State of the Bank Market 2. Typical Project Finance Structure 3. Project Structure Mitigates Project Risk 4. Project Capitalization 5. Successful Financing Requires Systematic Approach 6. At Financial Close Project Finance Execution 1. Sources and Uses 2. Private Placement Memorandum 3. Independent Engineer Report 4. Timetable 5. Project Finance Waterfall 6. Case Study – Project Finance Credit Quality Stern Brothers & Co. – Alternative Energy Finance Group 1. Overview 2. Current Projects 3. Biographies

Doug Dudgeon  
In a time of limited capital availability, bolt-ons are all the rage in biorefining. The cash to be saved from shared infrastructure with the host facility and from smaller size can be significant when compared with the cost of a standalone installation. That said, with all their inherent interdependencies, bolt-on projects are necessarily more complex than standalone projects. When considering a bolt-on application, it is important to remember first and foremost to do no harm to the host. Additional questions must be addressed in developing a

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bolt-on application, in order to avoid unintended consequences from integrating new technology with an existing, revenue-generating asset. These questions concern the compatibility of core technologies, economic trade-offs between the existing process and the new process, infrastructure availability, and the commercial affinity of the host's current business with the potential new business. Drawing on experience from Harris Group's decades of experience in advanced biofuels and retrofit plant design, this paper examines the critical questions of bolt-ons for implementing advanced biofuels technologies in three contexts: a dry-grind ethanol plant, a sugarcane mill and ethanol plant, and a pulp mill.

**Tuesday, December 10, 2013 |  
2:00pm – 3:30pm**

### **Progress in Production and Commercialization of Alcohol Fuels**

Moderator: James J. Spaeth, U.S. Department of Energy

*Production of Biobutanol from Jatropha Seed Cake*  
Jasmine Isar, Reliance Industries Limited

*Clean technology innovation to produce advanced biofuels, reduce landfilling and simulate regional economies*  
Sam Park, Enerkem

*Advances in DOE supported Cellulosic Ethanol Projects*  
James J. Spaeth, U.S. Department of Energy

*PROESA™ technology: Break-through Technology for Producing Advanced Bio-Fuels and Renewable Chemicals from Cellulosic Biomass*  
Kevin Gray, BetaRenewables

### **Abstracts**

Jasmine Isar  
Butanol production by *Clostridium acetobutylicum* ATCC 4259 was studied using the renewable feedstock jatropha seed cake. Chemical mutagenesis for improvisation of the strain for better butanol tolerance and production was done. Optimization of the physiochemical parameters resulted in about 14.3 g/l of butanol in 120 h using acid treated jatropha seed cake hydrolysate (7% w/v) along with AnS components. The process was scaled up to 15 L level yielding 18.6 g/L of butanol in 72 h. This is the first report wherein high yield of butanol has been obtained in single batch fermentation using acid pretreated jatropha seed cake. The strain was found to be tolerant to 3.0 % butanol under optimized conditions and the tolerance could also be demonstrated by the strain's ability to accumulate rhodamine 6G. The strain was found to be having high expression of the stress-response protein GroEL, the reason behind the tolerance and growth of the strain in presence of 3.0 % (v/v) butanol in the medium.

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### Sam Park

The commercialization of advanced biofuels is driven by the need to reduce dependency on oil, increase energy diversity and reduce carbon footprint. Public policies around the world contribute to the expansion of biofuel production by stimulating both the supply and the demand for renewable fuels. Despite the global recession, the advanced biofuels industry now operates facilities, and a first wave of technologies is now ready to be commercialized, as large scale facilities currently being built. A wide variety of biomass and residues are used as feedstock for these projects, including municipal solid waste. Three main categories of technologies are being developed and commercialized in this sector. The ones capable of using municipal solid waste (MSW) as a feedstock provide an alternative to landfilling and incineration, thereby offering additional environmental benefits. MSW feedstock has a large potential, given its abundance, and the fact that it is already collected. In addition to providing a new source of energy, advanced biofuels facilities stimulate regional economies, create high quality jobs, provide synergies with traditional sectors such as forestry, agriculture and waste, and contribute to revitalize the industrial manufacturing sector.

The presentation will talk about the context for the growth of this sector and for the use of MSW as a feedstock for the production of advanced biofuels. It will describe the main

categories of technologies that were developed over the last decade and give an example of a technology using non-recyclable municipal solid waste as a feedstock. The benefits of commercial-scale advanced biofuels facilities, as well as the advantages of using municipal solid waste will also be presented. Finally, the presentation will explain why public policies around the world play an important role in stimulating innovation and creating a business climate that attracts private investment to allow for the commercialization of these innovations.

### James J. Spaeth

DOE will provide an update on progress and developments in DOE supported cellulosic ethanol projects.

### Kevin Gray

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,

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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, which opened in Jan 2013, is designed to produce approximately 20 MM gallons of ethanol from a combination of agricultural residues (wheat straw) and energy crops (*Arundo donax*). 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.

**Tuesday, December 10, 2013 | 4:00pm – 5:30pm**

## **Overcoming Challenges in Regulation and Intellectual Property**

*Regulatory Strategies for Use of Genetically Modified Organisms in Biofuel Production*

Moderator: David J. Glass, D. Glass Associates, Inc.

William Kenealy, Mascoma Corporation

*What is patentable in the biotech sector?*

Konrad Sechley, Gowling Lafleur Henderson, LLP

*The Here and Now of TSCA Regulations on Biobased Chemicals*  
Kathleen Roberts, Bergeson & Campbell Consortia Management, LLC

## **Abstracts**

David J. Glass

William Kenealy

Genetically modified microorganisms are broadly coming into use for the production of biofuels or bio-based chemicals. These include modified yeast strains with improved or more efficient ability to ferment ethanol, photosynthetic microorganisms to produce fuels from solar energy, and microbes that can synthesize alkane fuels or fuel precursors. These are in addition to the numerous industrial enzymes used in ethanol fermentations that are now produced in modified production strains. Many

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of these GMMs will need to navigate the unique regulatory regimes that have been established in the U.S. and elsewhere in the world to provide oversight over industrial uses of biotechnology, and a growing number of companies have successfully completed the process for their novel strains.

David Glass will begin this panel with an overview of regulatory requirements for industrially-useful genetically modified organisms in the U.S. and internationally, including regulations applicable to the uses of modified microorganisms, algae or plants. This will be followed by presentations of case studies illustrating the paths to regulatory approvals for under several different regulatory regimes, including approvals for use of GMMs in contained manufacturing and for use of inactivated biomass as an animal feed ingredient.

David Glass will present a case study of Joule Unlimited's successful navigation of the U.S. biotechnology regulatory system for a novel microorganism for the production of ethanol. This is a strain of cyanobacteria modified to produce ethanol from sunlight, carbon dioxide and water, which was the subject of a Microbial Commercial Activity Notice submitted to EPA, and approved through a voluntary consent order allowing use of the organism at Joule's Demonstration Plant in New Mexico.

Next, William Kenealy will discuss Mascoma's experience with its

advanced yeast products which reduce the cost and increase the yield of grain-based ethanol production. TransFerm expresses glucoamylase (GA) during the fermentation, which can reduce the cost of separately purchased enzymes as well as increase the rate of ethanol production. TransFerm Yield + is an advanced yeast that in addition to GA also makes less glycerol thereby resulting in a higher yield of ethanol per feedstock compared to conventional yeast. These are the first bioengineered yeast both fully reviewed by U.S. regulatory authorities and broadly-utilized in the grain ethanol industry. The genetic modifications used to create TransFerm and TransFerm Yield + are built in a robust *Saccharomyces cerevisiae* host, utilize genes from safe and well-characterized donor organisms, and are very stable. TransFerm and TransFerm Yield + have been favorably reviewed by the FDA's Center for Veterinary Medicine (CVM) for inclusion in the Association of American Feed Control Officials (AAFCO) official publication of feed ingredients. In addition, both products have been reviewed by the EPA under the MCAN program.

### Konrad Sechley

The challenge to obtain patent protection for technology in this sector has never been greater. Are nucleotide sequences still patentable? Is the yeast strain we developed patentable? What data is required to protect biotech related inventions? Much uncertainty exists in the

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requirements to protect innovation development within the biofuel and bioenergy sector. These and other issues that impact what is protectable, the scope of protection, and information required to support protection of innovation will be reviewed within the US, Europe and Canada. The take home messages arising from recent decisions in the US, Europe and analogous cases in Canada, pertaining to the types of subject matter that can be protected, the extent of experimental support, and what is required for utility will be discussed. This presentation will compare trends within the European, US and Canadian Patent Offices, with an emphasis the biotech sector and how these trends impact protection of biofuel and bioenergy innovation.

### Kathleen Roberts

How do the evolving biobased and renewable chemical technologies fit within the current Toxic Substances Control Act (TSCA) framework? The short answer is “not well.” The longer answer involves detailed evaluation, probable notification, reporting, and recordkeeping requirements. This session will focus on helping manufacturers understand the journey their biobased product will face in the U.S. Environmental Protection Agency’s current system of TSCA regulation, including potential delays, detours, and pitfalls. We will consider strategies, tips and helpful hints that companies can use as they navigate the TSCA gauntlet. Without this knowledge, commercial success will be virtually unachievable.

