Pacific Rim Summit
on Industrial Biotechnology and Bioenergy
December 7-9, 2014 • San Diego, California
Building Innovative Collaborations Across the Pacific

Feedstock Production and Utilization

Monday, December 8, 2014 | 8:30am – 10:00am

International Connectivity to Building a Bio-Based Products Industry

Moderator: Murray McLaughlin, Bioindustrial Innovation Canada

Murray McLaughlin, Bioindustrial Innovation Canada

Robert Speight, Queensland University of Technology

Tom Browne, FP Innovations

Jim Wynn, Michigan Biotech Institute

Abstract

This session will explore how two countries connected through a bioindustrial MOU are developing various aspects of their bioeconomy to become a key part of their future economy. Canada and Queensland, Australia have a lot of similarities - excellent research, strong agriculture and biomass base, and a focus on future business opportunities in the bioeconomy. The presenters in this panel will discuss their research efforts in the bioindustrial area and relate that work to national and international opportunities from a research and business perspective. Biomass and it's conversion to value is a strong focus in Canada and Australia and we will hear from the presenters how this is progressing toward commercial opportunities in energy, bio-based chemicals, biomaterials, and other opportunities for international partnerships. The research with a strong commercial focus on green and sustainable technologies is focused on new job creation and business opportunities with international linkages.

Monday, December 8, 2014 | 10:30am – 12:00pm

Optimizing Waste Feedstocks to Advance the Bioeconomy

Renewable Chemicals and Biofuels Made From Urban Waste
Moderator: Tim Cesarek, Enerkem

Transforming waste into business opportunities
Tim Hitchman, DSM

Optimum Supply Chain for Municipal Solid Waste Energy Recovery in Nevada
Mohamed Leila, McGill University

DME, a clean burning diesel replacement made from cellulosic biomass
Brittany Syz, Oberon Fuels Inc.

Abstracts

Tim Cesarek
This presentation will talk about the context for the growth of the advanced biofuels and renewable chemicals sectors and the use of municipal solid waste (MSW) as a feedstock for the production of these

valuable products. It will describe the main categories of technology innovations that were developed over the last decade for the production of advanced biofuels and the development of advanced biorefineries. It will use the case of Enerkem's game changing waste-to-chemicals and biofuels facility in Edmonton to demonstrate the benefits of using MSW as a feedstock. Finally, it 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 clean technology innovations. The demand for advanced biofuels and renewable chemicals is driven by the need to reduce dependency on oil, increase energy diversity, reduce carbon footprint for everyday products and stimulate economic growth. Governments around the world are mandating the use of biofuels in order to increase energy security and diversity as well as to reduce greenhouse emissions. Over 60 countries around the world have established mandates or targets for the use of renewable fuels in their conventional fuel pool. Large chemical groups around the world are increasingly interested in adding renewable alternative chemicals to their hydrocarbon-based product lines. Their customers, the large multinational consumer goods manufacturers, are looking for solutions to meet consumers' demand for greener everyday products. According to the International Energy Agency's Bioenergy Task 39, 102 projects are being pursued worldwide for the production of advanced biofuels using different transformative technologies. These projects include pilot and demonstration facilities that are already in operation, as well as commercial projects that are either planned, under construction or already in operation. The one recently built by Enerkem in Edmonton, Alberta, is joining the list of commercial facilities. This facility is dedicated to the production of biomethanol and ethanol and uses the non-recyclable and non-compostable portion of the household garbage from the residents of the City of Edmonton as feedstock. By unlocking the value of MSW, technologies like Enerkem's take advantage of the abundant supply of municipal solid waste to profitably and sustainably produce advanced biofuels including cellulosic ethanol. They represent a sustainable alternative to landfilling and incineration, and also provide a new source of energy. They open the door to the creation of a strong biorefinery sector that generates economic prosperity and helps transition to a bioeconomy.

Tim Hitchman

Reducing waste in the agribusiness value chain - When the issue becomes a valuable opportunity. Within today's agricultural value chains, over 50% of all food products are lost along the way. In order to help provide solutions for addressing the pressing needs of supplying sufficient quantities of food to the growing global population, Tim Hitchman will present concrete steps that can be undertaken to create a more sustainable agribusiness chain.

He will include examples of effective strategies used to transform waste into valuable product streams by those in the sector.

Mohamed Leila
The United States Air Force and Navy have set ambitious targets to acquire aviation biofuels by the year 2016. Municipal Solid Waste (MSW) has been proposed as a potential feedstock for military jet fuel creation. This research paper aims to use linear programming techniques to evaluate the optimum supply chain for waste to energy recovery in the state of Nevada. The results will shed light on the crucial barriers to generating jet fuel from Municipal Solid Waste.

Brittany Syz
Our company, Oberon Fuels, is the first company to bring DME, dimethyl ether, to North America as a cleaner alternative to diesel fuel produced from biomass. Brittany will present information on the advantages that DME provides as a replacement for diesel and the various feedstock that can be used to produce the fuel. 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 (cellulosic) 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_2 emissions with companies such as DHL using DME in commercial operations. 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. Oberon's first such production facility is located in Imperial Valley, in Brawley, CA. In 2013, 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 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. DME is the smart choice for an alternative fuel to diesel made from wasted resources such as food waste and plant waste.

Monday, December 8, 2014 | 2:30pm – 4:00pm

Innovative Method for Increasing Biomass Availability

Global Feedstock Availability and Cost
Moderator: Julia Allen, Lux Research

Scaling cellulosics: Innovative methods for providing feedstock to produce 2G sugars
James Hettenhaus, CEA Inc.

Increasing biomass with reduced inputs using symbiosis
Sharon Lafferty, University of Washington

Global Feedstock Logistics – from Field to Facility
Jay Van Roekel, Vermeer Corporation

An Empirical Analysis of the Emergent Industrial Bioeconomy
Brandon Morrison, Duke Center for Sustainability & Commerce

Abstracts

Julia Allen
As biofuel and bio-based chemical producers commercialize, feedstock considerations take center stage in any discussion on economic viability. In order to compete with petroleum, bio-based must be at cost parity, and the biomass input is the largest component of any bio-based cost structure. For long term growth, feedstocks for bioproducts need to be cheap, plentiful, secure, and can't compete with the food supply. Governments, investors, and corporations have begun driving the industry to next-generation, non-food feedstocks, but the technical and supply chain challenges have caused missed milestones and unmet government mandates. On the front end of the value chain, this presentation will separate hype from reality, digging into the economics, availability, and main challenges for first generation feedstocks like corn and vegetable oils, cellulosic feedstocks like corn stover and palm residues, waste resources like municipal waste and animal fats, and energy crops such as jatropha and switchgrass.

James Hettenhaus
Once the economic feasibility of the large, second generation ethanol plants in the US and Brazil now underway are known in the 1st half of 2016*, innovative methods will be described for accelerating growth—facilitating rapid scaling of a reliable, sustainable and economic feedstock for supplying additional plants with the proven technology. The 2014 US corn crop produced 14 billion bushels and had 330 million dry tons of stover in the fields after harvest. The 200 corn ethanol plants, surrounded by corn, will process 25% of the corn grain, five (5) billion bushels, but use less than 0.3% corn stover, just one (1.0) M dt in 2015. The table below shows the 2014 stover fraction relative to the feedstock that meets the RFS cellulosic ethanol equivalent. Stover % Required to Meet RFS Year RFS, gal B Feedstock dt M Stover % 2016 4.3 53 16% 2018 7.0 88 26% 2020 10.5 124

37% 2022 16.0 188 57% Brazil, the world's largest sugar cane producer is similar to the US--more than 400 sugar cane mills, processing 600 M tons of sugar (sucrose). Bagasse, the remaining stalk, 600 M dry tons, provides energy for the sugar refining and generates electricity for sale. The excess bagasse and up to 70% of the sugar straw are collected and used for 2G sugar feedstock. Sugar straw, the cane tops and leaves, is about 14% of the cane ds. * Three US plants started in the 3rd Q using mostly corn stover and a small amount of straw: Abengoa (25MGY), DuPont (30MPY) and Poet-DSM (20MPY). In Brazil, GranBio (20MGY) and Iogen-Raizen (10MGY) finish construction in 2014 and will use cane straw and bagasse early next year.

Sharon Lafferty

The use of biological N2 fixation rather than chemical fertilizer could offer a more environmentally and economically sustainable means of increasing biomass production. We previously reported the presence of endophytic Rhizobium from hybrid poplar grown in greenhouses. A variety of diazotrophic (N2 fixing) endophytes were subsequently isolated from wild poplar at a natural riparian area dominated by cobble. These endophytes were shown to be mutualistic symbionts by adding them to other plant species, including grasses, corn, Douglas-fir and a variety of crop plants for increased growth and improved health in nutrient-limited conditions. When the endophytes from wild poplar were added to hybrid poplars under greenhouse conditions, there was a significant increase in nitrogen fixation. Using fluorescent tagging, we have demonstrated that the endophytes can migrate throughout the host plants. Not only do the endophytes from wild poplar fix N2, they also produce plant hormones, solubilize phosphate, and increase drought tolerance of the host plant. By using symbiosis with natural microbial partners of wild poplar on cultivated poplar, substantial increases in biomass can be attained with reduced inputs.

Jay Van Roekel

Vermeer has a great deal of global experience in harvesting, collecting, transporting and processing various biomass materials ranging from corn stover to energy grasses to straws and sugarcane residue for use in a variety of biofuel and bio-energy applications. We propose the sharing of experiences – the good and the bad – along with field and customer-validation best practices. Our market presence within the core topics contained within the conference can show examples of CHST of world-wide activities. Should you have any questions or require additional information, do not hesitate to contact me. 1710 Vermeer Road East | Pella, Iowa, USA 50219, 641-621-8299 | vermeer.com

Brandon Morrison

The bioeconomy spans the production and utilization of renewable biological feedstocks, including their conversion into food, feed, energy, chemicals, and other industrial end-use products. The movement towards greater utilization of biobased materials and products illustrates a shift towards increased environmental performance of both firms and products to meet demands set by consumers, the investment community, institutional buyers, and government policies. The quantification of the bioeconomy has proven difficult to date, owning to its multifaceted and interdisciplinary nature. At a time where the global population is rapidly increasing and urbanizing, coupled with an expected dramatic expansion of the global middle class, there is a need to better understand the volumetric and spatial flows, as well as the industrial uses of, biological feedstocks. Gaps in the literature exist in regards to deciphering the global flow of biological resources into both intermediate and end-use outcomes. Greater analysis is also needed into the future development of the bioeconomy; specifically, research aimed at analyzing the potential risk and unintended consequences that may result from shifting to the use of more biological feedstocks. While corporations and governments are increasingly requiring Life Cycle Assessment (LCA) modeling of the environmental trade-offs of utilizing biological feedstocks, LCA methodology many times lacks the incorporation of broader spatial systems evaluation in the final analysis. The primary objective of this research was to develop a framework in which one could examine the flow of global agricultural and forestry production into intermediate and end-use outcomes. We undertook an empirical analysis of existing databases, as well as published literature and first-hand interviews with representatives from global public and private sectors, to quantify the global bioeconomy. In addition to documenting the primary flows (e.g., food, feed), this research further extends prior works by identifying and quantifying secondary industrial uses of agriculture (e.g., energy, chemicals). The empirical analysis we undertook provides a broader understanding of biological resource flows, as well as a qualitative exploration of potential implications resulting with the expansion of the bioeconomy. In addition to deciphering the flow of commodities, we also sought to quantify the spatial distribution of key bioeconomy feedstock crops within the global economy via exportation and importation. With the results of this examination, researchers, industry, and government alike will be in a better position to address the multi-dimensional feedbacks and synergies of the bioeconomy, as well as predict areas of risk and areas ripe for future production increases. Growing industrial demands for biological feedstocks have already increased demand for foreign land acquisitions and infrastructure development, most notably in areas with vast tracts of arable land (i.e., Africa). Future demands of the bioeconomy have the potential to create new emergent markets and shipment hubs, thereby altering maritime shipping lanes as emergent biobased feedstock producers are connected with industrialized nations and/or intermediate processing hubs. Greater LCA analysis of the distribution phase of biobased energy and/or products will be necessary to understand the larger system implications of shifting to biobased feedstocks.

Tuesday, December 9, 2014 | 2:00pm – 3:30pm

Optimizing 2nd Generation Energy Crops

Optimizing Sorghum Hybrids for Biorenewables Feedstocks
Daniel Erasmus, NexSteppe

Comparative evaluation of some brown midrib sorghum mutants for the production of food grain and second generation biofuels
Yadhu Guragain, Kansas State University

Cellulosic Ethanol Production from Energy Cane
Tim Brummels, Canergy

Christian Tobias, USDA Agricultures

Abstracts

Daniel Erasmus
Though biofuels, biopower and bio-based products are rapidly emerging and maturing industries are providing significant contributions to growing energy needs around the globe, these industries will not reach the desired level of scale or sustainability without a set of feedstocks optimized for these end-uses1. Several of the major technology providers have modeled their commercial scale technologies on specific feedstock sources to validate the technology’s capability. However, there is growing evidence that in many areas where such technologies could conceivably be implemented, such specific feedstocks are unlikely to provide the year-round supply of quantity and/or quality required for optimized and economically viable bio-refinery operations. NexSteppe, a global renewable feedstock seed company based in California, is dedicated to the development of next generation feedstock solutions for the biofuels, biopower, and bio-based products industries. In the quest for such products, NexSteppe is combining advanced breeding techniques, multiple breeding cycles, analytical technologies and agronomic practices in the development of dedicated energy crops tailored for these industries. And while there is continuing debate concerning the advantages and disadvantages of various feedstocks crops, NexSteppe is proposing that at least for the foreseeable future, the focus should be on the development of comprehensive supply chain plans incorporating several complementary qualified feedstock crops combined with sound agronomics to meet the potential demand for suitable biomass. While ongoing breeding efforts continue to make significant headway in yield and composition quality improvements and agronomic guidelines continue to be refined, NexSteppe has bred commercial sorghum hybrids on both sweet and high biomass platforms. Commercialization is providing validation of not only a scalable, reliable and cost-effective feedstock but also one that is complementary to other feedstocks such as sugarcane bagasse and residues. Breeding and development efforts are focused on

providing and refining sorghum hybrids tailored for various biorenewables industries including advanced first generation biofuels, cellulosic biofuels, biopower and biobased products. 1Biofuels & Biochemicals Then and Now: Innovation Trends from Feedstocks to End Products. 2014. Leo Zhang, Research Analyst, Cleantech Group

Yadhu Guragain

Sorghum is considered a model energy crop because of its high photosynthetic efficiency, abiotic stress tolerance, and wide applications as food, feed, and fuels. Various brown midrib (bmr) mutants of sorghum have recently been produced globally to develop the new sorghum lines to improve agricultural productivity and bioprocessing efficiency of lignocellulosic residues for biofuels. In this study, Early Hegari, Atlas, and Kansas Collier cultivars of sorghum and two bmr mutants of each cultivar (bmr 6 and bmr 12), were evaluated and compared for agricultural productivity and bioprocessing efficiency. Agricultural productivity was evaluated based on flowering time, plant height, stover yield and grain yield. Bioprocessing efficiency was evaluated on composition of raw and alkali pretreated biomass, enzymatic hydrolysis yield of monomer sugars, and fermentation yield of 2,3-butanediol from released sugars. Time of 50% flowering ranged from 53 (Early Hegari) to 85 days (N595- bmr 12 of Kansas Collier) and plant height from 1.1 (N596- bmr 6 of Early Hegari) to 2.2 m (Atlas); there was a moderate positive correlation between flowering time and plant height (m) ($R2 = 0.77$). A similar correlation was observed between plant height (m) and stover yield (t/ha) (($R2 = 0.68$). However, no correlation of grain yield was observed with plant height and stover yield. Atlas and N598 (bmr 6 of Atlas) were found as the most promising sorghum lines based on the stover yields (6 and 4.8 t/ha, respectively) and grain yields (4.7 and 4.1 t/ha, respectively). The highest stover yield (7.1 t/ha) was observed on N595, but with very low grain yield (0.2 t/ha). The bmr mutants of Early Hegari (N596- bmr 6 and N597- bmr 12) were found the least attractive sorghum lines due to low grain and stover yields. Biomass composition analysis indicated that bmr mutants had 10 to 23 % less lignin content and 1 to 10% less total glucan and xylan content than their parent cultivars, except N597. The highest reduction in lignin content (23%) and minimum reduction in total glucan and xyaln content (1%) was observed on N596 mutant. However, direct relationship between reduction on lignin content and total carbohydrate content was not observed. Sugar released was 19, 16 and 14 % (W/W) more in bmr 12 mutants of Early Hegari (N597), Atlas (N600) and Kansas Collier (N595), respectively. However, sugar yield increment in the corresponding bmr 6 mutants was either zero (N598) or 5 to 6% (N596 and N594). No correlation between the lignin content and sugar yield was observed. For example, N595 and Atlas had equal lignin content (14%), but sugar yield (g total sugars released/g raw biomass) in N595 was 25% more than Atlas. This indicated that factors other than total lignin content also significantly affect the enzymatic hydrolysis of pretreated biomass. These factors could be lignin structure, crystallinity of cellulose and linkages in monomer of cellulose, hemicelluloses and lignin units. Currently, the quality of released sugars are being evaluated for 2,3-butanediol production using Bacillus licheniformis. Additionally, lignin structure in these sorghums lines will be characterized by NMR and FTIR for the evaluation of the most promising sorghum lines. These data will also be discussed at the conference presentation.