**Wednesday, December 11, 2013 | 8:30am – 10:00am**

### **Advances in Aviation Biofuels**

Moderator: Michael Vevera, Mercurius Biorefining

*Is there a renewable aviation fuel feedstock?*

Eric Mathur, SGB

*Liquid Phase, Catalytic Conversion of Biomass to Bio-Jet*

Karl Seck, Mercurius Biorefining

*Alaska Airlines Investments in Aviation Biofuels*

Jay Long, Alaska Airlines

*Highlights of Two Aviation Biofuel Research Efforts by USDA NIFA and the FAA*

Ralph Cavalieri, Washington State University

### **Abstract**

#### Eric Mathur

Drop-in replacements for petroleum liquid fuels represent a major component of the growing energy demand. Although cellulosic ethanol will mitigate the depletion of oil reserves used for gasoline and algal biofuels hold long term promise as jet fuel substitutes, there are no obvious present day renewable feedstocks available. Candidates include canola, oil palm, rapeseed and soybean; yet

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redirecting agricultural resources toward energy feedstocks negatively impacts food security and is not sustainable. A promising alternative is the non-edible oilseed shrub, *Jatropha curcas*. *Jatropha* is native to Central America and was distributed by Portuguese sailors to colonies three centuries ago in the Cape Verde Islands. The primitive crop was recognized as heating oil and as a result, 35,000 tons of *Jatropha* seed was exported from Cape Verde to regions throughout Africa, Asia and Latin America. The spread of a few *Jatropha* cultivars from the center of origin to the pan tropics created a genetic bottleneck in the diversity of *Jatropha* found outside Central America. Recent attempts at domestication of *Jatropha* failed because the plantations used undomesticated landraces derived from Cape Verde germplasm which limited genetic improvement through breeding and selection. The inability to commercialize *Jatropha* as an energy feedstock was not a reflection on *Jatropha*, but rather of the business getting in front of the science of domestication. Here, we present evidence that *Jatropha curcas* germplasm is not genetically depauperate; but rather encompasses a substantial pool of genetic variation sufficient to propel breeding efforts designed to achieve economic yields. It is well known that the highest degree of genetic diversity within a species is typically found near the center of origin. High genetic diversity at the origin of a species is a consequence of population density and

maximized time for accumulation of selected and unselected mutations. *Jatropha* planting materials were collected throughout Central America in order to create a germplasm repository which now comprises over 12,000 genotypes derived from ~600 accession families. The plant collection exhibits high levels of phenotypic diversity including variation in flowering time, oilseed content, fruit yield, plant architecture, susceptibility to fungal pathogens, pest resistance, drought, heat, flood and cold tolerance. Our results now confirm that genotypic diversity underlies the observed phenotypic variation. Small sequence repeats (SSR) and genome-wide SNP markers were used to analyze diversity of the germplasm collection. The results conclusively demonstrate that the genomic variation residing in the Central American germplasm collection positively correlates with the phenotypic diversity. Moreover, the analysis reveals that virtually all *Jatropha* land races cluster tightly, confirming genome scale homologies and derivation from a common ancestor. In contrast, the germplasm collected near the center of origin of the species forms eight divergent clades, punctuated with a wide spectrum of genotypic variance within each clade. Thus, our findings suggest that *Jatropha curcas* possesses the genetic potential necessary for crop improvement. Considering the short generation time of this perennial and the ability to propagate both by sexual and vegetative methods, there are no apparent genetic obstacles preventing

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Jatropha from becoming the preferred oilseed feedstock for renewable jet fuel.

## Karl Seck

Mercurius Biorefining was established to produce profitable drop-in fuel (Jet Fuel and Diesel) from non-food biomass feedstocks. Mercurius is developing a “faster ~cheaper~ better” method of producing profitable drop-in biofuels. Mercurius uses a proprietary, patent pending technology called Renewable Acid-hydrolysis Condensation Hydrotreating (“REACH”) to make profitable drop-in hydrocarbon liquid fuels.

## Jay Long

This presentation describes strategies, motivations, and future plans for integrating aviation biofuel into normal airline operations. As aviation biofuel technologies evolve, driving production rates up and unit costs down, the use of alternative aviation fuels as part of the standard fuel sourcing operation is drawing closer to a reality. Alaska Airlines flew 75 flights using an 80/20 blend of HEFA based bio-jet in 2011 as part of a “proof of concept” initiative aimed to support the aviation biofuel production efforts. The airline is currently engaged in a project with Hawaii BioEnergy for a project to produce aviation bio-fuel in an area that currently sees very high jet fuel prices and significant supply constraints. It is Alaska’s opinion that identifying these types of markets will not only allow the producers to compete on an economic basis, but will

also provide much needed supply integrity.

## Ralph Cavalieri

In recent years, the USDA National Institute of Food and Agriculture (NIFA) and the Federal Aviation Administration (FAA) have competitively awarded funding that directly supports the development of alternative jet fuels, with emphasis on biologically derived jet fuel. Two such projects are being led by Washington State University.

The USDA NIFA Agriculture and Food Research Initiative Coordinated Agricultural Project entitled Northwest Advanced Renewables Alliance (NARA) seeks to develop a sustainable supply chain that uses forest residues as the primary feedstock and biochemically produces jet fuel and other valuable products. The five-year project is in its third year and involves about 180 individuals from multiple universities and companies. Important progress has been made in addressing technical as well as sociological and economic challenges. Four pretreatment pathways have been examined as well as methods for economically collecting and transporting feedstock and developing valuable co-products. Industrial partners, such as Weyerhaeuser and Catchlight, are working closely with the project’s university researchers on feedstock, pretreatment, and co-product issues. Conversion of the hydrolysate to isobutanol and ultimately jet fuel is advancing through the efforts of Gevo, another of the project’s industrial partners. A parallel conversion

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pathway to jet fuel, known as BioChemCat, is also being explored. Educational programming, a major objective of the NIFA program, is addressing needs for STEM education related to biofuels at all levels from primary grades to secondary and undergraduate education.

The second project, very recently awarded by the FAA, is the Center of Excellence for Alternative Jet Fuels and Environment. This new Center of Excellence (COE) is co-led by the Massachusetts Institute of Technology and involves a total of sixteen research universities. The COE also engages nearly fifty companies and other organizations as research collaborators and stakeholders. The FAA identified ten overarching research objectives in the solicitation that address all aspects of development, testing, and certification of alternative jet fuels as well as environmental impacts of aviation related to noise, emissions, and energy consumption.

## Synthetic Biology, Algae and Marine Biotechnology

Monday, December 9, 2013 |  
8:30am – 10:00am

### Optimizing Algae Production for Beneficial Bioproducts

*Microalgal-based Food and Feed  
Ingredients – Crop of the Future?*

Moderator: Jim Flatt, Synthetic  
Genomics

*Marine Microalgae for a Sustainable  
Future-Production and Application of  
Algae for Fuel, Feed and  
Nutraceuticals*

Valerie Harmon, Cellana LLC

*Algae, beyond energy*  
Jose Sanchez, OriginOil

*Commercialization of New Algae-based  
Omega-3 Supplements with High  
Bioavailability*

Brian L. Goodall, Valicor Renewables  
LLC

### **Abstracts**

Jim Flatt

Projected growth in world population and changes in dietary trends, coupled with constraints on arable land, water and carbon emissions will increasingly tax conventional agricultural food production methods and drive development of alternative crops which can augment traditional sources of food and feed products. Large-scale cultivation of microalgae has the potential to meet a significant portion of future needs, however, requires significant development to establish cost-effective and robust production of strains with desirable nutritional properties. Microalgae are relatively unique in their ability to produce a large fraction of their biomass as high-quality protein as well as other nutrients including oils and carotenoid pigments. Researchers at Synthetic Genomics have assembled a vast and

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diverse collection of microorganisms including algae collected globally through its bioprospecting programs. Hundreds of these have been evaluated for nutritional quality, leading to the identification of microalgal lead strain candidates which provide similar amino acid profiles for various plant and animal proteins, including soy, canola and pea, dairy proteins and fishmeal proteins. Algal protein ingredients must meet a number of other requirements in order to be considered mainstream sources for human and animal nutrition, including desirable olfactory profile, neutral color, low allergenicity and compatibility with different food matrices, which may require either aqueous soluble or insoluble proteins. The use of microalgal biomass as a partial replacement for fishmeal in aquafeeds represents another promising market application. Specific microalgal strains provide proteins and lipids similar in composition to those of current sources such as menhaden as well as desired micronutrients. Microalgal cultivation currently is still too expensive and limited in scale to compete on price and availability today. However, advances in synthetic biology and genomics offer powerful new tools to improve strain performance and robustness, which are required for attractive production economics. Strains exhibiting improved photosynthetic efficiency relative to wildtype ancestor strains are required to achieve higher biomass productivity and titer associated with economical production

of commodity food and feed products. SGI has recently developed algal strains allowing significantly improved light availability in a mass culture setting, without compromising photosynthetic performance of the strain, providing the foundation for improved production economics.

### Valerie Harmon

Cellana is a leading developer of algae-based bioproducts, and its pre-commercial production of marine microalgae takes place at the Kona Demonstration Facility (KDF) in Hawaii. KDF is housing more than 80 high-performing algal strains for different bioproducts, of which over 30 have been grown outside at scale. The patented ALDUOTM algal cultivation technology allows Cellana to successfully tackle the issue of contamination, one of the biggest technical challenges for algal mass cultivation in open ponds. As a result, algal mass cultivation is no longer limited to a few extremophiles, instead, we are able to tap into the hugely diversified algal strains in nature and significantly expand the catalog of algal strains for mass cultivation. 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's research and production at KDF have addressed major areas that are crucial for the commercialization of algal biofuels: yield improvement, cost reduction, and the overall economics.

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Commercially acceptable solutions have been developed and tested for major factors limiting areal productivity of algal biomass and lipids based on years of R&D work conducted at KDF. This talk will share some of the highlighted R&D work, including areal yield improvement, oil optimization, Omega 3 fatty acid development, flue gas utilization and feed application of defatted algal biomass for both aquatic species and livestock. Improved overall economics was achieved through a holistic approach by integration of high-value co-products in the business model.

### Jose Sanchez

Algae is one of nature's most efficient and versatile photosynthetic factories. With a short growing cycle, algae is not only an attractive and healthy source for natural feed and fertilizer, but also is a viable petroleum alternative in fuels and chemicals. Unlike petroleum, algae absorbs rather than emits CO<sub>2</sub>. The main challenge for implementation of this resource is overcoming cost barriers associated with algae production, since often times the same amount, if not more energy is expended generating algae than is generated by it. Current methods for separating algae from water such as centrifuge and membranes are simply too expensive for large-scale implementation. Centrifuge in particular can also damage the algae product rendering it useless in many applications. Enter OriginOil, developer of a chemical-free, high-speed technology that efficiently

harvests algae with just finely tuned electrical pulses. In the first stage, the pulses cause the algae to coagulate. In the second, a cloud of microbubbles gently lifts the algae to the water surface. In 2012, OriginOil teamed up with their first customer, MBD Energy to pilot the algae harvesting technology, freshly patented in Australia. Since then the company has expanded rapidly, recently installing its technology in the La Defense building complex in Paris as a part of a dual water clean-up and energy generation system. The team also recently completed tests validating an increase in the shelf life of the algae feedstock when harvested through OriginOil's EWS technology. Additionally, OriginOil has found that EWS can accelerate the production of astaxanthin by sanitizing and concentrating the *Hematococcus pluvialis* (HP) algae for more effective stressing. In this session, VP Jose Sanchez will discuss the need in the algae industry to reduce production costs and solutions available to do so. In the second half of his presentation, Mr. Sanchez will address the burgeoning market applications for high quality algae. Using the recent OriginOil findings regarding shelf life and astaxanthin production, Riggs will educate attendees about new growth sectors in the algae industry.

Brian L. Goodall, co-authors Isaac Berzin, Sam Couture, Kiran Kadam & Brian Waibel Qualitas Health and Valicor Renewables have partnered to commercialize the first algae-based pure EPA supplement.

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The integrated production facility (from algae cultivation to finished product) is located in the Southwest United States and enjoys many critical benefits – excellent climate and high insolation, essentially unlimited access to brackish water, land availability, infrastructure and abundant local sourcing of carbon dioxide thanks to the local oil and gas industries. The strain used is *Nannochloropsis*, a natural strain not genetically engineered. The strain exhibits exceptional EPA production capacity, has been grown in multiple locations including Israel and 2 different states in the Desert Southwest. Crop protection methodologies for this strain have been developed over the last several years giving us a proven, robust growth platform. Two additional major benefits of the selected strain are the fact that it delivers EPA in the absence of other omega-3's such as DHA, and that the bulk of the EPA resides in the biomass as polar lipids e.g. phospholipids and glycolipids. Since EPA and DHA compete for the same receptors in the body, for adults seeking the health benefits of EPA (such as anti-depression) it is pure EPA, rather than an EPA / DHA mix, that is important for effectiveness. In addition, the EPA dose taken is less critical than how much of the EPA ends up in the blood stream and target organs – the bioavailability of the EPA source. In the market today, there are products that are more bioavailable, like Krill oil, which is premium priced and enjoys market growth of 40% per annum. It is important to remember

that the polar lipid composition that makes Krill oil so bioavailable comes from the algae in their diet. Together Valicor Renewables and Qualitas Health have developed a new patents-pending nutraceuticals platform which permits the efficient recovery and fractionation of these EPA-containing polar lipids from their naturally-occurring algae source without hydrolysis or other degradation.

**Monday, December 9, 2013 |  
10:30am – 12:00pm**

## **New Approaches to make Biology Easier to Engineer**

*Reliable parts to make biology easy to engineer*

Moderator: Vivek Mutalik, Lawrence Berkeley National Lab

*Developing designable genetic control systems for applied synthetic biology*

James Carothers, University of Washington, Seattle

*High-throughput, one-pot gene synthesis and characterization*

Tim Hsiau, University of California, Berkeley

*Ginkgo Bioworks: A factory for engineering organisms*

Jason Kelly, Ginkgo BioWorks

## **Abstracts**

Vivek Mutalik

Our ability to routinely engineer genetic networks for applications is limited by the scarcity of a well

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characterized compendium of diverse regulators that are orthogonal (that is, do not inadvertently cross-react), homogeneous (operate with similar kinetics, thermodynamics and other structural properties) and have predictable functionality in variety of contexts. There is also a need for developing methods to study the performance variability of characterized part in a particular sequence, environmental and host context, so that genome scale engineering efforts can be realized.

In this talk, I will present a series of examples in developing large compendium of genetic parts that function reliably in different contexts, methods to characterize the context effects on part performance, sequence-activity models to understand the mechanistic details of a part operation and the development of software tools to aid in sharing and visualization of these open source parts collections. These tools are valuable in building bio-manufacturing platforms to produce key components of fuels, medicines and biochemicals.

## James Carothers

Naturally-occurring metabolic systems have evolved control circuitry that minimizes the accumulation of toxic intermediates and maintains homeostasis. At present, engineering even rudimentary control circuitry for synthetic biological systems remains very difficult. We have successfully formulated design-driven approaches that use mechanistic modeling and kinetic RNA folding simulations to

engineer RNA-regulated genetic devices with quantitatively-predictable activities that can control gene expression in metabolic pathways producing industrial chemicals in *E. coli* (Carothers et al. & Keasling. *Science* 2011). We have also demonstrated that models and simulation tools can be used to inform the design of microbial dynamic sensor-regulator systems (DSRS) engineered to produce fatty acid-based chemicals and fuels (Zhang, Carothers, & Keasling. *Nature Biotech.* 2012). At the University of Washington, we are developing designable RNA-based systems to solve control problems in a 15 gene pathway engineered to produce substituted styrenes for advanced polymer applications in photonics, photolithography and biomedicine. Here, I will present these results and discuss the potential for using the conceptual and experimental frameworks that we have established to create full-fledged design platforms for applied synthetic biology.

## Tim Hsiao

Cheap and reliable gene synthesis methods will benefit the field of biotechnology. To address this problem, we have developed an inexpensive gene synthesis and error correction method. In our system, we can make hundreds of genes in a one-pot fashion from microarray-synthesized oligos. We then developed a genetic system for selection of correct sequences from the background pool of error-containing genes. Our genetic system

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can also provide information about the solubility of the synthesized gene. Such solubility information will be useful in ranking algorithms for rational design of genetic and metabolic pathways.

## Jason Kelly

Organism engineering firms are emerging that build microbes to specification for customers. Making heavy usage of robotics and computer-aided design (CAD), these companies are closer to semiconductor chip fabrication plants than to biotech labs. Ginkgo Bioworks opened a new 11,000 sq ft organism fabrication facility in early 2012. This talk will describe the facility and our work designing and building microbes for the production of chemicals and fuels -- including our development of organisms engineered to use C1 compounds as feedstocks.

**Monday, December 9, 2013 |  
2:30pm – 4:00pm**

## **Metabolic Engineering of Aquatic Photosynthetic Organisms**

Moderator: Peter Heifetz, Heifetz BioConsulting

Mark Hildebrand, University of California, San Diego

Margaret McCormick, Matrix Genetics, Inc.

David Stern, Cornell University

Farzad Haerizadeh, Life Technologies

## **Abstract**

Aquatic photosynthetic organisms, including cyanobacteria, micro algae, diatoms and macro algae (seaweeds) have attracted significant interest as potential sources of photosynthetically-derived and carbon-neutral renewable feedstocks, specialty chemicals and biofuels. The application of metabolic engineering tools and approaches to cyanobacteria and algae creates opportunities for coupling bioproduction to direct solar energy conversion without the need for organic carbon feedstocks. Recent advances in the understanding of biological circuitry and its regulation in these organisms has now enabled effective application of technologies for strain selection, targeted genetic manipulation and synthetic biology to chemicals, food, feed and medicines. In parallel, product-optimized cultivation and product isolation strategies have evolved rapidly as projects have moved toward commercialization. This session will highlight significant advances in metabolic engineering of cyanobacteria / algae as well as the development of integrated and scalable platforms for specialized bioproduction.

**Tuesday, December 10, 2013 |  
2:00pm – 3:30pm**

## **Updates on Algal Biofuel Production in China**

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*Production of Biofuels and Chemicals  
Using Microalgae Isolated from the  
Coastal Regions of China*

Moderator: David Guangyi Wang,  
Tianjin University

*Development of Jet Biofuels for a  
Sustainable China Aviation Industry*  
Pengcheng Fu, Beijing University of  
Chemical Technology

*Synthetic photosynthetic  
Cyanobacteria to produce fine  
chemicals directly from CO<sub>2</sub>*  
Weiwen Zhang, Tianjin University

*Large-scale Production of Microalgal  
Biomass and Chemicals in China:  
Current Status and Perspective*  
Zhongxin Yang, Hangzhou Xinwei  
Low-carbon Tech R&D Ltd, Hangzhou,  
Zhejiang, P. R. China

**Abstract**

David Guangyi Wang

Microalgal biomass has been considered as one of the most promising feedstocks for biofuel (e.g., ethanol and biodiesel) and industrial chemical production. However, selection of microalgal strains with desirable physiological and biochemical features from local habitats has been one of major challenges in the large-scale production of microalgal biofuels and chemicals. Although the coastal areas of Pearl River Delta have long been

known for huge diversity of aquatic life surprisingly only little work has been done to use local microalgae resources for biofuel and chemical production. Recently, we have isolated 18 marine microalgal strains, belonging to the families Chlorellaceae, Scotielloccystoidaceae, Neochloridaceae, Selenastraceae and Scenedesmaceae. Of isolated strains Mychonastes afer PKUAC 9 and Scenedesmus abundans PKUAC 12 were selected for biomass and ethanol production analysis. Comparison of three cultivation modes (stationary, shaken and aerated) resulted in the highest biomass productivity obtained for aerated cultures that yielded 0.09 g and 0.11 g dry weight per day per liter of medium for M. afer PKUAC 9 and S. abundans PKUAC 12, respectively. Carbohydrate accumulation monitored by FTIR showed that early stationary phase is optimal for biomass harvest. Microalgal biomass was successfully used as a carbohydrate feedstock for fermentative bioethanol production. Scenedesmus abundans PKUAC 12 was superior feedstock for bioethanol production when pre-treated with the combination of dilute acid treatment and cellulase. Additionally, two strains, Chlorella Sorokiniana 21 and Monoraphidium 3s35, were found to produce large amount of long-straight chain alkane and fatty alcohols under the optimized cultivation conditions

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and can be used to produce important supplements for petrodiesel-like fuels and chemicals used in the cosmetics and food industries.