Tim Brummels
Canergy LLC is embarking on a biofuels project that will be the first in North America to rely on a dedicated energy crop. Canergy's 30 MMGPY ethanol plant in the Imperial Valley of California will use locally grown energy cane, from which low carbon-intensity ethanol will be produced from both the juice and fiber fractions of the plant. The facility is slated for construction in 2015, and operation in early 2017. The second generation technology is provided by Beta Renewables. Canergy has worked extensively to evaluate growth characteristics and compositional characteristics of 17 varieties of energy cane, and tested bioconversion of the juice and fiber fractions to fuel. Attributes such as crop yield, juice and fiber content, cellulose content, hemicellulose content, and lignin content have all been evaluated. The Imperial Valley of California has some of the world's best conditions for crops. The area receives the most sunlight of any area in the United States, and its irrigation-fed water supply allows for precise control of water to crops. The main crops in the area include a wide variety of fruits and vegetables, but also, hay, grasses and other feed crops. Canergy chose this area to develop its energy cane varieties, in rotation with other crops in the area. Canergy can harvest its cane for nearly 11 months per year, for direct delivery to its nearby biofuel facility, avoiding the challenge of a short harvest window and long term storage that is encountered with many other lignocellulosic feedstocks. In this presentation, Canergy will present data from field trials with different varieties of energy cane. We will also describe key factors and outcomes from ongoing compositional and conversion tests with the different varieties, which have led to the selection of optimal varieties for its commercial plant.

Tuesday, December 9, 2014 | 4:00pm – 5:30pm

Advancements in Fuel Technologies Driving Commercialization

Advancements in Cellulosic Ethanol Technology that are Driving Commercialization
James Croonenberghs, Novozymes

Cellulosic Ethanol: commercialization and application
Martin Mitchell, Clariant

Cellulosic Ethanol from Corn Fiber
Jeff Scharping, ICM

Novel Viable Hydrogen-free BioRefining Technology Platform for Renewable Aviation & Diesel Fuels
Inder Singh, SBI BioEnergy Inc.

Abstracts

<u>James Croonenberghs</u>
Novozymes continues its unprecedented effort to develop new enzymes that enable the cost effective conversion of a variety of lignocellulosic substrates to simple sugars. This effort involves not only the development of new enzymes, but also the integration of key process steps; namely pretreatment, hydrolysis and fermentation. Our vision of a commercially competitive and environmentally sound biofuels industry is the driving force behind for our biofuel efforts. In fact, the development and integration of the enzymes needed for the conversion of biomass to biofuels has become the single largest project in company history. Novozymes continues to launch new enzymes that dramatically lower costs to hydrolyze biomass substrates into simple sugars. The new enzymes are characterized by increased catalytic activity, improved tolerance to inhibition, and the ability to work in a variety of processes and conditions. The enzymes have been tested on agricultural residues and woody based substrates. In addition to developing new enzymes, Novozymes continues to work with industry leaders to integrate the technologies both upstream and downstream of enzymatic hydrolysis. This needed integration of technologies drives our open and broad approach to partnering, with a focus on innovative companies representing varied cellulose based substrates and cutting edge technologies. These companies have reach commercialization and are driving the industry forward. Our presentation will briefly discuss existing and new enzyme technology developed and launched by Novozymes. We will also touch upon the value of partnering and how our partners have moved Advanced Biofuels to commercial reality.

<u>Martin Mitchell</u>
The energy industry is currently undergoing a paradigm shift. More and more economies are looking at possibilities to substitute fossil resources by renewable ones, both to secure energy supply and to increase sustainability. The transport sector in particular will continue to be largely dependent on liquid energy sources and advanced biofuels are the preferred way to efficiently reach renewable substitution goals in the short to medium term, and increase energy security. The key technical hurdle in the past has been how to access the sugars bound in the lignocellulosic material. In recent years technological breakthrough has been achieved through a variety of technologies, in particular enzymatic conversion. The matter now is to validate production processes and optimize the efficiency for large scale competitive production, in particular through high process yields combined with low OPEX and CAPEX. Clariant’s sunliquid® technology overcomes these challenges through a thorough and entirely integrated process design

and innovative technology features offering a one-stop shop solution flexible to be used to convert different feedstock and adopt to various plant concepts. The production cost can compete with those of first-generation bioethanol and the greenhouse gas savings of the sunliquid® ethanol are 95% compared to fossil fuels. Realisation on an industrial scale is no longer merely a dream. Since July 2012 Clariant has successfully been operating a demonstration plant in Straubing, Germany, with an annual capacity of 330,000 gallons (1.25 million litres, 1,000 tons), converting approximately 4,500 tons of lignocellulosic feedstock per year. After focussing on wheat straw for the first months of operation, in May 2013, first runs with corn stover and sugarcane residues also showed good results and validated the technology further. This was a new milestone reached by the project, confirming that the technology can be implemented worldwide. The results obtained in the demonstration plant have been incorporated into the recently finished sunliquid® Process Design Package. The package includes detailed planning principles for a commercial plant that can be quickly adapted to the prevailing situation. The flexibility of the modular concept makes it easy to account for site or project specific boundary conditions during the planning process. It thus delivers the technological blueprint for commercial facilities between 20 and 60 million gallons of ethanol production per year. A one-year fleet test by Clariant, Haltermann and Mercedes-Benz brings cellulosic ethanol onto the road as an E20 blend for the first time. All three companies contribute with their expertise to the project. sunliquid20 is a premium-grade petrol containing 20% cellulosic ethanol. The cellulosic ethanol is obtained from wheat straw and produced at Clariant's sunliquid demonstration plant in Straubing, Germany. The ethanol is then blended with standard gasoline by fuels specialist Haltermann to yield the final product – an innovative and highly sustainable E20 blend. Its high octane rating of over 100 enables optimum efficiency through higher compression ability. The fuel is tested in Mercedes-Benz series vehicles: the current BlueDIRECT gasoline standard engines from Mercedes-Benz can already run on petrol with 20 % ethanol easily, confirming that the fuel is ready for market and technically compatible with in series vehicles.

Jeff Scharping
Cellulosic Ethanol from Corn Fiber is the first easiest step for a Generation 1 corn starch plant to move into cellulosic ethanol. Patriot Renewable Fuels is the first plant that will be running ICM's Gen 1.5 technology to produce 14mmgy of Cellulosic Ethanol.

Inder Singh
The DROP-IN fuels of renewable diesel and jet fuel are generally produced using an external source of hydrogen as a key reactant in the process. The need for an external source of hydrogen greatly increases both the operating and capital costs of a refinery; thereby limits its economic viability. The availability of hydrogen gas can be another deciding factor in

whether to set up a refinery. It is therefore desirable to have a process that does not require the addition of hydrogen from external sources. SBI is in the process of commercializing a unique proprietary technology platform that produces petroleum equivalent DROP-IN renewable fuels from a blend of cheaper natural oils and high free-fatty acid feedstocks. SBI's simple manufacturing process is based on proprietary heterogeneous catalysts that function in a way that does not require the addition of hydrogen, consumable chemicals, or water to the reaction. SBI's catalysts are also inexpensive, have a long active life, and are easy to rejuvenate. SBI combines these catalysts in a process intensification and continuous-flow through reactor that was originally designed by the company for manufacturing pharmaceuticals and fine chemical intermediates. Finally, its process is feedstock-flexible allowing it to use the most economical form of natural oil and free fatty-acid feedstock including vegetable oils, animal fats, algae oil, etc. Currently available renewable fuels very often consist of straight chain alkanes or iso-alkanes. SBI's renewable jet and renewable diesel contain a full spectrum of hydrocarbon molecules critical for maintaining cold flow properties, oxidation stability and combustion qualities that is comparable to petroleum derived fuels. SBI has its fuels tested by competent third party labs and is confident that they exceed ASTM and European fuel standards. SBI's renewable fuels can be used as B100 replacing petroleum jet fuel or diesel fuel, and can be blended with petroleum fuels in any desired proportions to meet regional Renewable Fuel Standards (RFS) requirement. SBI's technology also generates pharmaceutical grade glycerol and high purity colorless biodiesel (FAME) with favorable cold flow properties as an intermediate product in the process. SBI plans to sell its highly pure glycerol to offset much of the costs to produce its renewable fuels. SBI is currently building a 10 million litre refinery in Edmonton, Alberta to demonstrate the efficacy to produce large quantities of renewable diesel and jet fuel. SBI expects to begin operations of this demonstration refinery by the end of 2015. A full-scale commercial plant that can manufacture 240 million litres of renewable fuels is planned for 2018.

Renewable Chemical Platforms

Monday, December 8, 2014 | 8:30am – 10:00am
The Pathway to Commercialization - Lessons Learned

An insider investor's perspective on renewable chemistry industry and development
Denis Lucquin, Soffinnova Partners

Accelerated Development of Biobased Processes: A How-To
Mark Burk, Genomatica

Leading biosynthetic chemicals being used in motor oil and industrial lubricants sectors.

Allen Barbieri, Biosynthetic Technologies

Amyris: Lessons Learned in Launching our First Product
Michael Leavell, Amyris

Abstracts

Denis Lucquin
• Sofinnova's experience and lessons learned in this space
• Why we invest in some companies and not in others?
• What should you think about before coming to VC for money?

Mark Burk
Genomatica will share insights on how it achieved certain milestones in its butadiene process development program more than twice as fast as it delivered them in its commercial GENO BDO™ process. The discussion will walk through examples of use of Genomatica's integrated biotechnology platform, the 'intertwining' of process engineering, computational modeling and lab experimentation, and more.

Allen Barbieri
Biosynthetic Technologies (BT) is commercializing technology invented and patented by the USDA, and exclusively assigned to BT. BT manufactures very high-performance biosynthetic chemicals that are used throughout the automotive and industrial lubricants sectors to potentially displace the existing petroleum lubricant feedstocks. The Company is funded by Monsanto, BP and several other strategic investors. BT is currently operating a demonstration plant that was built and is operated by Albemarle Chemical at their headquarter chemicals plant in Baton Rouge. BT is currently working with virtually every major motor oil & industrial lubricant company in the U.S. to test, evaluate and ultimately incorporate these biosynthetic oils into many of their existing motor oil and lubricant product lines.

Michael Leavell
Amyris is an integrated renewable products company focused on providing sustainable alternatives to a broad range of petroleum-based or environmentally sourced products. Our industrial bioscience 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, lubricants, and fuels. This presentation will address the lessons we learned in scaling up our renewable emollient squalane (normally sourced from deep sea sharks) and bringing it to market. Bringing a new product to market has significant challenges, including: transferring technology from the laboratory to manufacturing scale, establishing storage and shelf-life, managing regulatory requirements, and customer acceptance criteria. Challenges in each of these phases of product development will be outlined and discussed and lessons learned will be presented. Amyris' squalane has shown remarkable market growth, currently has a 17% market share in its third year.

Monday, December 8, 2014 | 10:30am – 12:00pm

Advancements in Renewable Chemical Manufacturing and Commercialization

Moderator: Cenan Ozmeral, Myriant Corporation

Strategic Alliances to Commercialize "Green" Chemicals in Asia Markets
Cenan Ozmeral, Myriant Corporation

Performance Plastics and the Mission for Bio-Based Polymers.
Olga Selifonova, Reluceo Holdings LLC

Hi-Tech Specialty Oils from Waste Carbon
Lisa Dyson, Kiverdi

Greg Keenan, Penford

Chris Cassidy, USDA Rural Development

Abstracts

Cenan Ozmeral
Myriant Corporation (Myriant), a U.S. based chemical company developing technologies for the production and manufacturing of biobased chemicals, is transitioning from a successful start-up company to an integral part of a major corporation. PTT Global Chemical Public Company Limited (PTTGC), Thailand's largest integrated petrochemical and refining company, expanded its "green" chemicals production capabilities after acquiring the majority of Myriant's shares to achieve the integrated production of bio-based chemicals in markets globally. Myriant currently produces bio-succinic acid at its commercial facility located in the Port of Lake Providence, Louisiana, producing 30 million pounds per year. Cenan Ozmeral, Ph.D., Myriant's President and CEO, will expand on Myriant's early days, financing strategy, manufacturing and commercialization strategy, and will share lessons learned.
As stewards for the sustainable development of green chemicals, PTTGC has a portfolio to include additional partnerships with Natureworks in Minnesota and Emery in Malaysia/US/Germany. Dr. Ozmeral will convey PTTGC's green chemicals strategy, current and future strategic alliances focused in using Thailand's abundant agricultural resources for the production of chemicals. Myriant's speaker will communicate important opportunities and lessons encountered from development to commercialization of bio-based chemicals.

Olga Selifonova
Ubiquitous use of modern plastics is associated with a recognized set of global problems related to their disposal, environmental recalcitrance, health risks and non-sustainable means of production. Nature offers many important clues for the technologists interested in design of sustainable bio-based industrial polymers with valuable functional features across the pallete of thermal, mechanical and optical properties, as

well as the environmental, health and other essential requirements. Incorporation of hydrolytically sensitive bonds, inclusion of well-placed non-aromatic ring structures, and complete avoidance of harmful or non-biodegradable building blocks become important tools in shaping the evolving concepts for the "benign by design" polymeric backbone structures. This approach offers a non-incremental path to enabling industrial processes with immediate use of the non-food cellulosic biomass sources available from worldwide agricultural practices. We will discuss our path to discovery, stringent project selection and development of performance plastics, functional polymers and polymer system components admissible for the sustainable production and use in the post-petroleum age.