### Pengcheng Fu

Viable and alternative fuels for commercial aviation uses can play an important role for the emission reduction and sustainable economic development. PetroChina and Boeing are leading respective teams in China for exploring second-generation biofuel feedstocks and processes that have the potential to reduce greenhouse gases throughout their entire lifecycle.

Three interactive efforts have been made in order to develop a sustainable aviation fuel industry in China: 1) Industrial evaluation of feedstocks, processing and demand/distribution for aviation biofuels; 2) demonstration flights and 3) Supply-chain R&D focused on algae feedstock and processing techniques.

The study may result in the environmentally progressive fuel solutions (bio-jet fuels) that differ from traditional biofuel development. Jet biofuels will incorporate second-generation methodologies relative to sustainable feedstock source selection and fuel processing, which are uniquely suited for aerospace applications. These jet biofuels can potentially be blended with traditional kerosene fuel (Jet-A) to reduce dependency on petroleum-based fuels. Additionally, sustainable feedstock sources may avoid deforestation

practices and potential competition with global food resources, while helping to lower aviation carbon dioxide outputs. Details will be discussed.

### Weiwen Zhang

Photosynthetic Cyanobacteria have attracted significant attention as a "Microbial factory" to produce biofuels and various fine chemicals, mostly due to their abilities to utilize CO<sub>2</sub> and sunlight directly as carbon and energy sources, respectively. We will report our recent progress on engineering cyanobacterium *Synechocystis* sp. PCC 6803 to produce 3-hydroxybutyrate (3HB) and butanol directly from CO<sub>2</sub>, and on strengthening *Synechocystis* for better tolerances against toxic products.

### Zhongxin Yang

The focus of my presentation is the industrialization possibility of microalgae as a fuel in China. From a business perspective, the cost is the main limiting factor to the microalgae fuel industrialization. The cost consists of the following four technical aspects: algal species, cultivation site and cultivation technique, harvesting and extracting technology, converting technology and final products application. Furthermore, my presentation will explore the possibility of algae cultivation in various regions in China, as grouped by energy adequacy, water and land availability, the abundance of carbon dioxide, the suitability towards factory farming, and so on. To be considered the most ideal location for microalgae

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biofuel production, the following criteria have to be met: 1) There has to be an abundance of energy, i.e. a large variance in temperature throughout the day, and algae cultivation can be maintained throughout the year in nature condition; 2) Virtually no rainy days, and more clear days. This is conducive to year round algae farming; 3) Adequate water resources, including fresh water, salt water, waste water, and sea water; 4) The availability of economical land as granted for such purposes.

**Tuesday, December 10, 2013 | 4:00pm – 5:30pm**

### **Biosynthetic Pathways to Desired Renewable Chemicals**

*An industrial biotechnology pipeline for optimizing isoprenoid production in Saccharomyces cerevisiae*

Moderator: Sunil Chandran, Amyris

*Systematic level engineering E. coli and S. cerevisiae efficiently producing aromatic amino acid and ethanol*

Liu Shuangping, Jiangnan University

*Renewable Chemicals from Waste Carbon*

Lisa Dyson, Kiverdi

*Clarification of succinic acid fermentation broth by ultrafiltration and the associated membrane fouling mechanism*

Jianmin Xing, Institute of Process Engineering, CAS

### **Abstracts**

#### Sunil Chandran

Amyris is building an integrated renewable products company to apply industrial synthetic biology to genetically modify microorganisms, primarily yeast, to serve as living factories. These modified yeast strains convert plant-sourced sugars into potentially thousands of isoprenoid molecules, providing a broad range of renewable chemicals and transportation fuels. Synthetic biology approaches at Amyris have accelerated creation and improvement of *S. cerevisiae* strains that make high levels of isoprenoids. This presentation will describe our integrated yeast strain engineering and phenotyping pipeline and its application for combinatorial exploration of thousands of complex genotypes to continuously improve isoprenoid-producing strains over the past five years.

#### Liu Shuangping, co-authors Liang Zhang, Guiyang Shi

National Engineering Laboratory for Cereal Fermentation Technology (NELCFT) is an advanced biomass biotransformation research center in China. Some microorganisms producing organic acids and ethanol are being built by applying industrial synthetic biology in NELCFT. In microorganisms, the control architectures of the biosynthesis pathway for desired chemicals always

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form a system, it is difficult to release all the drawbacks generated by high intracellular concentrations of desired chemicals or intermediates. With the development of contributing tools (hosts, vectors, genetic controllers, and characterized enzymes) in synthetic biology, the costs and time are decreased to develop an effective cell factory at systematic level. The biosynthesis of aromatic amino acids (like phenylalanine, tyrosine and tryptophan) is one of the most complicated amino acid synthesis pathways. In this study, the engineering of a L-phenylalanine (Phe) producer was carried at systematic level: modify the PTS system to moderate the glucose uptake rate decreasing the acetic acid formation; genetic switch on or off the expression of target genes to conciliate cell growth and precursors synthesis; attenuate the shunting pathway to construct non-growing cells; and also increase the enflux by expression the exporter. After these works, an industrial *E. coli* with high productivity and yield was constructed which was comparable or even better than existing producing strains. Based on the chassis above, the organisms producing D-phenylalanine, L-phenylglycine and tryptophan are being constructed. Besides, our team also constructed a super *S. cerevisiae* at the system level with the abilities of higher permeability resistance, lower

glycerol formation, cellobiose and protein utilization, and intracellular redox balance.

### Lisa Dyson

Kiverdi is an advanced sustainable oils and chemicals company developing bio-based replacements to oleochemicals and petrochemicals using our proprietary Carbon Engineering Platform, a bioprocessing technology capable of converting low-cost, abundant waste carbon in form of syngas or industrial waste gas into high-value renewable chemicals at a fraction of the cost of current approaches. Kiverdi's technology uses a class of proprietary chemoautotrophic microbes that act as "whole cell" biocatalysts in our innovative 1-step bioprocess for the direct production of renewable organic molecules from gaseous inorganic carbon and hydrogen for a wide variety of product applications such as surfactants, polymers and fuel additives.

With increasing levels of waste generation and decreasing available space for disposal, each year 134M tons of landfill, 170M tons of agriculture and 60M tons of forest waste generated in the United States is an untapped biomass resource to produce high value chemicals. Kiverdi's solution fills a "scale gap," that enables localized, low CapEx deployment of waste-derived syngas conversion plants to target diverse chemistries to make drop-in and custom renewable chemicals with mid to long carbon chain lengths, varying

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levels of unsaturation, and added functional groups that serve as sustainable intermediates for surfactants, polymers or fuel additives that can compete on cost and performance. Our feedstock flexibility and high-yield bioprocess enables us to produce drop-in and custom renewable chemicals allowing us offer downstream customers predictably higher margins and supply certainty for achieving their sustainability goals.

#### Jianmin Xing

Ultrafiltration was investigated to clarify succinic acid fermentation broth in consideration of integrating fermentation and separation process and removal the product in situ. Different membranes (PES 100kDa, PES 30kDa, PES 10kDa and RC 10kDa) were used and two models were applied to analyze the fouling mechanism. Results indicate that ultrafiltration is feasible in clarifying succinic acid fermentation broth. Almost all the microorganism cells (99.6%) were removed from the fermentation broth. Proteins were also removed effectively by all membranes selected in this study. The removal rate was 79.86% for PES 100kDa, 86.43% for PES 30kDa, 86.83% for PES 10kDa, and 80.06% for the RC 10kDa. After ultrafiltration, clearer permeate was obtained comparing with the centrifugation. Among all tested membrane, PES 10kDa gave the best results which showed relative higher initial flux (18.86 L/m<sup>2</sup> h), highest protein removal rate (87%), and less flux drop rate. Resistance-in-series model was applied to determine

the main factor that caused the operation resistance. Results showed that most membranes selected in this study tended to be fouled by cake layer or concentration polarization. Hermia's model, which is composed of four individual sub-models, was used to analyze the predominant fouling mechanism for the used membranes. Results showed that the fouling of RC 10kDa and PES 30kDa was controlled by the complete blocking mechanism, while PES 100kDa was controlled by the intermediate blocking and PES 10kDa was controlled by cake layer. This conclusion was also proved by SEM photos. Membrane characteristics were analyzed before and after ultrafiltration by AFM and goniometer. Both contact angle and roughness of most membranes increased after ultrafiltration.

**Wednesday, December 11,  
2013 | 8:30am – 10:00am**

#### **Techno-economic and Physical Analysis for Microalgae Growth**

Moderator: David Hazlebeck, Global Algae Innovations

##### *Algal Lipid Trigger and Grow Tank System*

Mark Randall, T2e Energy, LLC

##### *Techno-economic and Fluid Dynamics Analysis for Growing Microalgae with the Intent of Producing Biofuel Using a System Model*

Leah Raffaelli, University of Denver

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*A Dynamic Imaging Cell Monitoring System for Real-Time Analysis of Algae-to-Biofuel Production*  
Victoria Kurtz, Fluid Imaging Technologies, Inc.

*Algae Testbed Public Private Partnership (ATP3): An Experimental Framework for Performing Long Term Cultivation Trials Across Different Regional, Seasonal, Environmental, and Operational Conditions*  
John McGowen, Arizona State University, AzCATI

## **Abstracts**

Mark Randall

T2e Energy Holdings ("T2e") is a start-up alga to biofuels company located in San Diego dedicated to use its patented and third party verified LipiTrigger™ technology to produce high value algae products. T2e's LipiTrigger™ and grow tank technology not only increases algae biomass productivity and lipid content but also improves algae lipid profiles favorable for biofuels production. T2e's novel intellectual property uses a highly sustainable, waste resources-based mixotrophic approach in conjunction with our patent pending grow tank system to provide certain strains of algae with the most optimal controlled growing environment. This approach has achieved unequalled results including greater algal density while favorably changing the oil profile that serves the biochemical/bioplastics and biofuels industry. Controlling every variable of the algal growth process

enables T2eH to produce a predictable level of oil and biomass production with a symbiotic modular system co-located near existing coal, gas, or wood fired power plants. With this approach T2e can produce a gallon of algal oil at scale for less than \$1.00/gallon anywhere in the world.

The presentation will cover results from UCSD's Center for Algae Biotechnology, US Department of Agriculture and Ohio State's Ohio BioProducts Innovation Center that verify the level of algal growth including total biomass, lipids and the resulting change in oil profile. We will also discuss our unique methodology for our mixotrophic system that includes our patented algae grow tank system that enables T2e to grow algae adjacent to existing power plants anywhere in the world.

Leah Raffaelli

Techno-economic and systems studies to date are abbreviated and missing a number of important variables. By including these variables in a detailed model integrating biology, chemistry, engineering, and financial aspects, a more defined fluid dynamics and financial analysis are possible. Through optimizing the model productivity based on the resulting net profit, the system analysis results in a more accurate assessment of environmental and economic sustainability of specific algal growth scenarios. Photobioreactor algal growth scenario optimization in the system model has resulted in realistic engineering design requirements. Results show feasibility for

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photobioreactor growth scenarios to be economically sustainable when co-products are included, but definite technological advancements and growth improvements must be made. The main factors inhibiting a cost effective photobioreactor growth scenario are culture density, temperature, and lighting distribution for solar lit photobioreactors, and lighting cost for artificially lit photobioreactors. Open pond algal growth scenarios do not show any prospect of economic or environmental sustainability with current technology due to the large amount of surface area required, inefficient water use, and low culture density. All algal growth scenarios are inferior to petro-diesel regarding energy inputs, carbon emissions, and environmental sustainability. No algal growth scenarios included in this study come close to meeting the U.S. requirement of biofuel emitting at least 20% less carbon emissions than diesel from crude oil.

## Victoria Kurtz

This presentation will detail a novel new system (patent applied) for real-time analysis and monitoring of algae production. The system uses in-flow digital imaging to capture images of all representative cells or other microorganisms in photo bioreactors or raceway ponds. Sophisticated image processing algorithms are used in real time to segment each microorganism from the background, and record over 30 size, shape and gray-scale measurements for each microorganism. Cell size and

concentrations are produced in real-time, and are used for trend analysis. This system can be hooked into any part of the production flow loop for analysis at any point in the process. The system is Class I, Div. I compliant, and automatically cooled to maintain proper working temperature on-site. Using a unique auto-dilution system, the concentration is adjusted for optimum presentation of the microorganisms to the imaging system. Since every particle image and its measurements are saved by the system, it creates an ironclad audit trail for how data was recorded. Test data collected in the field will be shown illustrating typical results from the system. A short video will show how the system works in real-time, including how the particle images are acquired and measurements made. Finally the results of the analysis will be shown, detailing how the system can be used to monitor microorganism size and concentration, and in particular, identification of predators.

## John McGowen

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

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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), and Georgia (Georgia Institute of Technology), ATP3 provides comprehensive cultivation and harvesting facilities along with the required experience of operating an open testbed system.

A primary objective is to utilize our team's 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 is implementing an experimental framework named "Unified Field Studies" (UFS).

Through our UFS framework, we will generate 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. System and scale variation has the potential to induce unwanted non-geographical-related variability between testbeds as a function of differences in system design and scale of operation between sites. In Phase 1 of our project, this variation is being minimized at the five testbed sites via the adoption of a uniform design of outdoor raceway pond systems with respect to: size, geometry; volume; depth and hydrodynamic mixing. Identical outdoor raceway ponds have been installed at each site using a standard geometry, paddle wheel design, and monitoring and control systems. As it is also essential that each site operates to a standard set of protocols with respect to experimental study timing and duration, operating conditions, as well as sampling and analytical protocols for a fixed set of parameters, we have spent Phase 1 harmonizing protocols across all sites. Without these controls for our cultivation systems and protocols, the inter-site variability would be more difficult if not impossible to interpret. For this presentation, we will present current progress on the initial

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experimental design framework, our harmonization and alignment activities and results from our initial cultivation trials.

## Technical and Research Presentations

**Monday, December 9, 2013 | 8:30am – 10:00am**

### Development of New Industrial Enzymes

*The biotech (r)evolution in oilseed crushing & refining*

Moderator: Tim Hitchman, DSM

*Computational Design: Enabling New Products, New Enzymes, and New Pathways*

Eric Althoff, Arzeda Corp.

*Developing novel strategies on laccase production and purification: Recent advances and new trends*

Feng Wang, Institute of Process Engineering, CAS

*An Industrial Scale Platform for Enzymes and Other Proteins*

Danai Brooks, Dyadic International, Inc.

### Abstracts

Tim Hitchman

Degumming is the removal of phospholipid impurities from oil. This step results in the majority of loss of oil yield associated with purification of crude soybean and canola oils, among

others. The reason for the yield loss is that phospholipids are emulsifiers and drag oil with them as they are removed. Enzymatic degumming with Purifine® PLC is a unique process that results in increased yield of degummed oil by reducing heavy phase oil losses and release of the DAG component of the gums. The value of implementing Purifine PLC degumming is further enhanced by integration into downstream refining (for edible oil or biodiesel end uses) and improvements in quality of co-products such as meal. Enzymes for degumming of vegetable oils are hardly a new thing, having first been introduced to the industry in the early 80's. Since then, a steady stream of technical publications has demonstrated that degumming using enzymes is feasible and can be applied to deliver oil that exceeds standard quality specifications. Only in recent years, the economic benefits of enzyme degumming have also been quantified, those processors who have taken the lead in adopting the technology for degumming of crude soybean oil are now documenting yield gains of up to 2%. These data now allow the industry to establish benchmarks for overall economics of investment for future implementations. This practical case about technology developments in the oilseed crushing & refining industry will illustrate how to fast forward evolution into revolution and provide direction for successful biotech commercialization.

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### Eric Althoff

The recent emergence of industrial and synthetic biotechnology has the potential to radically transform the chemical industry. Despite significant success, the availability of efficient biocatalysts is one of the major limitations to design novel cell factories that can produce valuable chemicals renewably. To this end, we developed novel computational methods and applied them to rationally engineer enzymes with a wide range of activities, including the de novo design of enzymes catalyzing a retro-aldol reaction, a Kemp elimination reaction, and a Diels-Alder reaction. 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 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. The demonstrated success and wide applicability of our methods open the way for the design of a variety of novel biocatalyst necessary for the efficient development of

biosynthetic pathways for the industrial scale synthesis of high value chemicals.

### Feng Wang

Laccase belongs to a group of copper-containing blue oxidases with the capability of catalyzing one-electron oxidation coupled to the reduction of molecular oxygen to water. Because laccase can oxidize phenolic compounds, aromatic amines, and even nonphenolic substrates in the presence of redox mediators, it is widely used for various purposes such as pulp delignification and bleaching, wastewater treatment, dye decolorization, food processing, biopolymer modification and biosensors. However, low enzyme yield in laccase production and high-cost in laccase purification limit its use in industrial applications. Great efforts have been made to enhance laccase production, involving isolation and breeding of high-producing strains, medium optimization, inducers utilization, reactor design and recombinant expression of laccase genes in different hosts. Methods for laccase purification have been developed for different microbial species, including ammonium sulphate precipitation, ultrafiltration, three-phase partitioning, gel filtration, ion exchange, and affinity chromatography. It is crucial to improve laccase production and purification for the high demand of laccase from industry. In this paper, we critically review recent advances in laccase production and evaluate the advantages, drawbacks, and

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scalability of developing technologies for laccase purification. In addition, novel strategies for the enhancement of laccase yield and magnetic separation methods for simple and efficient purification of laccase are put forward according to our recent achievement on laccase research.

### Danai Brooks

What is C1? A robust and versatile fungal platform for gene discovery, expression and the production of enzymes and other proteins. Based on the *Myceliophthora thermophila* fungus, a soil-borne saprophyte that secretes cellulases. Developed in the early 1990's through a fortuitous UV-induced mutation and continuously bioengineered since. Addresses the critical bottlenecks of protein discovery, development, scale-up and commercialization. Enables new product introduction with less time, cost and risk. Enables new product introduction with less time, cost and risk. Broad platform capabilities validated through 17 years of R&D and 15 years of product sales and partnerships with key players.