Lisa Dyson
Kiverdi is a specialty oils and chemicals company developing bio-based replacements to oleochemicals and petrochemicals using our proprietary Carbon Engineering Platform. Kiverdi "brews" valuable specialty oils using proprietary microbes that consume CO2 and waste off-gases or waste-derived syngas to make oils used to produce non-food consumer products and industrial goods, including detergents, plastics, personal care items, soaps, animal feed, candles, etc. Global and local chemical manufacturers and consumer goods companies alike are emphasizing sustainability as a key element in their growth strategy. However, many have come to associate sustainability with higher cost. On the other hand, 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. At Kiverdi, we address the dual problems of waste management and price volatility of chemicals by utilizing biomass as feedstock and demonstrating that sustainability can be cost-competitive as well as profitable. 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 that serve as sustainable intermediates for surfactants, polymers and fuel additives or high-energy density jet fuel that can compete on cost and performance.

Monday, December 8, 2014 | 2:30pm– 4:00pm

The Future of Renewable Chemicals

Karl Sanford, DuPont

Abstract

Tuesday, December 9, 2014 | 2:00pm– 3:30pm

Strategy for Building Differentiated Renewable Chemicals

Moderator: Bill Baum, Genomatica

Corbion: Creating new businesses based on core competences build for lactic acid
J.F. van der Pol, Corbion

Direct fermentation for Isobutene, Butadiene and Propylene production: a platform to renewable plastics, synthetic rubber and fuels
David Gogerty, Global Bioenergies

Scaling up Non-food Biomass-derived Benzene, Toluene and Xylenes (BTX) as a Low Cost Drop-in Replacement for Key Chemicals
Daivd Sudolsky, Anellotech Inc.

Technology and Commercialization of DDDA
Jose Laplaza, Verdezyne

Abstracts

J.F. van der Pol
Based on over 80 years of experience in biobased products, 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 Corbion's business. Based on the strong foundation in technology, sustainability and cooperation, Corbion has a strategy of building a differentiated biobased products portfolio. Thus, Corbion products are designed by science, powered by nature, and delivered through dedication. This presentation will focus on the biobased chemicals landscape, the analysis that was made to select new products and the recent expansion of the Corbion products and project portfolio -into area's such as L and D- lactides, succinic acid, FDCA, Propionates and second generation feedstock developments- and highlight key partnerships, capabilities and technologies that form the basis for the development into a full-fledged biobased products company.

David Gogerty
Most industrial bioproduction processes are based on naturally existing metabolic pathways, limiting the scope of industrial biology, and preventing access to many of the chemistry's largest markets. The purpose of Global Bioenergies is to develop innovative metabolic pathways for the production of light olefins from renewable resources, by direct fermentation. Light olefins (ethylene, propylene, linear butene, isobutene and butadiene) are the core of the petrochemical industry, and each represents multi-billion dollar markets. These markets have been shaped by the historical co-production of light olefins from fossil oil in steam crackers. Recent shale gas expansion in steam crackers, resulting mostly in the production of ethylene, has changed the market landscape, emphasizing the need for alternative production routes for the other olefins. However, light olefins are not naturally produced by microorganisms and no bioprocess to convert renewable resources to these molecules has been industrialized so far. Global Bioenergies has developed an artificial metabolic pathway to isobutene. Importantly, production of

a volatile compound such as isobutene by direct fermentation presents two major advantages: first, in contrast to ethanol or isobutanol production, the product is spontaneously removed from the culture broth, which alleviates the significant challenges associated with product toxicity. Second, the purification process is considerably easier and cheaper since no energy consuming methods such as distillation or phase separation are necessary to purify the end product. Scale-up and commercialization is moving forward, and Global Bioenergies continues to expand its partnerships, which now include Audi and Synthos. Global Bioenergies also actively investigates advanced sources for feedstock to our enzymatic pathway, which could include waste gasses as shown in our feasibility study with LanzaTech. Finally, while isobutene production moves towards industrial scale, Global Bioenergies is also developing new artificial pathways enabling direct bio-production of Butadiene and Propylene.

David Sudolsky
Anellotech has grown tremendously in the past two years, from 4 employees in 2012 to 24 engineers, chemists and chemical industry professionals today as we continue to expand our laboratory, pilot plant and HQ facility outside New York City in Pearl River, New York. The first shipment of BTX for partner evaluation, from a small pilot plant, was made in June 2013, as the Company moves ahead with rapid development of its technology. These BTX samples are being used by our strategic partners for business development as well as for small-scale conversion into downstream products. With funding in place from [unannounced] strategic partners, Anellotech's process for direct conversion of non-food biomass into BTX is now moving from this small pilot plant stage into the small-scale demonstration phase. Anellotech's thermochemical catalytic process has very favorable economics compared to conventional, petroleum-derived BTX. Anellotech uses inexpensive feedstock (wood chips, bagasse, and stover), no pretreatment other than drying and grinding, and performs all chemistry in one fluid bed reactor with an economical zeolite-based catalyst, requiring no external hydrogen or enzymes. Anellotech has also made further advances in developing catalyst improvements that increase the p-xylene selectivity within the xylene fraction of its product stream. This advance increases the amount of desirable p-xylene coming out of the process. In addition to p-xylene, Anellotech produces low-cost benzene and toluene, which can be used as drop-in feedstocks for making low-cost commodity chemicals such as caprolactam (for nylon), polyurethane, SBR, etc. Our presentation will provide an update on our progress and future plans. About Anellotech Inc. Founded in 2008, Anellotech (www.anellotech.com) has developed a clean technology platform for inexpensively producing petrochemicals and transportation fuels from renewable non-food biomass. Anellotech's mission is developing renewable products that are less expensive to manufacture than their identical petroleum-derived

counterparts. Anellotech's technology achieves superior economics and increased sustainability by performing all chemical conversions in one reactor, using low cost catalyst and non-food biomass feedstocks. Anellotech's first platform application, Biomass to AromaticsTM will produce "green" benzene, toluene, and xylenes ("BTX") that can be sold into an existing $100+ billion market. Anellotech plans to license the technology and may own and operate its own plants.

Jose Laplaza
Dodecanedioic acid is a twelve carbon linear diacid that has multiple applications in the chemical industry. The biggest application is in the polyamide market to produce Nylon 6,12 used in engineering plastics with high performance demands such as in the automotive industry. The majority of the DDDA produced today is derived from non-renewable feedstocks such as butadiene or alkanes. Verdezyne has developed technology to produce DDDA from lauric acid, a twelve carbon fatty acid found in coconut oil and palm kernel oil. In the last couple of months we have demonstrated our process at pilot scale and produced over a metric ton of polymer-grade, renewable DDDA. In this presentation, we will give an update on our technology and path to commercialization. In addition, we will discuss new technology that will open the use of additional renewable feedstocks for the production of DDDA and other long chain diacids.

Tuesday, December 9, 2014 | 4:00pm – 5:30pm

What are the Performance Drivers for Biobased Product Producers?

Driving the Bioeconomy: Biobased Products in the International Marketplace
Moderator: Marie Wheat, USDA

David Saltman, Malama Composites

Christian Johnson, Bio Fiber Solutions International

Abstract

Marie Wheat
Now estimated around $2.6 billion, the biobased products industry is growing by approximately 15 percent annually. Biobased products are rapidly becoming part of the product mix for many industries including automotive and mass transit, chemicals, plastics, biomedical, construction, and electronics. Promising developments in research and finished goods from biobased feedstock are helping to fuel economic growth.
The USDA BioPreferred® program's mission is to encourage the development of new biobased products and serve as a catalyst for the success of biobased products in

the marketplace. Learn about recent developments in the BioPreferred® program as they partner with other U.S. federal agencies, international entities, business, and industry stakeholders to drive the biobased economy. Find out the latest on TPP negotiations, as well as, transatlantic progress on regulatory coherence related to biobased. Learn about progress on the economic impact analysis of biobased products mandated in the 2014 Farm Bill. Learn more about the mandatory requirements for U.S. federal agencies to purchase biobased products and tips for complying with the new regulations for designating your complex product or biobased intermediate. Receive updates on the progress being made in the voluntary labeling initiative resulting in USDA certified biobased products from over 40 countries.

Advanced Biofuels and Biorefinery Platforms

Monday, December 8, 2014 | 8:30am – 10:00am

Spotlight on India: Advancing the Bioeconomy

Bio-ethanol from non-food energy resources : A potential and economically viable fuel for future
Moderator: Arvind Bhatt, Himachal Pradesh University
Narender Sharma, Kuantum Papers, Ltd.

Lab scale biorefinery using an oleaginous red oxidative yeast strain for low cost biodiesel production with zero waste production technology
Debarati Paul, Amity University

Evaluation of sugarcane bagasse as a carbon source for the growth and lipid production potential of garden soil yeast
Saima Shahzad Mirza, Lahore College for Women University

Abstracts

Arvind Bhatt
Narender Sharma
Bio-ethanol, the most potential first generation biofuel, made by fermenting sugar extracted from starch-laden crops is gaining global attention as a promising approach for sustainable development and also for world energy security. It reduces our dependence on fossil fuels besides minimizing the green-house-gas emissions and is an established oxygenates increasing the octane value of fuels. The global consumption of petroleum and other fossil based fuels, particularly oil is increasing tremendously with transportation sector dominating the consumption. In view of the current scenario of dwindling fossil reserves and increased demand of the non-petroleum based fuels, an inevitable endeavor is required to balance the demand and supply of transportation fuel through cost effective and alternative fuels such as ethanol, butanol and biodiesel. Ligno-cellulosic

biomass because of their abundance, low cost and since these are not being used as food, besides sugarcane, starches, rice, wheat etc derived from plant residues represent the most prominent renewable and alternative sources for the production of fuel-grade ethanol. Extensive research and development efforts put in during the last few decades for utilization of lignocellulosic materials (wood, grass, forestry, agricultural waste, paper/ pulp industrial waste) as potential feedstock for bioethanol industry have shown promising results. Recent data suggest that leading producers around the globe are using a significant amount of traditional food and feed crops for bio-fuel production. The United States has the world's largest corn based bioethanol industry while sugarcane is used in Brazil for bioethanol production. Such large-scale production of bio-fuels i.e. use of primary sources of food and feed as raw material would be undesirable specially from food security point of view. Hence considerable research and development is required to find low cost technology to produce economically viable next generation bio-ethanol. Hemicellulose besides cellulose contain reasonable amount of fermentable sugars but is generally regarded as a waste product in the paper industry since only cellulosic present in the raw material is used for the paper manufacturing. An innovative way to convert this waste from paper industry to useful product i.e. bioethanol is through the use of advanced biotechnological processes utilizing enzymatic hydrolysis (xylanases). This on one hand will reduce the use of chemicals like alkali and chlorine while on the other will facilitate the easy removal of lignin from cellulose. Thus the development of bioethanol production process from agro-industrial byproducts of non-food sources will not only provide agro-based industries a techno-economically viable option for recycling the waste and overcome the problem of waste accumulation in the environment, but will also ease pressure on fossil fuel reserve due to its use as potential fuel for power vehicle for transportation. Various possibilities / opportunities of using low cost, non- food energy resources of plant origin as a potential and economically viable fuel for future based on our past efforts in this direction at laboratory scale and also with some preliminary experiments with Industrial partners will be discussed during the presentation.

<u>Debarati Paul</u>
An oleaginous (fat accumulating) oxidative red yeast that can accumulate lipids and ß-carotene to more than 50% of its biomass when grown under different carbon and nitrogen ratios was isolated in the lab. It showed the capability to grow in various cheap agricultural raw materials such as sugar cane juice, molasses, extracts of vegetable and fruit peels for lipid production. There was concomitant production of beta-carotenoids (antioxidant) and finally biocompost was produced as an additional useful product. The technology aims at recycling of all the waste/effluents generated during lipid or carotenoid production. Since the organism uses glycerol as a carbon source, this by-product of biofuel

production will also be utilized for further cultivation of the organism. The other waste effluents (cell mass and liquid discharge) would be utilized as nutrient (nitrogen) source for re-generation of the yeast biomass in the bioreactor. The proposed technology is novel bioprocess for the production of an important biofuel along with other invaluable components as by-products. It is also a low-cost technique in terms of availability of raw materials and processing. The zero-discharge aspect of this method makes it further lucrative because it is environment friendly. Preliminary results reflect upon the efficiency of the process as 'good return on investment' (ROI). It has an advantage over algae, mycelial fungi and bacteria due to its unicellular and relatively high growth rate with utilizing low cost fermentation media and is also a good source of proteins, lipids, and vitamins if used as animal feed.

<u>Saima Shahzad Mirza</u>
Biodiesel is considered a "carbon neutral" fuel, as carbon dioxide released from its burning was previously captured from the atmosphere during the growth of the vegetative crop that is used for the production of biodiesel. This study aimed to evaluate agro-industrial waste especially sugarcane bagasse as a carbon source by the oleaginous yeast for the production of bio-lipids. A subset of isolates was cultivated in sugarcane bagasse for screening their qualitative bio-lipid production potential with the Sudan Black B staining. Cultivation of yeast isolates in sugar cane bagasse at pH 7 and 28 ± 2.0 °C revealed maximum bio-oil production upto 93.03g/L and 87.07g/L by G2 and G4, respectively. However, maximum dry mass upto 9.33g/L and 8.67g/L was determined by the isolates G2 and G4 with optical density of 1.85 and 1.82, respectively at 660nm. This study proved that further research directed towards utilization of various cheap carbon sources, such as agro-industrial waste, industrial effluent and crop residue as substrate for oleaginous yeast hold high promise and could serve as sources for biodiesel production in future. These approaches could allow development of compatible processes for biodiesel production from cheap feedstock which might prove promising to save energy and operational cost.

Monday, December 8, 2014 | 10:30am – 12:00pm

Converting GHG's to Achieving a Fuel and Climate Solution

Moderator: Kasiviswanathan Muthukumarappan, South Dakota State University

Link Globally, Think Locally... Achieving Aviation Biofuels Carbon Neutral Growth 2020 Goal
Richard Altman, Commercial Aviation

Converting a CO2 Problem into a Fuel Solution
Dan Robertson, Joule

Food and Energy Security via Biological Conversion of Methane
Josh Silverman, Calysta Inc.

A novel continuous oil seed extraction method for jet fuel production
Kasiviswanathan Muthukumarappan, South Dakota State University

Abstracts

Richard Altman
Aviation Biofuels have made significant strides to develop and deploy technologies qualified for safe use as drop in jet fuels. With three process pathways approved and more on the way in the next two to three years the focus is increasingly on deployment to achieve the the aviation industries goal of carbon neutral growth by 2020 and a 50 reduction in carbon footprint by 2050. This paper focuses on communicating the "farm to Fly" model for establishing local aviation biofuels initiatives and illustrating how these efforts are being linked to similar global efforts in the Pacific Rim and elsewhere to achieve both industry goals and development of local liquid fuel and co-product supply benefiting the entire biofuel supply chain.