**Monday, December 9, 2013 |  
10:30am – 12:00pm**

### **Stabilization in Fermentation Processes**

*New Yeasts for Grain Based Ethanol*  
Moderator: William Kenealy, Mascoma Corporation

*Study on anaerobic fermentation of different organic wastes for biogas production*

Priyanka Gupta, Birla Institute of Technology, Mesra, Ranchi

*Production of ethanol from pine needles using different fermentation strategies*

Parvez Singh Slathia, Shri Mata Vaishno Devi University

*Continuous Fermentation: Prospects for Future Rollout*

Joshua Velson, Nexant

### **Abstracts**

#### William Kenealy

Mascoma has developed advanced yeast products for grain-based ethanol production. By expressing starch-degrading enzymes in situ during the fermentation, TransFerm™, can reduce the cost of separately purchased enzymes as well as increase the yield and rate of ethanol production. The genetic modifications used to create TransFerm™, are built in a robust ethanol production strain, resulting in a yeast product that is truly a "drop-in" solution for ethanol producers. TransFerm™ Yield + is an improved strain that also makes less glycerol thereby resulting in a higher yield compared to conventional yeast. The increased yield was enabled by introduction of an alternative metabolic pathway designed to complement the reduction in glycerol. The in situ production of glucoamylase also reduces the concentration of glucose present initially during

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fermentation. As a result, TransForm Yield + is able to rapidly produce ethanol while producing less glycerol in response to osmotic stress. The combination of these interactions provides the basis for ethanol yield improvements of up to 5%. Mascoma has teamed with industry leaders ICM and Lallemand Biofuels and Distilled Spirits to bring TransForm products to the ethanol production industry.

### Priyanka Gupta

Priyanka Gupta, Raj Shekhar Singh, Ashish Sachan, Ambarish S. Vidyarthi  
Current environmental problems have led to an increased interest in anaerobic digestion of solid wastes. Solid wastes can be stabilized by the process of anaerobic digestion thereby generating biogas, which can be used as renewable energy source. Biogas is a source of energy which will leave less of an environmental footprint, as compared to coal or oil. The objective of this study was to see the performance of the selected organic wastes in a single stage anaerobic reactor for biogas production. In this study, experiments were conducted using different organic wastes i.e. vegetable waste, garden waste, saw dust, fruit wastes, and sugarcane bagasse, under anaerobic conditions. Mine water (from Jitpur colliery, India) was used as inoculum for the biogas production. The study was carried out in a specially designed laboratory scale reactor for a period of two months. Amongst the considered biomasses the highest methane yield of 212.87cc was observed with vegetable waste.

### Parvez Singh Slathia

Today most energy demands are met by non-renewable energy resources that may lead to resource depletion and environmental problems. A demand to develop novel renewable energy harvesting technologies is urgent and the production of ethanol from biomass has been focused upon recently. Most ethanol has been produced from starch based plants causing high food and feed prices. Ethanol from lignocellulosic biomass such as wood, agriculture wastes, energy crops, wastes from pulp and paper industry is seen as future technology.

In the current study we have tried to use pine needles from *Pinus roxburghii* as a source for ethanol production. Pines are coniferous, evergreen, resinous tree belonging to the genus *Pinus* of the family Pinaceae. Pine needles from *Pinus roxburghii* were obtained from forests of Tikri, Udhampur, J&K (India). The Pine needles were dried and comminuted to pass through a sieve of 2 mm. Different concentrations of acid ( $H_2SO_4$ ) and alkali (NaOH, KOH, aqueous  $NH_3$ ) were used in conjunction with steam and pressure for varying time intervals for maximizing the sugar release. The biomass stream after pretreatment was filtered and pH adjustment was made in the filtrate. The solid fraction was dried and again treated with steam for obtaining sugar from this fraction. Enzyme treatment of both the fractions was done using

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commercial cellulase and pectinase enzymes and optimization of time for maximum sugar release was done. Enzyme loading of 5U/g of biomass was used. For fermentation *Saccharomyces cerevisiae* and *Kulyveromyces marxianus* were used in two different modes SSF (Simultaneous Saccharification and Fermentation) and SHF (Separate Hydrolysis & Fermentation). Ethanol concentration was measured by Gas chromatography at regular intervals.

In our study acid treatment proved to be better in releasing sugar than alkaline. Both the methods were used to carry out the further study. Enzyme treatment with the both the enzymes combined gave better sugar yield when enzymes were used in isolation. In fermentation studies, it was seen that SSF proved to be a better method yielding more ethanol concentration than SHF. While using biomass loading of 5g in the pretreatment processes fermentation yield of .2g of ethanol was obtained as the best production. Further, *Kulyveromyces marxianus* was a better producer of ethanol having a higher conversion efficiency of 52% than that of *Saccharomyces cerevisiae* which is 47%. The biomass residue left after first pretreatment has considerable amount of sugar which was used for ethanol concentration. Thus, pine needles are an attractive source for ethanol production and technology needs to be developed for their use in fuel ethanol industry.

Joshua Velson

Continuous fermentation is an oft-cited but rarely realized goal for commercial biotech processes. Despite the possible benefits of continuous fermentation configurations, the critical problems in maintaining steady state production with living organisms introduce many risks with scale. This presentation will present a brief overview of the history of continuous fermentation, its advantages and disadvantages, and the prospects for the future, focusing on emerging process configurations, how they mitigate many of the problems with older designs, and finally which bioproducts are most likely to see commercialization of continuous fermentation processes in the future. Designs including free-cell chemostats, immobilized cell bioreactors and membrane-integrated bioreactors will be discussed.

**Monday, December 9, 2013 |  
2:30pm – 4:00pm**

### **Advancements in Biological Processes in Industrial Biotechnology**

Moderator: Bhima Vijayendran,  
Redwood Innovation Partners, LLC

*Performance of a Carboxydotherrmus hydrogenoformans gas lift reactor for syngas upgrading into hydrogen*  
Mathieu Haddad, University of Montreal

*Thermophilic Consolidated Bioprocessing of Cellulosic Materials to Biohydrogen*

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Lew Christopher, South Dakota School of Mines and Technology-CBRD

*Synthetic Biology - A Tool for Self Replicating In Situ Resource Utilization*  
John Cumbers, NASA Ames Space Portal and SynBioBeta

### **Abstracts**

Mathieu Haddad, co-author Serge R. Guiota

Gasification of biomass and waste is a way to produce non-fossil hydrogen (H<sub>2</sub>) through high temperature (750–1500°C) conversion of carbonaceous material into a synthesis gas (syngas) mainly composed of carbon monoxide (CO), carbon dioxide (CO<sub>2</sub>) and H<sub>2</sub>. The final calorific value of syngas, that is its H<sub>2</sub> to CO ratio, varies depending on the feedstock type used and gasification conditions.

One typical way of achieving augmentation of the H<sub>2</sub> content of syngas in industry is through the use of the water-gas shift (WGS) reaction:  $\text{CO} + \text{H}_2\text{O}_v \rightarrow \text{CO}_2 + \text{H}_2$ . The WGS is catalyzed using catalysts that are highly intolerant to sulfur and need regeneration, and the process is energy-intensive, namely for steam generation.

Alternatively, biologically-mediated WGS reactions possess several advantages over conventional chemically mediated WGS mechanisms; such as enzyme specificity, improved yields, overall

cost efficiency and environmental attractiveness.

Of interest to this study is the obligate anaerobic *Carboxydothemus hydrogenoformans*, an extreme thermophilic (70°C) bacillus that uses CO as sole source of carbon and energy and catalyses the WGS reaction.

We investigated and optimized, in a 35 L gas-lift reactor, H<sub>2</sub> conversion of CO by a pure culture of *C. hydrogenoformans*. The reactor was operated with a continuous supply of gas for 3 months. Reactor performance was evaluated under various operational conditions, such as gas recirculation (0.3 and 1.5 L/min), CO loading rate (from 0.05 to 0.46 mol.L<sup>-1</sup><sub>reactor</sub>.d<sup>-1</sup>) and nutrient addition to the medium. Overall, results indicated a constant H<sub>2</sub> yield of 95±1% and 82±1% (mol<sub>H<sub>2</sub></sub>.mol<sup>-1</sup><sub>CO</sub>) in a supported and unsupported growth medium respectively regardless of the operational condition tested. Once the biomass concentration was sufficiently dense, a maximum CO conversion activity of 0.17 mol<sub>CO</sub>.L<sup>-1</sup><sub>reactor</sub>.day<sup>-1</sup> or 3.79 L<sub>CO</sub>/L<sub>reactor</sub>.day<sup>-1</sup> was achieved. One major parameter that impacted both biological activity and volumetric mass transfer was the gas recirculation/CO feed ratio. We here demonstrate that, as long as this ratio is higher than 40, mass transfer limitations are bypassed resulting in maximum conversion efficiency (90.4±0.3%) and biological activity (2.7±0.4 mol<sub>CO</sub>.g<sup>-1</sup><sub>volatile suspended solid (VSS)</sub>.day<sup>-1</sup>).

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### Lew Christopher

An environmentally friendly and potentially viable alternative for sustainable H<sub>2</sub> production is presented through the utilization of renewable cellulosic materials. Switchgrass (SWG) is viewed as one of the most promising energy crops for the U.S. conditions with low nutrient/water requirements and high adaptability to any weather/soil/land conditions. Production of SWG is projected to increase ten-fold within next ten years. Furthermore, its high carbohydrate (>65%) and low lignin (<20%) content favors a fermentation route for SWG utilization. On the other hand, the handling and disposal of municipal solid waste (MSW) is of growing global concern. In the U.S. only, MSW recently reached 5 lb per capita per day. However, MSW contains approximately 60% of biodegradable material which can be utilized for bioenergy production. The H<sub>2</sub> production capabilities of the extreme thermophile

*Caldicellulosiruptor saccharolyticus* DSM 8903 were examined on SWG and MSW, with glucose and microcrystalline cellulose (MCC) serving as references. It was demonstrated that *C. saccharolyticus* can ferment SWG bioH<sub>2</sub> in a single step at 11.2 mmol H<sub>2</sub>/g without any physicochemical or biological pretreatment. In comparison, the bioH<sub>2</sub> yields on MSW were approximately 4-fold lower. BioH<sub>2</sub> production from glucose reached the theoretical maximum for dark fermentation of 4 mol H<sub>2</sub>/mol glucose,

whereas MCC produced 9.4 mmol H<sub>2</sub>/g cellulose at 7-fold higher H<sub>2</sub> production rates than SWG. The consolidated bioprocessing (CPB) capabilities of *C. saccharolyticus* present opportunities for cost savings of capital and operational expenses in excess of 50%. The advantages of utilizing thermophilic microorganisms on low-cost carbon feedstocks without prior thermo-chemical treatment for bioH<sub>2</sub> will be discussed.

### John Cumbers

Gravity is the force of nature that drives the cost of space exploration and our eventual settlement of the solar system. To send a kilogram of mass from the Earth into low earth orbit currently costs between \$5,000 to \$20,000 depending on the launch vehicle. Every human breathes out one kilogram of CO<sub>2</sub> every day. Currently we do not have technologies capable of fully closing the CO<sub>2</sub> loop in space, meaning that we are wasting valuable resources.

*In situ* resource utilization (ISRU) is a set of technologies developed by NASA and others for reducing the cost of access to space by utilizing the resources on other planetary bodies and in closed loop life support systems. Biology offers an advantage over physical and thermal systems because a small initial mass can be sent into space and replicate into a much larger mass from the resources around it.

In this talk I will outline a new concept of resource utilization for planetary

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sustainability. I will then outline the resources that are available in different space environments and discuss some of the technologies that could be used for the production of food and other consumables that could be made there.

**Tuesday, December 10, 2013 |  
2:00pm – 3:30pm**

### **Feedstock Conversion Technologies**

*Novel Solid Catalysts for Production of Renewable Sugars and Chemicals from Lignocellulosic Materials*

Moderator: Brian M. Baynes, Midori Renewables, Inc.

*Lignocellulosic biomass as potential substrates for the white biotechnology*  
Joachim Venus, Leibniz Institute Agricultural Engineering (ATB)

*Characterization of an adapted microbial population to the bioconversion of carbon monoxide into butanol using next-generation sequencing technology*

Guillaume Bruant, National Research Council Canada

*Evaluation of second generation biofuels production from native halophytes by chemical-characterization of *Salicornia sinus-persica**

Alassali, A., Cybulska, I., Thomsen, M. H., Masdar Institute of Science and Technology

### **Abstracts**

Brian M. Baynes

Solid catalysts, despite their broad applicability as the workhorses of the petrochemical industry, have seen little penetration in biomass hydrolysis, because the required catalytic power, lifetime, and costs have been difficult to achieve. Here we introduce a family of novel, chemically-defined materials that are highly potent and reusable biomass hydrolysis catalysts. Mild operating temperature, relatively short residence time on the order of an hour, and easy catalyst reuse combine to enable biomass to be converted to sugar and other products at very low cost. Product selectivity can be tailored to yield (1) fermentable sugars, (2) soluble fiber, or (3) sugar dehydration products such as HMF/furfural. This talk will highlight Midori's work in invention and development of this technology through 100 kg/hr pilot tests over the past four years and its potential to make ultra low cost sugars and other renewable products a reality.

Joachim Venus

Renewable feedstocks (e.g. crops, lignocellulosics, green biomass, residues) can be utilized directly, e.g. as energy carriers, as packaging materials, as fibres, for the production of colouring agents or as lubricants. However, they can also be converted biotechnologically by enzymes and microorganisms, giving us access to a multitude of new, biocompatible products and possible uses. Often the

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economy of bioprocesses is still the problem because in the case of bulk products the price is affected mainly by raw material costs. The production of environmental friendly, climatically sustainable basic chemicals based on renewable raw materials can help to save limited fossil resources and to increase the economic potential of rural areas. The cultivation and utilization of renewable resources for the non-food application become an alternative source of revenue in agriculture and forestry. Even though the manifold potential is used only for a small part today the production and processing of renewable resources will be a growing economic factor in future. The sugars after the pre-treatment of several agricultural and forestry feedstocks can be converted by lactic acid bacteria to produce lactic acid. Lactic acid, its salts and esters have a wide range of potential uses and are extensively used in diverse fields, e.g. bioplastics. The goal is to develop a fermentation process based on the substitution of expensive nutrients and supplements by cheaper materials from renewable resources due to their main proportion of the whole process costs. Besides the basic research projects respecting the screening and characterization of microorganisms, phenotypic optimization, down-stream processing of fermentation products, application and refining of lactic acid, economic assessment of bioconversion processes the scale-up to a technical scale of several processing steps have to be developed for transferable solutions of bioconversion

technologies of renewable materials. For that purpose a multifunctional pilot plant was planned and built at the site of ATB to investigate different raw materials and products. The construction of a pilot facility for the production of lactic acid from renewable resources consequently fills a gap in the various phases of bioprocess engineering from applied fundamental through application research to the launch of biotechnological processes in practice. First results of lactic acid fermentation in a 450-L-bioreactor will be presented. 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 down-streaming 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 electro dialysis with monopolar and bipolar membranes.

### Guillaume Bruant

Microbial production of butanol is still conventionally based on the utilization of carbohydrates as carbon feedstock.

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More and more companies are interested in using alternative carbon sources, such as biomass, notably by combining conversion of biomass into syngas via gasification and microbial fermentation of syngas components. To date, only few syngas-fermenting microorganisms that can produce butanol are known. Discovering new microorganisms or microbial consortia capable of fermenting syngas into liquid biofuels, and engineering them to make them commercially attractive is primordial in a strategy to develop an economically viable platform for biobutanol production.

This presentation will discuss the use of next-generation sequencing technology to perform microbial community analyses of anaerobic undefined mixed cultures, with the objective to identify microbial species particularly adapted to the bioconversion of carbon monoxide (CO), a major component of syngas, into butanol. CO enrichment experiments were performed with an anaerobic granular sludge treating agricultural wastes. The sludge was incubated during two months at mesophilic temperature, with continuous CO injections in the headspace, creating an atmosphere of 100% CO. Liquid samples were collected after one and two months of operation, for subsequent microbial community analyses. Presence of volatile fatty acids (VFA) and alcohols was verified by gas chromatography after two months. DNA was extracted from the initial sludge and from the samples collected during enrichment

experiments. Bacterial and archaeal 16S rRNA genes were amplified and then sequenced using the Ion Torrent sequencing technology.

Data generated were analyzed using the ribosomal database project (RDP) Classifier web tool. Notable differences were observed in the microbial community structure between the initial anaerobic microbial consortium and the CO-adapted population obtained after enrichment experiments. Diversity of the bacterial population notably decreased after CO enrichments, with 17 different phyla identified in the initial sludge, as opposed to 14 after one month and 11 after two months of operation. Bacteroidetes, Actinobacteria and Proteobacteria, which were the dominant phyla in the initial sludge, significantly decreased after CO enrichments. On the opposite, Firmicutes became ultra dominant, representing more than 82% of the total bacterial sequences after two months. The order Clostridiales became the most important in number, with *Acetobacterium* being the most representative genus (49.8%). Archaeal diversity was less important since almost all the sequences were classified into the same phylum, Euryarchaeota, for both the initial anaerobic microbial consortium and the CO-adapted population. After CO enrichments, the class Methanobacteria increased, with *Methanobacterium* becoming the most representative archaeal genus (44.6% of the total archaeal sequences). On the opposite, the class

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Methanomicrobia, initially dominant, decreased. The Ion Torrent sequencing technology allowed us to monitor the evolution of the microbial population during CO enrichments, and gave insights on microbial species particularly adapted to the bioconversion of CO into butanol. Future work will now include using known bacterial solvent producers to bio-augment the adapted consortium, to improve its butanol production and optimize and stabilize the performance of the process.

#### Ayah Alassali

1. Introduction: Abu Dhabi exemplifies a coastal desert, where seawater could be used for salt-tolerant crops (halophytes) cultivation. The produced halophyte biomass could be utilized in feed, food and/or energy production, depending on its chemical composition. In this study the UAE native halophyte *Salicornia sinus-persica* was studied for its potential to be used as a feedstock for bioethanol production. Fresh *Salicornia sinus-persica* contains more than 65% of water. For such green biomass direct fractionation and fermentation can be advantageous. This allows for water preservation and the ability to run at lower dry matter in the fermentation step. Chemical characterization and ethanol potential of the juice and fibers of the fractionated *Salicornia sinus-persica* was examined in this study.

2. Methodology: Two batches of *Salicornia sinus-persica* (washed and unwashed) were juiced, where two

main fractions were obtained (juice and fibers). Washing of the fresh biomass aims to reduce or remove the nonstructural ash (salt deposits). Both fractions were tested for their total dry matter and ash content. Sugar monomer composition was tested for both fractions applying acid hydrolysis as described in (Sluiter et al., 2008a). The extent of glucan-to-glucose convertibility was tested for the juice by enzymatic hydrolysis (by simultaneous saccharification and fermentation (SSF)) and acid hydrolysis followed by fermentation by baker's yeast. High Performance Liquid Chromatography was used for ethanol, sugars and other metabolites analysis as described in (Sluiter et al., 2008b).