Dan Robertson
Today there is mounting evidence that human-attributed CO2 emissions are linked to global climate change, and multiple solutions to reduce such emissions are being explored with increasing urgency. Apart from carbon capture and sequestration, a costly and complex approach, there are newer technologies that enable the utilization and monetization of carbon – converting waste emissions directly into valuable products. Chief among these new technologies is the solar conversion of waste CO2 to liquid fuels, a process that can simultaneously cut carbon emissions and enable sustainable transportation for generations to come. Upon wide-scale implementation, the process could convert 150 million metric tonnes of CO2 into 300 billion gallons of drop-in fuel per year, with no dependence on biomass feedstocks, arable land or fresh water. This presentation will focus on the progress and potential of this direct CO2-to-fuels conversion platform, which is now operating at demonstration scale. How does the process differ from biofuel production? Unlike fuels produced from agricultural or algal biomass, Joule produces fuels directly and continuously from sunlight and waste CO2 – avoiding costly raw materials, pretreatment and downstream processing. The company's technology platform applies photosynthetic biocatalysts to produce and secrete fuel, not as intermediates to produce lipids or sugars that are subsequently converted to fuel. The products are inherently infrastructure-ready, including fuel grade ethanol and hydrocarbons for diesel, jet fuel and gasoline. As recently announced, Joule has successfully co-opted the natural photosynthesis process at unprecedented efficiencies for direct, continuous fuel production. This includes the engineering of a photosynthetic biocatalyst able to divert 95% of fixed carbon normally converted to biomass directly to fuel, and the industry first improvement of its photon energy conversion efficiency. This means that Joule has not only effectively re-channeled photosynthesis, but improved its

overall energy capture efficiency by nearly 100% in outdoor testing at Joule's demonstration facility in Hobbs, New Mexico. The entire process takes place in a custom-engineered SolarConverter system, from photon capture to product creation and initial separation, with no requirement for arable land, fresh water or crops. Furthermore, this uniquely modular system can achieve replicable productivity whether installed across 250 or 25,000 acres, mitigating scale-up risk. The application of this technology will consume otherwise harmful CO2 emissions from industrial facilities, such as coal or gas fired power plants. Particularly in light of carbon mandates proposed in the US and abroad, this technology represents an ideal option for such facilities, converting their waste emissions direclty into renewable fuels and helping them meet carbon reduction goals while also adding a potential revenue stream.

Josh Silverman

The recent rise in domestic production of methane has driven the cost of natural gas to record lows. Calysta has developed a genetic engineering platform for host organisms (methanotrophs) capable of metabolizing this abundant domestic feedstock to a variety of biofuels, biochemical, and nutrients. 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. Methane's 34x higher greenhouse gas contribution relative to CO2 implies that capturing these sources will have a significant environmental benefit. Longer term, biomass-to-methane strategies may eventually enable a fully renewable carbon cycle if 'green' methane-based technologies are developed. Calysta's proprietary BioGTL platform utilizes genetically engineered methanotrophs. Methanotrophs are prokaryotes that utilize methane as their sole source of carbon and energy. Calysta has developed a suite of tools for the expression of heterologous proteins in methanotrophs, as well as tools for the efficient targeted manipulation of the methanotroph genome. In addition, Calysta controls the world's only commercially-validated gas fermentation technology, a critical requirement to the scale-up of a viable methane-based process. Calysta is moving rapidly to commercialize high-quality, non-GMO protein based on this validated technology addressing a wide range of issues in global food security and sustainability.

Kasiviswanathan Muthukumarappan

The majority of oil seeds are extracted using hexane; however, this method has major drawbacks. Hexane emissions negatively impact the environment, and hexane's low flash point, explosive vapor, and toxic residuals create costly safety issues. Thus, finding an alternative oil extraction method to achieve the lowest environmental impacts and to further develop green chemistry is of utmost importance. A novel continuous oil seed extraction process will be carried out by simultaneous application of a single-screw extruder and two biodegradable solvents, d-Limonene from citrus peels and a-Pinene from pine gum, for three types of oil seeds. A 23 full factorial design for the 2 levels of barrel temperature (T) (80 and 120 °C), 2 levels of screw speed (SS) (80 and 150 rpm), and 2 levels of solvent to oil-seed ratio (R) (10 and 15% w/w) were conducted for the two proposed solvents separately to investigate the effect of extrusion condition and R on the oil content of the extruded seeds. Oil content was determined using an appropriate apparatus depending on the solvent boiling points and quality of the extracted oil in terms of free fatty acid profile, heating value, and Elemental analysis were determined using GC, Bomb calorimeter, and Elemental analyzer, respectively. It was clearly observed that at higher SS of 100 rpm and T of 120 °C, application of 10% Ethanol led to 28% and 8% increase in oil contents of the one-time extruded seed and doubled extruded seeds, respectively compared to those of the seeds extruded at the same condition without Ethanol treatment.

Monday, December 8, 2014 | 2:30pm – 4:00pm

Enhancing the Biorefinery through the Development of Innovative Enzymes and Yeasts

On-site Enzyme Production Using the C1 Expression System
Danai Brooks, Dyadic International Inc.

Continuous liquefaction and enzyme reutilization of pretreated ligno-cellulosic material
Rodolfo Romero, Andritz

Enhanced microbial oil production for renewable diesel raw material with genetically modified oleaginous yeast and mould
Kari Tapio Koivuranta, VTT Technical Research Centre of Finland

Advanced Yeast Biocatalysts for 2nd Generation Ethanol Production
John McBride, Mascoma Corporation

Development of High-Efficiency Yeast Strain for Cellulosic Bioethanol Production using Non-recombinant Approaches
Yoshiki Tsuchida, Honda R&D Co., Ltd.

<u>Abstracts</u>

<u>Danai Brooks</u>
What is C1? Of the leading expression systems in used to produce enzymes for second generation biofuels and bio-based chemcimals, Dyadic's C1 system could hold the most promise for the industry. C1 is an integrated enzyme production platform that is based on Dyadic's proprietary fungus strain Myceliophthora thermophile. C1 produces large variety of enzymes economically at very high yields that can hydrolyse a wide range of feedstocks, perform at higher temperature and broader pH ranges, and is proven at industrial scale in fermenters upwards of 500,000 litres in size. The C1-based strains produce an enzyme mix in a single fermentation that can efficiently hydrolyze a wide variety of ligno-

cellulosic feedstock. These strains are being continuously improved by overexpressing genes, increasing the level of key enzymes in the mix. As a result of these efforts, the efficiency of the enzyme mix has been significantly improved and the amount of enzymes necessary to hydrolyze pretreated corn stover has reduced by a factor of more than five over the past five years Further, C1 is already proven to produce enzymes at large manufacturing scale. Abengoa, is scheduled to open the one of first commercial second generation plant worldwide in Hugoton, Kansas in 2014 is using C1 technology for their process under a Dyadic licence. The next generation of enzymes developed at Dyadic have promise to decrease costs further. Focused on the on-site manufacturing approach, Dyadic's CMAXtm enzymes can be tailored towards different substrates and pretreatment technologies. C1's genome is over twice as rich as Trichoderma reesei, a leading alternative expression system for this application, and can be genetically modified to produce specific homologous enzymes. Genes used within the genome, versus inserting genes from other organisms, are often more productive and sometimes meet non-GMO requirements in certain countries. We believe that using C1 technology to turn biomass into cheap sugar, combined with an on-site manufacturing strategy, promises to make production of second generation biofuels and bio-based chemicals cost competitive.

Rodolfo Romero

Commercialization of second generation cellulosic ethanol is a reality. It's has taken several years for some companies to develop the technology for commercial production. There are different technologies used to produce this fuel. However further optimization are required to make this commodity more affordable. One of the common steps in the process that can be further optimized is a faster liquefaction of the already pretreated slurry as well as enzyme reutilization which both would contribute greatly on capital and production cost reduction. Faster liquefaction not only can reduce the time and simplify the equipment of the process but also provide a possibility to increase the final concentration of sugar. In this study a newly developed technology reduces liquefaction time to 3 hour or less as compared to 8-20 hours as reported by others by using a continuous mixing device for instantaneous, uniform enzyme distribution in high-solids concentration of biomass and a specially designed plug flow reactor where the liquefaction takes place. Figure 1 show a fully automated newly developed pilot scale liquefaction system. Moreover, latest results using a chilled recirculation stream intended to recycle enzymes and cool down newly incoming material have shown that the time for liquefaction can even be reduced further to only 1-2 h. Rheology variables and sugar yields studies on this system have shown that the reutilization of pre-liquefied material and hence recycled enzymes perform better on newly incoming material than as if compared to a system without recirculation.

Kari Tapio Koivuranta

Some yeast and mould species can accumulate lipids, mainly triacylglycerols, as over 50% of their biomass. These oleaginous species can be used to produce microbial lipids, for example for renewable diesel production. Various approaches have been considered for improving microbial lipid production, including mutation, genetic modification and environment optimisation. We have enhanced the production of triacylglycerol in an oleaginous yeast and a filamentous fungus by genetic engineering. One to four exogenous genes were expressed both in the yeast and in the mould to enhance triacylglycerol production, especially the yield per carbon consumed. At the same time, a metabolic pathway which did not exist in these species was established. The genetically modified strains were cultivated in bioreactors with glucose or xylose as carbon sources. With glucose as carbon source, genetically modified yeast produced up to 25% more triacylglycerol (g/l) with 24% higher yield (per used glucose) than the control strain, which itself already had good triacylglycerol production. From xylose, modified yeast produced up to 19% more triacylglycerol with 12% higher yield (per used xylose). The modified filamentous fungus produced up to 9% more triacylglycerol with 19% higher yield (per used glucose) than the control strain and up to 18% more triacylglycerol with 7% higher yield on xylose, compared to the control strain. The highest triacylglycerol yield was 26% (per glucose consumed) with the modified mould.

John McBride

Mascoma Corporation has developed and deployed the first commercial genetically modified (GM) yeast to be widely used for fuel ethanol production. To date, we have released two products for corn ethanol which reduce enzyme requirements and increase yields for corn ethanol. These products are currently being used by 20% of corn ethanol manufacturers in the United States, and have cumulatively produced more than 3 billion gallons of ethanol. While GM organisms are sure to be the baseline deployed for 2G ethanol production due to the necessity of converting 5 carbon sugars, Mascoma envisions a yeast biocatalyst that will do much more to reduce costs and increase yields. We have developed a suite of technologies for enzyme reduction and yield improvement in the 2G process, including xylose utilization, arabinose utilization, glycerol reduction, acetate conversion to ethanol, and cellulase and hemicellulase enzyme expression. Among the technical achievements we have realized to date are strains that can ferment xylose at high rate and yield in a variety of pretreated substrates, strains that can consume >9 g/L of acetic acid and convert it to ethanol during anaerobic fermentation, and strains that secrete enzymes to displace as much as 30% of the cellulase enzyme required in the 2G process. In all, these technologies can increase the yield of ethanol during 2G fermentations by 25% relative to strains that only ferment glucose and xylose. They can also be used to enable novel process configurations where 1G and 2G sugars are co-fermented allowing even larger enzyme reduction for the processing of lignocellulosic sugars. We are using our experience with industrial hosts, process conditions, and advanced molecular tools to develop robust strains and novel process configurations for the 2G ethanol industry.

Yoshiki Tsuchida
Lignocellulosic ethanol is awaited as renewable energy source that does not compete with food, and the main obstacle is fermentation inhibitors that inhibit fermentation of sugars, especially pentose (C5), thus leaving C5 usage as the key to increase ethanol production. Genetic engineering is often used to endow yeasts with C5 assimilation capability or C5 fermenting Escherichia coli with ethanol fermentation capability. Use of genetically modified microorganisms for industrial application requires additional capital investment and public acceptance, which is not established in some countries. Therefore, we focused to produce bioethanol from cellulosic biomass by non-recombinant microorganism. An ethanol fermentation yeast strain with xylose-assimilation capability was successfully isolated from natural yeasts and designated N strain, closely related to yeast Meyerozyma guilliermondii as sequence analysis of 26S rDNA-D1/D2 region indicated. A major obstacle in improving bioethanol production is varieties of fermentation inhibitors generated from biomass during the pretreatment process that cause to terminate fermentation either completely or partially, leaving fermentable sugars unused, especially xylose. For N strain to overcome the same obstacle, the in-house Disparity Mutagenesis Technology of Neo Morgan Laboratory, co-presenter, for selective improvement was employed, and 704C1 strain, a mutant of N strain, was obtained after repeating the mutagenesis-selection process for 10 times. Sugar hydrolysates derived from aqueous ammonia solution-pretreated rice straw were directly used for strain selection to gradually improve the microbial tolerance to inhibitors. Fermentation performance of the resulting 704C1 strain achieved more than 90%, evaluated with sugar hydrolysate derived from aqueous ammonia solution-pretreated rice straw containing glucose and xylose of total ca. 10%. The amount of xylose remaining unused after fermentation in the wild-type strain was greatly decreased in the 704C1 strain. Further, 704C1 strain maintained its fermentation performance in jar fermentation test with sugar hydrolysate derived from pretreated rice straw, when the liquid volume was changed from 1L to 5kL. It is worth to note that neither the removal of fermentation inhibitors, nor dilution of hydrolysate with high sugar contents was employed for the said evaluation. Corn stover is another abundant cellulosic biomass source widely distributed in the world. The improved strain obtained from adaptation with rice straw-derived hyderolysate was used as a parent strain for further improvement using corn stover-derived hydrolysate. Pretreatment condition with dilute

sulfuric acid was studied along with the strain improvement, to decrease the content of inhibitors in hydrolysate in a practical condition. As corn stover-derived hydrolysate showed higher inhibition than that derived from rice straw, self-cloning method was employed to strengthen prospective genes, for resultant microorganism is treated as non-recombinant in many countries. Trans-aldolase (TAL) is known to improve fermentation performance when over-expressed in Saccharomyces cerevisiae, thus chosen as the first target. The TAL over-expression was confirmed, and the strain was further improved with 3-stage selective improvement to show 10% increase in ethanol yield with corn stover derived hydrolysate, both in liquid and slurry.

Tuesday, December 9, 2014 | 2:00pm – 3:30pm

Utilizing Strategic Partnerships to Grow Your Business

Moderator: Glenn Doyle, U.S. Department of Energy, Bioenergy Technologies

Commercialization Capability and Strategic Partnerships
Ally LaTourelle, BioEconomy Partners, LLC

Lessons Learned and Best Practices from the Department of Energy's Integrated Biorefinery Portfolio – Opportunity to Reduce Risk and Accelerate Commercialization of Biofuels and Bioproducts
Glenn Doyle, U.S. Department of Energy, Bioenergy Technologies

New Pathway Petitions under the RFS and California LCFS: Status of the Programs and Strategies for Compliance
David Glass, D. Glass Associates Inc.

The Innovation, Manufacturing and Materials Science Institute - American Manufacturing Reimagined
Michael Alt, Eastman Kodak Company

The Status Of Available US And International Debt And Equity Mechanisms For Advanced Biofuels, Renewable Chemicals And Bioenergy From Waste
Mark Riedy, Kilpatrick, Townsend & Stockton LLP

Abstracts

Ally LaTourelle
If the biobased economy is to grow at the predicted pace, commercialization strategies will continue to be a critical factor to hurdle commercialization gaps. This is especially true as more technologies approach building their first large-scale plants, where technology has been proven at pilot and demonstration scale, but has yet to reach commercial capacity. BioEconomy Partners is currently developing a $30M fermentation toll manufacturing plant called the Bioscience Manufacturing Center in Rochester, NY to help bridge the gap for these emerging companies and reduce their "front end" capital expenditures. With a $3M grant secured for the development phase, this facility will increase toll

fermentation capacity east of the Mississippi river, and enable the development and expansion of a new innovation ecosystem in the Northeast. This project is also currently being leveraged to assist with government funding for companies looking to co-locate at the site. This presentation will outline the development strategy of the Bioscience Manufacturing Center, it's capability, and the strategic partnerships that make it possible to move strategic technologies in tandem to commercial scale, at multiple points in the supply chain.

Glenn Doyle
Since 2003, the Department of Energy (Department) has been substantially involved in cost-sharing the development of over thirty integrated biorefinery projects to process biomass feedstocks into transportation fuels and biobased products. These projects range in scale from developing engineering designs for pilot operations, to constructing integrated pilot- and demonstration-scale biorefineries, as well as commercial biorefinery demonstrations. Under direction of public law (Energy Policy Act of 2005), the Department was authorized to "carry out a program to demonstrate the commercial application of integrated biorefineries ." Under that mandate and subsequent law (e.g., the American Recovery and Reinvestment Act of 2009), the Department's Biomass Program conducted three separate competitive Funding Opportunity Announcements (FOAs) from 2006-2009 to solicit applications from the community to develop integrated biorefineries at these various scales. In 2012, the Department ran a fourth FOA for innovative integrated pilot plants that resulted in three projects being awarded. This presentation describes some of the key lessons learned and best practices the Department has compiled during the intervening 8 years (2006-2014) and how that information could be used to demonstrate successful risk reduction and potentially be used to accelerate commercialization. In fact, the Department's overall objective is to foster development of a robust, commercially-competitive biorefinery industry and help reduce the risk associated with designing, constructing and operating first-of-a-kind biorefinery demonstrations such that the return on the investment can become attractive to private investment. In this vein, the overall benefit of the industry-federal partnerships will be discussed along with the practices and processes used to help both recipients of federal funds and the Department achieve successful commercialization of integrated biorefineries.

David Glass
In order for developers of novel renewable fuels to generate Renewable Identification Numbers (RINs) under the U.S. Renewable Fuel Standard (RFS), the fuel and its production pathway must be listed in the RFS regulations as meeting the applicable standards. For those novel fuels not covered under the regulations, such as photosynthetic fuels or fuels produced using novel feedstocks or process technology, it

would be necessary to file a petition with the U.S. Environmental Protection Agency, to have the agency review the pathway and its lifecycle carbon utilization, to determine whether it meets the requirements under the regulations. A similar petition requirement exists for fuels to be accepted under California's Low Carbon Fuel Standard (LCFS). As of March 2014, there was a backlog of over 35 petitions awaiting action at the EPA, causing the agency to announce that it was undertaking a thorough review of its procedures for reviewing pathway petitions, and asking fuel developers to voluntarily hold off on new pathway petitions until this review is completed. EPA is expected to announce new procedures for petition review, improved guidance for petition submitters, and end the voluntary moratorium on new submissions at some point in Fall 2014. The California Air Resources Board has also been faced with a significant backlog of petitions, and has also been considering changes to its pathway petition process to streamline certain of the more routine pathways needing review. This presentation will describe the requirements for having a new pathway approved under the RFS, and will analyze the new EPA proposals and procedures expected to be released this autumn. The presentation will review the nature of the petitions currently pending at EPA and will provide practical guidance to fuel developers for how to prepare new pathway petitions in light of the new guidance expected from EPA. A similar analysis will also be made regarding the status of the California LCFS pathway petition program, along with discussion of strategies for compliance. David J. Glass, Ph.D. is an independent consultant specializing in renewable fuels and industrial biotechnology regulatory affairs, with over 25 years experience with U.S. biotechnology regulations, and extensive familiarity with international biotechnology and renewable fuel regulations. He has consulted for numerous U.S. and international industrial biotechnology companies, and has formerly served as director of regulatory affairs for Joule Unlimited.

Michael Alt

Commercialization of American innovation is of critical importance to this country as it strives to compete globally in today's dynamic marketplace. Yet, while the U.S. remains one of the most innovative, creative and industrious nations in the world, it is challenged to find new ways to successfully bring next generation products to market. The Innovation, Manufacturing and Materials Science Institute (IMMSI) at Eastman Business Park (EBP) is a new national model created to meet that challenge. A unique commercial accelerator situated within the Genesee-Finger Lakes region of New York, IMMSI is set up to drive economic development and manufacturing jobs growth by providing critical infrastructure to enable commercialization of new technologies. At a high level, many new economy opportunities need materials science, chemistry, pilot testing and infrastructure support – especially in the energy and biomaterials sectors which hold the key to American competitiveness on the world stage. As the home of Eastman Kodak Company, Xerox, Bausch & Lomb and many other established, high-tech corporations, the western New York region is uniquely able to provide that infrastructure, the skilled workforce and commercialization capabilities to bridge the gap between TRL3/MRL 3 and TRL10/MRL 10. Anchored by the unique and valuable assets at Eastman Business Park – Eastman Kodak Company's primary film manufacturing hub for over a century – find out how the IMMSI initiative is already driving advanced manufacturing, product commercialization and jobs, while creating cost benefits through established transportation systems and capital assets, within the new economy.

<u>Mark Reidy</u>
Mr. Riedy will discuss available debt, equity and non-dilutive funding sources and mechanisms for advanced biofuels, renewable chemicals and bioenergy technology companies and their US and international projects using waste feedstocks. These funding sources and mechanisms include the use of credit enhanced debt; protective insurance products; strategic investor equity; non-dilutive grants and tax equity; regulatory incentives; capital and institutional markets; green funds from states and banks; and new capital equity expansion mechanisms such as MLPs, REITs, High Yield Bond Funds, Yieldcos and other hybrid structures.

Tuesday, December 9, 2014 | 4:00pm – 5:30pm

A Global Perspective on Growing the Bioeconomy

Malaysia: Hub For Industrial Biotechnology Linkages Across The World
Moderator: Zainal Abu Kasim, Malaysian Biotechnology Corporation

BIO-MAN: a site-specific, feedstock procurement project to switch from a fossil to a multi-purpose green refinery, in a circular economy perspective
Enrica Bargiacchi, Consortium INSTM

New open access Bioprocess Pilot Facility
Arno van de Kant

Exploring Biomass: The Bioeconomy Concept
George Sakellaris, Bioeconomy in Greece

Harry Baumes

<u>Abstracts</u>

<u>Zainal Abu Kasim</u>
Malaysia is the second country in Asia after China and the first in ASEAN to implement the Bioeconomy initiative called the Bioeconomy Transformation Program (BTP). Since its launch in October 2012, Malaysia's bioeconomy has delivered excellent performance, which becomes crucial contributor to the economic growth. The BTP program is essential considering

Malaysia's abundance in natural resources, which needs to be efficiently utilized as well as Malaysia's strategic location, which can act as one of the global hubs to boost the commercialization opportunity in biotechnology. Due to these factors, the government of Malaysia has made some major announcements in the recently released Budget 2014 for the biotechnology field, including an allocation worth RM 85 million for BTP projects until the end of the second phase of the National Biotechnology Policy (NBP), which is in 2015. In addition, the government will also offer tax deduction for company that invest to acquire technology platform in bio-based industry, exemption on import duty on R&D equipment for companies that invest in pilot plants for the purpose of pre-commercialization in Malaysia, and special incentive to companies to partially cover the operational cost for human capital development for Centre of Excellence for R&D.

Enrica Bargiacchi
The City of Mantova and its Lakes are a UNESCO site, and also one of the major "Chemical Poles" in Northern Italy. Loss of competitiveness of the traditional fossil fuel based industries, in part related to increasing stricter environmental regulations, has determined a growing interest in more sustainable and eco-compatible industrial cycles, based on ligno-cellulosic biomass, grown in the 70 km-radius area around the biorefinery, and preferably delivered by fluvial barge and railways. With these premises, potential stress on soil price and its crop destination could arise in this area, with competition for best soils between present traditional food production chains and the new project. To preliminarily avoid this conflict, and have a sustainable biomass supply in the long run, a site-specific project is under development by Politecnico Milano, National Interuniversity Consortium of Materials Science and Technology (INSTM-Firenze), Consortium Italbiotec, and local Governments and Agencies. The 2nd gen. biorefinery will produce ethanol, xylitol, biochar, fibers for lighter particle boards, and will channel energy and materials to the existing manufacturing industries in the area. Saline effluents will be used to formulate fertilizers, with the aim at producing a zero-waste, environmental-friendly, energy-sustainable biorefinery. Biomass sources (200.000 t/y DM) have been identified in the following: (i) a part of the available crop residues (corn stover, poplar tops and rice straw) having a limited or no use as a feed; (ii) poplar short-rotation coppice instead of the less competitive traditional poplar for timber; (iii) Arundo donax in less demanding and polluted soils; (iv) programmed harvesting of natural reeds (mainly Phragmites spp.) in marshlands, according to established plans of rejuvenation of the biomass stock. Previous activity carried out by the involved research groups in the area (crop field tests, plant biomass and by-product characterization, harvesting time and methods, logistic modeling, etc.) will be used as a reference point for the project. Authors: Enrica Bargiacchi and Sergio Miele.

Arno van de Kant

BPF: Situated at the Biotech Campus Delft, the Netherlands, the Bioprocess Pilot Facility B.V. (BPF) is a unique open access facility where companies and knowledge institutions can develop new sustainable production processes by converting bio-based residues into useful chemicals or fuels and production processes for Food and Pharma. The facility has been specifically designed to enable the transition from laboratory to industrial scale. The facility has a modular setup. BPF allows users to construct complex operations by linking the separate process modules: Pretreatment, Hydrolysis, Fermentation and/or Downstream Processing. About 30 people, mainly experienced process operators. Investing about 37 Million Euro in expanding the facilities with pretreatment and food grade capabilities and also building a modern state-of-the-art control unit from which all the pilot plants can be controlled. Based at the Biotech campus Delft all infrastructure is present to perform chemical/biotechnology upscaling processes. Biobased production today: Examples of products/product range BPF is a service provider with very flexible facilities to help our customers to upscale their process. We have experience with many different chemicals, food and pharma ingredients of which many cannot be disclosed because of confidentiality. Known examples are ethanol, FDCA and 7-ADCA. Because of its high quality standards, the BPF can also be used to produce kg-quantities of material for pre-marketing and application tests at customers and/or preclinical trials (for Food or Pharma applications). The BPF has a long standing historical track record in bioprocess piloting with an experienced crew. Research and innovation activities BPF is well equipped to be able to upscale the process of the technology holder, and use our experience to improve the process. We have 4 different pilot plants which can interact being: • Pretreatment (biomass, also lignocellulosic) on benchscale and pilot scale. • Fermentation from 10l up to 8m3. (ATEX, GMO) • Downstream processing, chemical processing (ATEX) • Food grade pilot plant. All product streams can be connected, to mimic a downscale of a commercial plant. The upscaling of the lab process can than be proven on pilot scale with a good prediction of the process on commercial scale. Future ambitions BPF wants to be the provider of scale up for biobased products that are or have been developed on labscale in EU projects or within companies to overcome the valley of death, by providing access to equipment, knowledge and experience of the BPF. Chemicals like ethanol, lactic acid, succinic acid, ethanol, FDCA, enzymes.

George Sakellaris

The expansive growth of global population has leaded into a rapid depletion of many resources, including energy and food and to environmental pressures and climate change. The current economic crisis in Europe and the United States, partially due to the above reasons guided both parts to release official documents in which they consider bioeconomy as a key element for smart and green growth. Advancements in bioeconomy research and innovation will allow to improve the management of the renewable biological resources and to open new and diversified markets in energy and in bio-based products. Establishing the bioeconomy concept, a great potential becomes achievable: it can maintain and create economic growth and jobs in rural, coastal and industrial areas, reduce fossil fuel dependence and improve the economic and environmental sustainability of primary production and processing industries. Modern Biotechnology has become a key factor in modern bioeconomy worldwide. This potential is increasing since is touching a wider areas of applications within various domains, especially in the area of GMO's and their connection to Bioenergy, Biomaterials, Agrifood, Molecular Farming, Environment etc. As long as the potential of modern Biotechnology expands, lateral issues like regulatory frames, harmonized legislation, public perceptions and communications, ethical or moral issues are becoming more demanding and requiring. This perspective must be examined in the frame of the new emerging markets and the need of sustainable considerations for all applications. A substantial research has been already done on Bioeconomy and its huge potential in modern societies and economies. In these researches is shown the way to a more innovative, resource efficient and competitive society that reconciles vital security with the use of biomass and renewable resources for energy, food and industrial purposes, while ensuring environmental protection. It is however imperative to carefully consider a number of parameters in order to make this model feasible: • Sustainability in all levels, economic, environmental and social • Globalization and Universality consideration in all aspects (scientific and business), respecting the particularities on a case by case basis • Science and Technology Governance in the new conditions • Technology Transfer and the new pols of know-how creation • Social issues considering the benefits for the community, the diversification in public perceptions, the risk and the safety of products and practices In this whole new context, obtaining the full benefits of the bioeconomy will require purposive goal-oriented policy both by governments but also by leading firms, to establish goals for the application of biotechnology to primary production, industry and health; to put in place the structural conditions required to achieve success such as obtaining regional and international agreements; and to develop mechanisms to ensure that policy can flexibly adapt to new opportunities.

Synthetic Biology, Algae and Marine Biotechnology

Monday, December 8, 2014 | 8:30am – 10:00am

Value Added Products from Algae

Conceptual Design and Preliminary Engineering for Capture and Reuse of CO_2 and NOx for Algae Production from Stationary Engine Flue-gas in California
Dominick Mendola, UCSD/Scripps Institution of Oceanography

Improving Plant Oil Content and Biomass Yields Through a Synthetic Two-Gene Trait Stack
Amy Curran, Algenetix Inc.

Engineering regulatory proteins to improve biofuel tolerance in cyanobacteria
Weiwen Zhang, Tianjin University

Processing of Crude Algal Oils and Production of EPA and DHA Concentrates
Udaya Wanasundara, POS Bio-Sciences

Novel Process to Fractionate Algal Biomass into Fuels and Value Added Chemicals: Pathway to a Flexible Biorefinery Model
Nick Nagle, National Renewable Energy Laboratory

Abstracts

Dominick Mendola

In California, electrical power generation and natural gas compression utilize large, stationary natural-gas fired internal combustion engines and turbines as the main motive sources for electrical energy generation. To meet stringent California air quality standards, such engines are efficiently operated to yield relatively low quantities of carbon dioxide (CO_2) in their exhaust flue gases (ca. 3-5% v/v typical). Such low CO_2 levels present a significant engineering challenge when designing algal-based culture systems for efficient absorption of flue-gas CO_2 emitted from such plants. As a test exercise, we designed a 32 acre water surface (12.5 ha.) Oswald-type paddlewheel-driven raceway pond-based microalgal culture facility sized to absorb 1,257 kg CO_2/hr peak rate from a single 3,200 hp natural gas fueled base-load internal combustion engine. We chose a real engine from an extant installation of Southern California Gas Company, located at a natural gas compression facility in a semi-arid region of Southern California. The demonstration-scale facility was designed for culture of a cold-tolerant Chlorella spp. Algae, which would be grown for sale for human consumption in the US market. In the chosen location, relatively low winter temperatures would limit efficient algae production to 9-months per year. The design estimated peak productivity of algae in the summer months (10-hr solar day) would reach 3.0 $g/m^2/hr$. Yearly average algal biomass productivity was estimated at 16 $g/m^{-2}/day$ with overall biomass productivity from the 29.7 acres (11.7 ha) of production ponds summed to 636 metric tonnes per year. Summing all estimated production costs yielded $8.30/kg (USD) on a dry weight basis. This figure included straight-line capital depreciation on an est. $16.6M total construction cost for the 12.5 ha. facility. When scaled to ca.115 acres (45 ha.) of production ponds

production cost was calculated to be approximately $5.00/kg d.w., showing significant economy of scale for the process.