3. Results: The juice fractions were found to represent  $68.86 \pm 1.78\%$  of the unwashed batch and  $74.09 \pm 3.68\%$  of the washed batch. The fiber fractions were found to contain 98.99% (unwashed biomass) and 99.52 % DM (washed biomass) of which 19.77% (unwashed biomass) and 19.73% (washed biomass) was ash. Dry matter content of the juices were found to be 13.06% (unwashed) and 11.58% (washed) of which 58.01% (unwashed) and 57.37 % (washed) was ash. Sugar analysis revealed relatively low concentration of glucose, xylose, and arabinose in the juice fractions (8.40 g/L glu, 5.97 g/L xyl, and 3.42 g/L ara in juice of unwashed biomass and 7.39 g/L glu 4.87 g/L xyl, and 2.17 g/L ara in juice of washed biomass) and not much difference was observed between the

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washed and unwashed biomass. The fiber fractions contained 15.6 g/100 g DM glu, 11.55 g/100 g DM xyl, and 14.04 g/100 g DM ara for the unwashed biomass and 16.67 g/100 g DM glu 13.01 g/100 g DM xyl, and 13.82 g/100 g DM ara for the washed biomass. This is comparable to the lignocellulose content of the mature (dry) plant (Cybulska et al., 2013). The highest ethanol yield in SSF with Baker's yeast was achieved in juice extracted from washed biomass after enzymatic hydrolysis. In this experiment 129.61% of the theoretical yield (based on sugar analysis) was achieved. This shows that fresh juice of *Salicornia sinus-persica* is a good medium for yeast fermentation - but more work is needed to identify all fermentable sugars in the juice.

**Tuesday, December 10, 2013 |  
4:00pm – 5:30pm**

### **Sustainability Performance Value in Industrial Biotechnology Processes**

*Life Cycle Assessment of  
Transportation Fuel Production from  
Hydrothermal Liquefaction of Algae  
Grown in Open Ponds*

Moderator: Benjamin Saydah,  
Sapphire Energy

*Is Wood Bioenergy Carbon Neutral?*  
Roger Sedjo, Resources for the Future

*The Commercialization of Readily  
Biodegradable, Biobased Fluids and  
Cleaners, and the Motivations Driving  
Their Adoption*

Mike Guggenheimer, RSC Bio  
Solutions

### **Abstracts**

#### Benjamin Saydah

Life cycle assessment (LCA) has been used widely in recent years to estimate the energy and environmental implications of deploying algae-to-energy systems at scale. Until recently, the emergent nature of the industry required that most LCA studies were based largely on assumptions about how the industry would develop, likely impacting the value of the results. For this study data from a pilot-scale facility is used to estimate the life cycle energy and emissions profiles of algae-to-biofuels production using hydrothermal liquefaction (HTL) as the conversion pathway. HTL is attractive because it is efficient, using the entire algae cell biomass rather than only the lipid fraction, and it is flexible, producing a nonaqueous phase liquid product that can be blended during petroleum refining and upgraded into drop-in fuels, such as gasoline or diesel. The pilot data is used to inform the scale up to commercial scale and a LCA is conducted on the estimates of commercial scale operation. The pilot scale and commercial scale processes are then compared to current bio-fuels process for both their Energy Return on Investment and Greenhouse Gas emissions. Results suggest that the algal biofuel production based on HTL can provide substantial greenhouse gas (GHG) emission reductions compared to petroleum-derived

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benchmarks. Sensitivity analyses reveal several bottlenecks currently holding back HTL-based algae-to-energy production including industrial CO<sub>2</sub>, and nitrogen fertilizer supply chains, biocrude yields and heat recycling. Efforts to address these in industry and the implications on anticipated life cycle impacts are discussed.

### Roger Sedjo

The view that wood biomass for energy is “carbon neutral” since its use has approximately zero net because carbon emissions released in its utilization for bioenergy are subsequently captured in forest regrowth has been challenged by the use of a static accounting view of the forest carbon. The challenge argues that the use of wood for biofuels will result in a decrease in the forest stock and a net reduction in the carbon captured in the forest, at least for an extended period. The argument of this paper is the static approach ignores dynamic economic factors. The anticipated future use of forest for energy provides incentives to increase forestry investments in forest stocks, which will increase carbon sequestration in the near term and offset carbon losses due to increased harvesting of forests for energy. This situation is demonstrated with a dynamic optimization forest management model which shows that investments in the creation of forest biomass can often anticipate future uses and thus forest stock, and thus sequestered carbon, can actually rise

in anticipation of higher future demand. Empirical support for this outcome is provided from the forest experience of the U.S. since the mid-1990s.

### Mike Guggenheimer

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.

**Wednesday, December 11,  
2013 | 8:30am – 10:00am**

**Microbial Synthetic Pathways  
to Renewable Chemicals and  
Biofuels**

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*Bright Science at DSM: Development of a Commercial Lignocellulosic Biomass-to-Ethanol Process*  
Moderator: John Perkins, DSM

*Screening and Analysis Pipeline for the Microbial Production of Renewable Chemicals and Fuels*  
Michael Leavell, Amyris

*DEINOCHEM: The Deino Way to Green Chemicals*  
Nagib Ward, Deinove SA

*Corbion: Developing a biobased products portfolio*  
Hans van der Pol, Corbion

## **Abstracts**

### John Perkins

Royal DSM N.V. is a global science-based company active in health, nutrition and materials. DSM is a frontrunner in applied industrial biotechnology to produce ingredients for food and feed, or in bio-conversion technology for fuels or bio-based chemicals.

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 offer excellent solutions to these challenges of today and even more for future generations.

During the last 5-10 years a tremendous progress was made to continuously drive down manufacturing costs which posed major hurdles for commercialization. It has been recognized that cost optimization needs an integral process view (feedstock, pretreatment, enzymatic hydrolysis, yeast propagation and ethanol fermentation using high performing yeast) rather than optimization of single technologies. This presentation will show how innovation and an integrated research approach are used to drive yeast and enzyme development programs. Latest results to improve thermo-tolerant enzyme mixes, their production as well as advanced yeasts with improved robustness and C5 sugar converting properties will be discussed.

### Michael Leavell

Amyris is an integrated renewable products company focused on providing sustainable alternatives to a broad range of petroleum-sourced products. Our industrial synthetic biology platform converts plant sugars into a variety of molecules that can be used in a wide range of renewable products, including ingredients in: cosmetics, flavors and fragrances, polymers, industrial lubricants, consumer products, and renewable fuels. To enable rapid strain development, a high-throughput strain screening pipeline is needed as well as an in-depth analysis of winning strains using omics technologies. Together these technologies allow identification and characterization of improved

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strains, enabling strain engineers to quickly iterate on strain designs. This presentation will focus on Amyris' high throughput screening platform and omics analysis tools, and present specific examples of how each technology enables strain development.

### Nagib Ward

DEINOVE (Alternext Paris: ALDEI) is a cleantech company that designs, develops and markets novel industrial processes using biotransforming platforms based on the untapped extraordinary and advantageous properties of the Deinococci. Originally founded in France, and principally located in Montpellier, DEINOVE is now offering its technology platforms globally, with commercial representation in both the EU and United States ([www.deinove.com](http://www.deinove.com)).

Taking advantage of the unique genetic properties and unusual robustness of this relatively unknown bacterial phylum, DEINOVE optimizes natural fermentation and metabolic capabilities of these bacterial "micro-factories" to produce renewable green chemicals. The company is structured in two groups: while DEINOL is focused on producing renewable advanced biofuels, DEINOCHEM is focused on green chemicals or intermediates. Of notice, two subsidiaries operate independently, yet falling within the DEINOCHEM group: new antibiotics research (DEINOBIOTICS SAS) and plastics bioremediation (THANAPLASTTM).

DEINOCHEM is focused on producing renewable chemicals and reduced sugars from a variety of starting materials including cellulosic and other renewable feedstocks. The main costs of biochemical production are concentrated on feedstock, enzymes and capital, with feedstock and enzymes often accounting to 50% or more of the total cost. DEINOCHEM's platforms leverage thermophilic organisms (45-55C) that are not subject to the common constraints or limitations often seen in industry, and release their own enzymes in solution during the fermentation/hydrolysis, a rare process advantage named Consolidated Bioprocessing (CBP). Having the ability of produce end-products and expressing the enzymes necessary to break down feedstock for biotransformation, all at higher temperatures, gives DEINOCHEM's solutions a unique and significant cost advantage in techno-economic analyses.

Due to the unique genetics of Deinococci and essential towards biochemicals production, DEINOVE developed specific proprietary molecular biology tools to both tailor the end products production and optimize the metabolism of its platforms. DEINOVE is the World leading synthetic biology group leveraging Deinococci for industrial applications. These properties are key success factors for DEINOVE's targeted industrial applications by allowing a platform customization for each commercial partner, independent

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of either the starting feedstock or pretreatment technology employed.

In summary, the exceptional properties (robustness, biodiversity and metabolic properties) of DEINOCHEM's platforms, and DEINOCHEM's developmental research results, demonstrate the huge potential for these microorganism's to become true bacterial micro-factories for the production of bio-based chemicals in a cost-effective, environmentally friendly way.

#### Hans van der Pol

Corbion (Purac) is built on a strong foundation of leading edge fermentation and down-stream processing technologies, deep market understanding, strong customer

relationships and a unique global supply chain. Sustainability is in the heart of our business. Based on the strong foundation in technology, sustainability and cooperation, Corbion is building a differentiated biobased products portfolio. Thus, Corbion products are designed by science, powered by nature, and delivered through dedication. This presentation will discuss the recent expansion of the Corbion products and project portfolio -into area's such as succinic acid, FDCA and CaPropionate- and highlight key partnerships, capabilities and technologies that form the basis for the development into a full-fledged biobased products company.

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