Amy Curran
Algenetix has developed a technology portfolio that addresses the growing global demand for industrial plant oils by increasing the yield and energy (oil) content of renewable energy crops and microbes. The novel technology, known as PhotoSeedTM, is a two-gene synthetic biological oil synthesis and encapsulation modification. The technology can be engineered into different plant tissues or overexpressed in microbes and results in the packaging of triacylglycerols (TAGs) into stabilized lipid droplets, which are protected from catabolism and accumulate to measureable levels. This technology comprises the co-expression of a diacylglycerol O-acyltransferase (DGAT1) gene (or Algenetix's improved Enhanced DGAT) with a cysteine modified, protease resistant oleosin gene (Cys Ole). The company has measured increased TAG content and productivity from 50%-400% over wild type lines in various tissues across a number of terrestrial plants (hemizygous ryegrass, clover, alfalfa and homozygous Arabidopsis) and 65-180% over wild type in microbes (Yarrowia lipolytica and Saccharomyces cerevisiae). In addition to increasing TAG content in all species investigated, PhotoSeedTM expression in the model plant, Arabidopsis, resulted in a 24% increase in CO2 assimilation per unit leaf area and a 50% increase in biomass yield. In perennial ryegrass a 25% increase in biomass was measured as well. The production of stabilized lipid droplets in photosynthetic tissues created a constant demand for de novo fatty acid biosynthesis, which resulted in an increase in CO2 recycling (as it is used by the pyruvate dehydrogenase complex to generate Acetyl-CoA for lipid biosynthesis) in the chloroplast, leading to an increase in CO2 assimilation and biomass. PhotoSeedTM will create new-dedicated sources of industrial plant oils that are non-competitive with food crops for land and resources and can be grown at lower costs. Outside of the seed, plant cells have very low levels of lipids, typically 1.5%-4% of the dry matter (DM). The increase in lipid productivity from PhotoSeedTM has increased lipid content up to 8% DM in leaves of Arabidopsis and ryegrass. With next generation improvements, the company estimates a further increase of up to 12% DM. In essence, the technology converts every single plant cell into a "photosynthetic seed". When engineered into high yielding biomass crop candidates (such as Arundo donax and Giant Miscanthus), oil yields per acre are forecasted to be 10x higher than soybeans, the primary oil crop in the United States. This unprecedented level of lipid productivity will drive down feedstock costs to make the production of oleochemicals, biodiesel and aviation fuel feasible in the absence of subsidies. Algenetix forecasts that even in the lowest value commodity scenarios, renewable fuels and chemicals can be produced at

comparable or lower costs to petrochemical alternatives.

Weiwen Zhang
Global transcription machinery engineering (gTME) approach has been applied to various microbe to improve biofuel tolerance. However, although synthetic biology efforts have led to successful production of various biofuels in autotrophic cyanobacterial systems, very little is known regarding the regulation related to production and tolerance. We report identification and characterization of a two-component signal protein Slr1037 involved in butanol tolerance and a transcriptional regulator Sll0794 involved in ethanol tolerance in Synechocystis sp. PCC 6803. As the first regulatory proteins involved in ethanol and butanol tolerance, they are useful targets for further improving ethanol tolerance through transcriptional machinery engineering approach in Synechocystis.

Udaya Wanasundara
Crude oil from algal sources contains considerable amounts of non-triacylglycerol (non-TAG) substances. Some of these compounds are beneficial (i.e. tocopherols as an antioxidant), however, majority (i.e. free fatty acid, colored substances, phospholipids, glycolipids, etc) are objectionable because they render off color and off odor in the oil and cause foam and precipitates during storage. Presence of non-TAGs decreases storage stability and shelf life of the oil. Therefore, the processing of crude Algal oils comprising a series of discrete steps such as degumming, alkali refining, bleaching and deodorization (RBD) is carried out to remove these impurities. The most prevalent polyunsaturated fatty acids of Algal oils are docosahexaenoic (DHA) and eicosapentaenoic (EPA) which are being used in products ranging from dietary supplements to infant formulas. Naturally, these fatty acids are associated with other lipophilic compounds of the oil and effective separation and isolation techniques are needed to recover them in concentrated forms to use in nutritional applications. At POS Bio-Sciences (Canada), we are capable to process highly unsaturated crude oil into stable RBD oil and also to produce concentrated forms of EPA and DHA in small- or large-scale quantities via different techniques. This presentation focuses on our process capabilities and new methodologies that we'll be able to provide.

Nick Nagle
Efficient conversion/upgrading pathways are required for all of the algal components into high-value fuels and chemicals to reduce both production cost for algal biofuels, and risk to stakeholders. Previous pathways have focused on solvent extraction of the algal lipids for biofuel production while valorizing the remaining extracted biomass using anaerobic digestion to provide both heat and electricity. These models suffered from excessive energy inputs required drying the algal biomass removing water prior to lipid extraction and for solvent recovery-post extraction. Thermochemical routes, such as hydrothermal liquefaction (HTL) have been investigated as a potential bio-oil

production process. While higher carbon retention efficiencies for the bio-oils are achievable using HTL these processes require high-pressure operating conditions making the process more costly and challenging for continuous operation, which may translate to potentially higher costs to refine the bio-oil material into finished fuels or blend stocks. The fractionation process as described here represents an alternative pathway for the selective removal of biomass components and their subsequent conversion into high value fuels and chemicals, an approach more amendable to a biorefinery setting. This process as described models a flexible biorefinery model where a well-defined products allow for more opportunity for revenue streams. For example Omega-3 oils can provide the major portion of the revenue stream; yet represent only 10-20% of the feedstocks volume. Other products such as algal fishmeal and biocrude oil make up the balance of the revenue stream. Previously, under the auspices of the DOE consortium we successfully demonstrated a dilute acid-based fractionation process for algal biomass. The results of the process produced monomeric sugars for fermentation, primarily glucose and mannose, algal lipids were recovered from the solid fraction using hexane extraction leaving an enriched protein stream. Solubilizing a portion of the algal protein into amino acids served to reduce nitrogen requirements during fermentation allowing recovery of majority of the protein for additional uses such tertiary fuel production, fishmeal or biogas. The potential benefit from this process allows for the separation of the algal carbohydrate, lipid, and protein components and upgrading those components into well-understood and valorized commodity products (ethanol, renewable diesel, and isobutanol). We applied the fractionating process using two green algal, Chlorella and Scenedesmus, grown in photobioreactors and open ponds at Arizona State University. The algal biomass was grown under these conditions representing early, mid and late periods of nitrogen depletion, resulted in accumulation of protein, carbohydrate and lipids, respectively. The initial results from the integrated testing demonstrated a rapid release of lipid from the algal biomass after pretreatment, over 80% ethanol process yield from fermentation of hydrolyzed algal carbohydrates. No inhibition during fermentation was observed at these conditions, suggesting that the algal carbohydrates are amendable for other fermentation processes/products. Hence higher value products such as succinic acid and other organic acids can be produced using this process.

Monday, December 8, 2014 | 10:30am – 12:00pm

Update on Research and Development of Marine Bio-Products in China

Moderator: David Wang, School of Environmental Science & Engineering, Tianjin University

David Wang, School of Environmental Science & Engineering, Tianjin University

Yuzhong Zhang, School of Life Sciences, Shandong University

Qiwei Qin, South China Sea Institute of Oceanology, Chinese Academy of Sciences

A Review on Recent Progress of Marine Bio-Industry in Tianjin
Xiaorui Pan, College of Research & Technology, Tianjin University

Abstract

With rapid growth of economy, China has significantly expanded national efforts in exploring high-valued bioproducts from marine environments, including deep-sea habitats. This panel focuses on recent advances in marine bioproduct research and development from several interesting marine habitats by the leading scientists from major research and education institutions in China. Particularly, national efforts in exploring of bioproducts from deep-sea environments will be discussed.

Monday, December 8, 2014 | 2:30pm – 4:00pm

Genomic Development Tools and its IP Protection

Martha Marrapese, Keller and Heckman LLP

Application of genomic tools to accelerate natural breeding: genetically selected organisms (GSOs)
Eric Mathur, Yulex Corporation

Functionalization of Diatom Biosilica as Next Generation Biomaterials
Guri Roesijadi, Pacific Northwest National Laboratory

Konrad Sechley, Gowling

Abstracts

Eric Mathur

Guri Roesijadi
Future materials will include composite 3-D nanostructures produced through self-assembly in genetically-modified organisms. Diatoms can be functionalized *in vivo* by transformation with chimeric genes that encode application-relevant receptors, enzymes, and single-chain antibodies fused to a silica–targeting motif that immobilizes the construct in the biosilica shell. The functional units are viable after the modified biosilica is extracted from the cells, thus creating a path to their incorporation into devices. Development of biosilica-immobilized sensors that can be routinely optimized for diverse applications will benefit from a better understanding of the fundamental science underlying the biosilica assembly process and detailed characterization of the assembled composite biosilica structure. We provided proof of concept for bioassembly of a diatom-biosilica-immobilized sensor by genetically-modifying the biosilica of *Thalassiosira*

pseudonana with a receptor-based FRET sensor. Our findings from affinity pull-down of recombinant silica affinity protein Sil3 bound to proteins in diatom cell lysate implicated the secretory pathway in trafficking of Sil3 to the silica deposition vesicle (SDV), the site of silica assembly in dividing cells. To query this trafficking pathway and facilitate the development of novel application-relevant expression vectors, we developed a modular Gateway-based expression system with peptide- and fluorescent-protein-tagged Sil3 or one of its derivatives fused to application-relevant functional proteins and peptides. These constructs incorporated the tetracysteine Cy3TAG for *in vivo* and *in vitro* labeling by the biarsenical probes AsCy3 and AsCy3e. When GFP was included in the construct, GFP, AsCy3e, and PDMPO fluorescence exhibited an apparent co-localization in the SDV. AsCy3e also labeled non-silica-related structures presumed to be part of a trafficking pathway to the SDV. In diatoms transformed with single chain antibodies against either the explosive TNT or S-layer protein of the pathogen *Bacillus anthracis,* screening purified frustules with either the AsCy3 probe or fluorescent antigen indicated frustule-localization of the antibody fragments. The ability to direct tagged recombinant proteins to the biosilica through use of the Sil3 silica-targeting motif is advancing our ability to better understand trafficking of the recombinant proteins to the biosilica and develop novel biosilica-based materials.

Acknowledgements:
The work of current and former postdoctoral associates Drs. Karen Hecht, Nicole Ford, and Kathryn Marshall (PNNL) has been central to this project. Other major contributors include Drs. Thomas C. Squier and Yijia Xiong (Western University of Health Sciences) and Dehong Hu, Galya Orr, and Errol Robinson (PNNL). Drs. Nils Kroger and Nicole Poulsen (CUBE Center for Molecular Bioengineering, Dresden, DE) kindly provided clones for Sil3 and the truncated derivative $Sil3_{T8}$. Dr. Ellen Goldman (Naval Research Laboratory, Washington, D.C.) kindly provided clones for scFvs and sdAbs.

Tuesday, December 9, 2014 | 2:00pm – 3:30pm

Algae Testbeds: Models for Accelerating Commercialization

Moderator: Philip T. Pienkos

John McGowen, Arizona State University

Guido Breuer, Wageningen University and Research Centre

Kimberly Ogden, University of Arizona

Makoto Watanabe, University of Tsukuba

Abstract

Commercialization of novel technologies requires significant effort at scale up and process development to demonstrate that laboratory results can be translated to production. This is a time consuming and expensive

endeavor and can be daunting to small technology developers who do not have access to pilot, demo, or production facilities. Nowhere is the need for pilot facilities more acute than in the algal biomass sector, and challenges in obtaining sufficient funding either from federal sources or from private investors makes this "valley of death" especially problematic for commercialization of novel processes based on algal biomass. One solution to this problem is the institution of testbed or users' facilities which can provide access to a wide array of cultivation and downstream processing equipment to technology developers who might not have the resources to develop their own. In this way, novel technologies can be evaluated at scales larger than lab scale under real outdoor production conditions. Involvement of algae testbeds at early stages of technology development can reduce uncertainty regarding scale up potential and reduce risk for investors, thus greatly accelerating the path towards commercialization. In this panel we will hear from representatives of four algae testbeds: AlgaeParc in The Netherlands, the Algae Testbed Public-Private Partnership (ATP3) and the Regional Algal Feedstock Testbed (RAFT) in the US, and the Algae Industry Incubation Consortium (AIIC) in Japan. They will compare and contrast their capabilities and their interactions with algae technology developers to provide insights into value of this approach not just for processes based on algal biomass but for technology development in general.

Tuesday, December 9, 2014 | 4:00pm – 5:30pm

Innovation in Microbial Conversion Technologies

Engineering Biology to Address Global Challenges: Synthetic Biology, Markets, Needs & Application
Moderator: Nancy Kelley, Nancy J. Kelley and Associates

The production of bio-based, renewable Vitamin D via fermentation of a non-GM yeast
Sean O'Connor, Nucelis LLC

DEINOVE: Building innovative micro-factories
Nagib Ward, DEINOVE S.A.

Computational Design of New Pathways and Enzymes to Enable New Bio-based Products
Eric Althoff, Arzeda

Function Generator: a novel synthetic biology and combinatorial genetic gain-of-function platform technology for microbial improvement
Helge Zieler, Primordial Genetics Inc.

<u>Abstracts</u>

<u>Nancy Kelley</u>
Synthetic biology—the design and construction of new biological parts, devices, and systems, and the redesign of natural biological systems for useful purposes—is contributing sustainable and innovative solutions to numerous, pressing human needs and global challenges. A nascent field that emerged around 2000, synthetic

biology continues to open up new possibilities in healthcare, agriculture, chemicals, materials, energy, and bioremediation. With an expected global market of $10.8 billion by 2016, synthetic biology will play an important role in the bioeconomy and has increasing implications for future competitiveness and employment. Though early synthetic biology development was centered largely in the US, America is falling behind countries with organized national research agendas and coordinated funding – specifically, the UK and China. How far the US and the world can go with this technology depends upon the ability to bring together diverse researchers and stakeholders with a big vision, carefully considered strategy and the support to carry it through. The frontier is still just beginning to be explored, and there is much to be done to fulfill the promise of engineering biology safely and responsibly. Foreseeing this challenge and opportunity, in July 2013, the Alfred P. Sloan Foundation and Synberc (the Synthetic Biology Engineering Research Center) initiated a one-year independent sustainability initiative led by Nancy J Kelley & Associates to develop a strategic action plan to advance the field of synthetic biology in the U.S. Preliminary findings, one of the most comprehensive surveys of synthetic biology to date, were published in February 2014. Based on these findings, an integrated set of recommendations was published in May 2014. Both of these documents were summarized in two companion articles published in Industrial Biotechnology in June, 2014. The findings and recommendations were the product of a broad range of primary and secondary research, spanning over 110 interviews with members of the synthetic biology community, three months of working group meetings with nearly 100 members of the synbio community, extensive secondary research and review of a library of over 500 reports, journal articles, presentations, and government and public documents, as well as attendance at major synbio events and targeted regional visits to relevant institutions. Based on this work, the breakout session proposed will explore the emerging synthetic biology market and the global synbio landscape, including regulatory frameworks, funding initiatives, and social and ethical aspects, in order to highlight what is needed, today and over the next ten years, to properly develop this space. Additional Information: These challenges and models for an organization that can act as a leader between government, industry, philanthropy and the public are detailed in our report here (http://bit.ly/NJKAsynbioplan). Our summary of findings report can be found here (http://bit.ly/NJKAsynbioreport). The two Industrial Biotechnology reports can be found here: 1) http://online.liebertpub.com/doi/pdf/10.1089/ind.2014.1515 2) http://online.liebertpub.com/doi/pdf/10.1089/ind.2014.1516

Sean O'Connor
Nucelis LLC is an emerging metabolic engineering company leveraging its proprietary precision gene editing platform, the Rapid Trait Development

System™ (RTDS), to develop high value, bio-based and sustainable products for the multi-billion dollar cosmetic, personal care, food ingredient, and flavor and fragrance markets. RTDS is a proprietary gene editing platform that allows specific desired changes to be made in a targeted gene. With this approach, no foreign transgenic DNA is inserted into the cell's genome. This powerful technology enables precise metabolic engineering without the creation of recombinant pathways. The result is the fine-tuned production of desired molecules in a non-GMO host organism with no undesired off-target effects. One of the first products being developed with the RTDS platform is ergosterol. Ergosterol is a natural sterol found in the cell walls of yeast and fungi. The production of ergosterol in a renewable, bio-based, fermentation process offers the potential to provide a stable, cost-effective source of ergosterol, the critical chemical precursor used in the production of Vitamin D2. Vitamin D deficiency is increasingly being identified as an important public health issue. Increased use of high SPF sunscreens and legitimate fear of prolonged sun exposure have had the secondary consequence of creating problems with Vitamin D deficiency as Vitamin D is typically made via exposure to direct sunlight. There are currently sources for supplemental Vitamin D3, however they are made from animal components (specifically sheep wool lanolin), making them unsuitable for use by persons who are sensitive to animal based supplementation, specifically vegetarians and vegans. Vitamin D2 is currently made from ergosterol that is extracted from fungi. This process is expensive, creating a cost-barrier to those who would prefer a non-animal source of Vitamin D supplementation. This project offers the potential to create sustainable, cost-effective, and non-animal source of ergosterol and Vitamin D2, making it easier and less expensive for the source-sensitive population to have access to an acceptable Vitamin supplementation. Nucelis has successfully induced this microorganism to increase its conversion rate of glycerol into ergosterol to commercially-relevant quantities. By developing an alternative production process for ergosterol from a waste carbon source (glycerol), we enable ergosterol users to choose a more sustainable and more predictable supply for use in conversion to Vitamin D2. In addition to the production of ergosterol, our lead process is also producing a renewable source of non-GM squalene and squalane. These valuable chemicals are used in the cosmetic industry as emollients. Future microorganism platforms that are targeted for development include programs in bacteria, fungi, and algae, with further development work planned in yeast.

Nagib Ward
DEINOVE is a cleantech company that develops breakthrough production processes based on a yet to be fully exploited bacterium: the Deinococcus. Taking advantage of the unique genetic properties and robustness of the Deinococcus, DEINOVE optimizes metabolic capabilities of these bacteria to produce bio-based molecules from

renewable feedstocks. The company is structured in two programs: DEINOL is focusing on second-generation biofuels; DEINOCHEM on chemicals such as isoprenoids for application in cosmetics, fragrances or food industry. Maximizing on biotransformation kinetics and reducing the energy consumption, DEINOVE's platform leverages thermophilic organisms (48°C) that are not subject to the common constraints often faced by the industry, and that can manage the hydrolysis and fermentation steps jointly, without or with less added exogenous (hemi)cellulolytic enzymes. Indeed, DEINOVE's researchers have built a strain using its metabolic engineering platform capable of hydrolyzing cellulose as fast as the reference microorganism, Trichoderma reesei, a filamentous fungus commonly used to produce many commercial cellulases. This rare process named Consolidated Bioprocessing (CBP), gives DEINOVE's solutions a unique and competitive cost advantage. Adding it all up, DEINOVE's organisms co-assimilate C5 and C6-sugars as well as acetic acid, and are particularly resistant to common process inhibitors in particular furfural and hydromethylfurfural which usually affect fermentation performance, thereby maximizing both yields and conversion rates. At the beginning of this year, DEINOVE's team has achieved a major step towards industrialization: the production of ethanol at 9% v/v titer within industry accepted conditions and timescale, world's first and best results for a bacterial process. The company is now running scale-up tests for the production of ethanol from C5- and C6-sugars in fermenters in the hundreds of liter size. Moreover, DEINOVE's researchers were successful in producing significant concentrations of three molecules of industrial interest from the isoprenoid pathway at the laboratory scale, well in advance of the expected date. In summary, the properties of Deinococcus bacteria, and DEINOVE's demonstrated capacity to optimize them, demonstrate the huge potential of these microorganisms to become true bacterial micro-factories for the production of molecules in a cost-effective, efficient and environmentally friendly way.

<u>Eric Althoff</u>
The recent emergence of industrial and synthetic biotechnology has the potential to radically transform the chemical industry. Despite significant success, the availability of biosynthetic pathways to high value and high utility chemicals is one of the major limitations to increasing synthetic biology's bio-based chemical offerings. As a solution to this, Arzeda has developed novel computational methods for the identification of biosynthetic (metabolic) pathways which works in conjunction with our enzyme design technology to access bio-based chemicals not available in nature and enable fermentation of them. Arzeda's enzyme design technology is able 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 which is subsequently designed to maximize the enzyme's activity. 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. Our partnership with INVISTA on bio-based butadiene and our proprietary gamma-valerolactone program demonstrate two initial chemicals in development.

Helge Zieler

We live at a time of increasingly widespread use of microbes - bacteria, fungi, yeasts, cyanobacteria and algae, for renewable and sustainable production of bio-fuels, chemicals, pharmaceuticals and other compounds. This trend is projected to accelerate over the coming decades as the global chemical industry shifts from petrochemical to renewable biological feedstocks. Optimizing production strains currently requires laborious and costly applications of classical and modern genetic and metabolic engineering methods. However, these approaches are often unable to achieve economic product yields and other required traits related to yield, growth and stress tolerance. Primordial Genetics Inc. is a synthetic biology startup biotechnology company focused on developing the Function Generator™ platform, a novel, combinatorial genetic, gain-of-function technology designed to generate microbial phenotypic diversity, improve product yields and enhance stress & product tolerances in microbes employed for production of a variety of chemical and biological compounds. Function Generator™ technology facilitates strain improvement and trait development by combining domains from different genes, a mechanism which is rarely observed in the laboratory, but which regularly occurs in nature, albeit on an evolutionary timescale. To achieve complex traits related to growth, yield and product tolerance, an entire genome’s worth of biological function is used as starting sequences to create a Function Generator™ combinatorial library. This transformative approach, which is distinct from gene shuffling and other methods for protein evolution or pathway engineering, increases the phenotypic diversity of an organism and enables the production of traits that are otherwise difficult to engineer. Function Generator™ has multiple advantages over current technologies, especially in relation to the probability of achieving a trait of interest, novelty of the newly-discovered genes, transferability of these traits to other strains and organisms, and the speed and cost of the discovery process. The technology will prove more flexible and more powerful than traditional methods in creating organisms with higher productivities. Because the technology is empirical in nature it does not require knowledge of genes and pathways relevant to a trait of interest; it can be applied equally well to established and to little-studied production organisms. Primordial Genetics has used Function Generator™ to engineer stress and alcohol tolerance traits in yeast. A yeast Function Generator™ library consisting of approximately 20 million sequence combinations was created, from which 63 mosaic genes were isolated that confer alcohol and stress tolerance. Several of these genes boosted butanol tolerance from <2% butanol for the wild type strain to >3.5% butanol. Comparison between the Function Generator™ library and a single-gene overexpression library showed that the Function Generator™ approach on average results in >200x higher levels of cell survival, demonstrating the superiority of this technology for generating transferable traits. In addition, 'deconstruction' of 6 active genes showed that their phenotypic effects surpass the additive activities of the individual component sequences. Function Generator™ technology therefore has the potential to broadly enable microbial improvement and to accelerate productivity gains of microbial production systems in multiple industries.

Technical and Research Presentations

Monday, December 8, 2014 | 8:30am – 10:00am

Industrial Biocatalysis Development

Moderator: Jonathan Burbaum, ARPA-E, DOE

Biocatalysis at DSM
David Ager, DSM ChemTech R&D BV

Optimization of the culture medium for production of polygalacturonase from a wild-type strain Aureobasidium pullulans
Catalina Giraldo-Estrada, EAFIT University

Microbial consortia from high temperature composting of sugarcane bagasse, as a key source of novel thermostable enzymes for the sustainable bioconversion of lignocellulose
Sergio Trejo-Estrada, Instituto Politecnico Nacional

Aequor's novel chemicals, discovered in the Sea, inhibit bacterial contamination, corrosion, fouling, etc. on all surfaces without toxicity, improving the performance of biocidal controls.
Marilyn Bruno, Aequor Inc.

Abstracts

David Ager
Biocatalysis at DSM David Ager
Principal Scientist DSM Chem Tech

R&D BV PMB 150 9650 Stricklsnd Road, Suite 103 Raleigh NC 27615 USA david.ager@dsm.com +1 919 870-7791 DSM relies heaving on bioprocesses to make over 40 products using this approach. In addition, and especially for life sciences, the methodology is employed for other parties. Rather than a comprehensive overview, this talk will concentrate on two topics: PLE and P450s. Pig liver esterase has been shown to provide some excellent resolutions. In fact it is a mixture of enzymes. There are problems with its use in pharmaceutical applications, as it is of animal origin. The various isozymes have been cloned in E coli and can now be used in pharma application, as the enzyme is no longer of animal origin. How scale up was achieved will be illustrated. The second topic on P450s is not as advanced. P450s can perform oxidations, which are either difficult or impossible to perform with current chemical methodology. They are also useful to obtain metabolites. There are many difficulties associated with the use of P450s ranging from the production of hydrogen peroxide to enzyme stability. An example will be given of an application.

Catalina Giraldo-Estrada
Polygalacturonases (PGase) are enzymes of industrial importance since they catalyze the hydrolysis of a common natural polymer family: pectins. The main field of application for these enzymes is in the food industry: food processing, wine production and clarification of fruit juice and natural beverages in general. Industrial production of pectinases is carried out from microbiological sources under controlled conditions. The aim of the present work was the optimization of a culture medium for the production of PGase from an isolated microorganism previously selected from a large number of isolates. Isolation of the microorganism was done by cultivation-dependent technique from samples taken from peels from Citrus paradasi collected in three climate zones of the Antioquia region (Colombia). PGase enzymatic activity of 91 fungal strains was evaluated after cultivation in liquid medium enriched with pectin. 73% of the strains presented positive enzymatic activity, where the most promising result corresponded to Aureobasidium pullulans. This microorganism was identified by 18S rRNA gene sequence analysis. The best cultivation conditions in the Abdel-Mohsen (AM) were determined by experimental design. The best values for pH, temperature, cultivation time, and orbital velocity of the shaker were 4.0, 30 °C, 44 hours, and 100 rpm, respectively. At these condition, the maximum PGase activity was 4.7 U·ml-1, and the yields were YP/X = 384 U·g-1 of biomass and YP/S = 235 U·g-1 of pectin. A further response surface experimental design strategy was used to improve the cultivation medium reaching an increase in PGase enzymatic activity of 17% (5.5 U·ml-1). The maximum growth rate (μmax) was 0.14 h-1, and the yields were YP/X = 509 U·g-1 of biomass and YP/S = 419 U·g-1 of pectin. The optimized medium contains 13.1 g/L commercial citric pectin, 5.9 g·L-1 yeast extract, 0.5 g·L-1 MgSO4, and 1

g·L-1 de KH2PO4 (OAM – optimized Abdel-Mohsen). Furthermore, the optimized culture medium (OAM) is 50% cheaper than the standard AM medium, it is 1.3 USD·L-1 for former and 2.6 USD·L-1 for the latter. In addition, supplementation of the medium with cations and vitamins did not increase the PGase enzymatic activity in comparison with the optimized OAM medium. Fe2+, Zn2+, Ca2+, Mn2+ and Mg2+ did not have any effect on PGase activity; however, Cu2+ caused a decrement of 55% (2.5 U·ml-1) in PGase when it was added in a concentration of 10mM to the medium, and the cell growth was inhibited when it was added in 20mM.

Sergio Trejo-Estrada

One key step in the development of a sustainable bioethanol industry, is the development of bio-catalytic technology for the conversion of lignocellulosic biomass. No such technology has been developed by mexican industry. A multidisciplinary group which involves private distilleries, academic institutions and funding from the Ministry of Energy, has obtained pure and mixed cultures, as well as consortia, which under high temperature (50-65 ºC), produce mixed enzyme activities with very high hydrolytic potential. A novel, proprietary method to transform sugarcane agroindustrial by-products in growth container media for horticulture, provides a unique environment for the selection of thermotolerant microbial communities well adapted to the high temperature biodegradation of lignocellulose. An in depth study of microbial ecology of hot compost, as well as an enrichment protocol was developed to obtain selected microbial cultures and consortia. Microbiological and molecular characterization of the microbial community allowed for the detection of unusual phyla. An extensive screening for extracellular enzymes was developed. Fungal strains belonging to Corynascus and Chaetomium; as well as thermophilic strains from Actinomycetes and Bacillus, have been selected for the fermentative production of cellulases and hemicellulases, as well as beta-glucosidase and pectinases. Under laboratory and pilot scale conditions, selected strains were evaluated for their ability to produce stable enzymes by either submerged or solid substrate fermentation. Novel xylanases and endoglucanases from Corynascus, as well as a novel beta-glucosidase from Brevibacillus thermoruber are under development for their use as commercial enzymes.

Marilyn Bruno

Aequor is first-in-market with proprietary chemical compounds that effectively inhibit bacteria’s ability to attach to surfaces – the first stage of industrial, consumer and clinical surface fouling, contamination and infection. (The scientific explanation is that Aequor's chemicals prevent bacteria from forming "biofilm.") Aequor's Founder, Microbiologist Cynthia Burzell, Ph.D., discovered novel chemical compounds in the Sea that target a broad range of bacteria: industrial bacteria that cause corrosion, scale, fungi and mold; marine bacteria that enable the attachment of foulers (barnacles, mussels, algae) to surfaces in contact with water; and Gram-negative and Gram-positive medically-relevant bacteria which cause surface contamination and 80% of all bacterial infections. As validated by Lonza (one of the top 10 chemical companies in the world), Aequor's chemicals also remove existing biofilms, which no other chemical is known to do at non-lethal doses. Aequor's lead chemical is not harmful to any plant or animal life and therefore does not trigger a resistance response or the emergence of MRSA, Superbugs and other drug-resistant pathogens. It can be used in liquid or powder form as a substitute for existing toxic biocides or as a "bio-booster" for existing products, extending the time between antimicrobial treatments. This saves time, manpower, and money in addition to environmental and health benefits. Demand for biocides in general is projected to continue rising with population growth and higher land temperatures that cause the proliferation of biofilm-producing pathogens. Currently, the agro-industrial, consumer, and healthcare sectors spend over $600 billion annually on biocides that are incorporated in sprays, washes, paints, coatings, and surface materials to control bacterial contamination and fouling. Demand for Aequor's chemicals in particular is growing as regulators restrict and ban the toxic biocides currently on the market, threatening companies on the industry value chain with losing market share: chemical companies that manufacture and distribute biocides (disinfectants, antimicrobials, antifouling agents, pesticides, antiseptics, etc.) and downstream product formulators. Some examples of Aequor's lead chemical's benefits can include the following: --Energy: reduce fouling in intake pipes for traditional and renewable power plants, on ship hulls, and in water treatment filters and equipment. --Environment: reduce operational inefficiencies that raise energy consumption and CO2 emissions, and the environmental load of millions on tons of toxic biocides that persist in ecosystems and organisms. --Global Development: boost efficiency and the bottom line of emerging cleantech industries currently prone to fouling (algae/biofuel, aquaculture) and contamination (water reuse, recycling, desalination). --Healthcare: provide remedies to 90% of hospital-acquired infections associated with biofilm, as well as diseases currently considered incurable (cystic fibrosis, endocarditis). --Discovery: inhibit bacterial contamination on underwater and aerospace sensors. (The International Space Station approved an experiment to test Aequor's lead chemical both inside manned craft and, via airlock, in outer space.) For her discoveries, Dr. Burzell was honored by TechAmerica as Researcher of the Year. She is a frequent panelist on her methods to reveal the benefits waiting to be found in the world's oceans.

Monday, December 8, 2014 | 10:30am – 12:00pm

New Developments in Pre-Treatment Processes

Moderator: Ali Manesh, American Science and Technology Corp.

Development of the Glycell tm Biorefinery - an effective low cost lignocellulosic biomass pre-treatment process
Alex Baker, Leaf Resources Ltd.

Pretreatment – The Key to a Sustainable Gen2 Industry
Regis Benech, GreenField Specialty Alcohols

Cost-Disruptive Liquid Phase Catalysis Produces Sugars and Biochemicals

Victoria Gonzalez, Glucan Biorenewables LLC

Gas Phase Catalysis of Pyrolysis Oil for Fuels and Chemicals
Brian Cooper, Hazen Research Inc.

Abstracts

Alex Baker
Development of effective low cost lignocellulosic biomass pre-treatment has been a significant challenge for the emerging biofuel and green chemical industries. Lignocellulosic processing is driven by the ability of the process to liberate sufficient cellulose, hemicellulose and lignin by either mechanical and/or chemical means to make low cost product streams. Here we present a biorefinery concept that uses glycerol as a renewable catalyst for effective de-structuring of a range of wood and grass lignocellulosic fibre sources, including hardwood, non woody biomass such as bagasse and empty fruit bunch (EFB) from the palm oil industry. High yield of cellulose and enzymatic digestibility of the cellulose polymer to reducing sugars are features of the process. Additionally we have worked towards improving processing time, lowering inhibitory residues, decreasing enzyme usage and energy input. Additionally the glycerol can be recycled or used for further valorisation as a carbon source. Techno-economic modelling of the process has been undertaken based on actual scale data and has estimated favorable economics for a number of process product outputs including a cellulose pulp, a fermentable sugar syrup and cellulose derivatives.

Regis Benech
The Gen2 ethanol industry is real, but is in its early stages of development. In large part, success will depend on the availability of sugars derived from a variety of biomass that can be produced in a technically efficient and cost-effective way. That means recovering the largest quantity of sugars that the biomass can offer, in their cleanest inhibitor-free form, using an opex and capex efficient combined process and equipment technology. GreenField has developed such a combined technology. Our superior pretreatment process

recovers the highest amount of available and inhibitor-free sugars from a wide range of biomass. And our equipment technology, which was also developed in-house, executes our process in a versatile, acid-free, and largely mechanical fashion. At the core of our equipment technology is a unique filtering system incorporated into a highly modified twin-screw extruder, which allows for the separation of the C6 and C5 fractions and the processing of the C6 fraction in 1 piece of equipment. We incorporated this Extruder Technology into our 1 tonne/day continuous Pilot and operated this system for over a year to establish proof-of-concept' and develop the basic KPI's. We then designed and built a larger pre-commercial, mobile system and have been successfully operating it for the past 3 months, developing technical data and the final matrices needed for commercial scale-up. We expect to commercially exploit our technology beginning early in 2015. Our model is to control the manufacturing of the equipment and to market on a worldwide basis; all protected by a patent strategy covering more than 50 countries.

Victoria Gonzalez
Glucan Biorenewables is commercializing a biomass conversion platform that delivers cost disruptive sugars and drop-in chemicals. The platform uses organic liquid phase catalysis to selectively depolymerize biomass into high concentration soluble C5 sugars and lignin while leaving the cellulose intact. Gamma-valerolactone, the green solvent used in the process, increases hydrolysis and dehydration reactions, minimizes degradation products and has a low heat of vaporization. The pilot unit will be a continuous process to prove the economics of delivering cost competitive furfural and glucose. The Company has commitments from three companies for 100% of the furfural production of the first commercial plant.

Brian Cooper
Pyrolysis oil from woody biomass is corrosive, unstable and often of low value as a fuel. Despite these issues, pyrolysis is of major interest as it represents a significant step in biomass energy densification as well as a prime candidate for further processing into complex hydrocarbons for use in industry. The presentation will focus on Hazen's experience with softwood pretreatment and hydrolysis as they relate to thermochemical feedstock, as well as data from our subsequent pyrolysis oil catalysis project. A study on process variables will be presented, which addresses control of upstream parameters and how they impact catalytic yields. Additionally, analytical challenges encountered in these operations and a summary of Hazen's solutions will be discussed.

Monday, December 8, 2014 | 2:30pm – 4:00pm

Leading Pre-Treatment Technologies for Production of Fuels, Chemicals and Feed

Moderator: Charles Wyman, University of California, Riverside

Charles Wyman, University of California, Riverside

Bruce Dale, Michigan State University

Michael Ladisch, Purdue University

<u>Abstracts</u>

This Panel focuses on recent advances in leading pretreatment technologies that can be coupled with enzymatic hydrolysis to convert lignocellulosic biomass to sugars for fermentation to ethanol or other products. The low cost of lignocellulosic biomass coupled with widespread domestic abundance, ability to dramatically reduce greenhouse gas emissions, and potential to spawn new rural manufacturing jobs make it an attractive resource from which to produce fuels and chemicals. However, converting this low cost resource into commodity products is expensive, with recalcitrance to sugar release being the key obstacle to achieving low prices by biological conversion routes. Most forms of lignocellulosic biomass must be pretreated prior to biological conversion operations to realize the high yields vital to economic competitiveness, and effective pretreatments can also lower loadings of expensive enzymes to economic levels, reduce costs of downstream operations, and produce valuable co-products that can improve overall process economics and provide additional benefits. Various studies have shown that thermochemical pretreatments that employ chemicals in combination with heat are most effective in realizing high sugar yields from the coupled operations of pretreatment and enzymatic hydrolysis. This Panel will include a presentation of recent work at Purdue University on reducing the amount of enzyme required for hydrolysis and the fundamentals of pretreatment related to changes in cell wall structure and chemistry. Increased severity of pretreatment exposes both additional lignin and cellulose. However, lignin adsorbs cellulase, so more enzyme must be added if the additional exposed cellulose is to be effectively hydrolyzed. Conversely, cellulase loading may be decreased by a factor of 10 while maintaining 80% glucose yield by diluting the enzyme with non-catalytic protein (BSA) that binds to lignin and decreases cellulase adsorption on lignin. More enzyme is therefore available for cellulose hydrolysis resulting in enhanced hydrolysis. Michigan State University is advancing Ammonia Fiber Expansion (AFEX) pretreatment, now being commercialized, to produce cellulosic biomass that can be used either for animal feed or as biofuel feedstock, thereby largely eliminating the "food versus fuel" issue. The AFEX presentation will briefly describe AFEX science and technology and how it can be performed in distributed processing facilities called depots. These depots greatly improve the logistics of cellulosic biofuel systems and allow local communities to capture part of the added value of AFEX processing. A presentation by the University of California at Riverside will describe a novel Co-solvent Enhanced

Lignocellulosic Fractionation (CELF) pretreatment that removes nearly all the lignin from biomass, recovers most of the hemicellulose sugars, and produces glucan-enriched solids that can be almost completely enzymatically digested to glucose with about one tenth the enzyme loadings typically required. Furthermore, CELF has been found to be effective with a wide range of hardwoods, grasses, and agricultural residues. Following the fate of major biomass components, kinetic modeling, and SEM imaging suggest that the high lignin removal afforded by CELF could play a key role in achieving such high sugar yields with extremely low enzyme loadings and lead to alternate strategies to improve pretreatment.

Tuesday, December 9, 2014 | 2:00pm – 3:30pm

Feedstock Flexibility and Product Diversity

Cane Molasses – A viable feedstock for Levulinic acid
Moderator: Pramod Kumbhar, Praj Industries Ltd.

Bio-chemicals and US shale gas: sharing the space in the new chemical industry economy
Lee Walko, Omni Tech International

Feedstock Flexibility and Product Diversification through Partnering for Process Optimization, Integration and Demonstration
Todd Pray, Lawrence Berkeley National Lab

Acetone-butanol-ethanol (ABE) Production from Cassava by a Fermentation-pervaporation Coupled Process
Yinhua Wan, Institute of Process Engineering, Chinese Academy of Sciences

William Bardosh, Terra Verdae Bio Works

Abstracts

Pramod Kumbhar
Commercialization of renewable platform chemicals has witnessed a significant quantum leap. Most of the front runners are at the semi commercial validation stage. Most of these technologies are staged for demonstration using glucose originating from corn as a feedstock during the initial stages. These technologies will then be adapted to cheaper feedstocks for better commercial returns. Cost of feedstock becomes a crucial cost contributor especially in the case of platform chemicals obtained via dehydration reactions due to reduced molecular weight of the resulting platform chemical. Levulinic acid is one such platform chemical. Extreme reaction conditions necessary for converting glucose to Levulinic acid, trigger numerous undesirable humin forming reactions which further

worsen the commercial economics. Fructose is a better sugar in comparison to Glucose for Levulinic acid formation. However fructose is an expensive commodity and inversion of glucose to fructose again involves cost. Praj has successfully exploited molasses as a cheap feed for Levulinic acid wherein fructose obtained as a result of sucrose hydrolysis can be converted selectively and quantitatively to Levulinic acid/esters and the glucose fragment can be recovered quantitatively to offer a significant cost advantage. The process can be plugged on to an existing molasses based distillery to ferment glucose to ethanol. The paper will hightlight the salient features of this new technology along with its techno-commercial aspects.

Lee Walko

Most analysis on the impact on the biochemical landscape from US shale gas focuses on the upstream feedstock view. It is relatively straight forward with many of the assumptions and calculations based on capacity, production and forecasted consumption. Basic conclusions are always the same as shale gas displaces naphtha cracking: shale gas provides more C1 and C2s, and less C3s, C4 and C5+. BTX's are also forecasted to be in short supply. Energy prices and hydrogenation are often cited as contributing a positive benefit for biobased chemicals, which is true, however that same benefit is enjoyed by petroleum-based chemicals. C4 and greater biobased chemicals should prosper is the universal conclusion. Our analysis flips this scenario to a downstream product centric view as that is where the collisions occur in the marketplace. Shale gas enables both ethane derivatives and often overlooked, chlor-alkali derivatives via cheaper ethane and electricity. In particular, the chlor-alkali value chain is expansive and not well understood as many chemical derivatives are made via a chlorine route such as propylene oxide, and isocyanates both main stays of polyurethane chemistry, yet do not contain chlorine in the final product. These two derivatives chains feed massive infrastructures and tens of thousands of products and applications. In our view, biobased chemicals will prosper in selected downstream markets; however, it is complex and requires critical needs/performance based analysis to identify the sweet spots. There are many tentacles of shale gas that have a profound impact on biochemicals. With the rapid construction of ethane only crackers, the re-shoring of the US as chemical manufacturing moves back, is putting a tremendous strain on human resources, and capital dollars. The biochemical industry has to compete for these same resources to grow. In addition, many analyses fail to take into account the increased crude oil production from the US as the same horizontal drilling techniques are being used to extract oil from older wells. This clouds the future of BTXs as well. A point often missed is the capacity for a competitive response by oil refiners. They are masters of molecular manipulation and process control, meaning that if certain derivatives start to increase in price due to lower production, e.g. benzene, they have the technical capability to manipulate the molecules catalytically or adjust process conditions to increase production of these derivatives. Our conclusions are:

• Biobased chemicals will prosper based on investigative due diligence to identify the openings that petroleum and shale gas leave.
  o Finding the sweet spots and partnering with early adopters is critical for success
  o Biobased companies must position themselves as chemical companies
• Sustainability across the value chain is driving interest in biobased feedstocks and chemicals
• US shale gas is becoming very disruptive globally and will continue for the foreseeable future
• Lower cost for energy and petroleum derived feedstocks will complicate the chemicals markets
• The proliferation of ethylene and chlor-alkali derivatives will collide at the downstream product level with biobased derived products
• Human resources and capital will be strained in the coming years in the United States

<u>Todd Pray</u>
Feedstock flexibility and product diversity will continue to grow as key drivers of the Bioeconomy. The Advanced Biofuels (and Bioproducts) Process Demonstration Unit (ABPDU) at the Lawrence Berkeley National Lab (LBNL) has a unique mission to partner with industry, National Labs, Bioenergy Research Centers, and academia to optimize, integrate and scale production processes for advanced biofuels, bio-based chemicals and biomaterials. The ABPDU is a pilot facility with state-of-the-art development and scale-up equipment and unit operations spanning biomass deconstruction, fermentation bio-processing, downstream recovery, purification, and analytical chemistry. Flexible process configurations and order of operations make the ABPDU a premier site and partner to address unique challenges of development, integration and scale-up of novel biological and chemical processes from an array of feedstocks such as agricultural residue, energy

crops and municipal waste streams. The facility was commissioned in 2012 and its construction was funded by DOE's Energy Efficiency and Renewable Energy Office (EERE) using monies from the American Recovery and Reinvestment Act. The ABPDU, working with EERE's Bioenergy Technologies Office, is playing an active role in enabling the companies and technologies to build the Bioeconomy.

Yinhua Wan

With the depletion of fossil fuels and increasing environmental concerns, bio-based butanol production by fermentation has attracted more and more attentions worldwide. However, one of the major obstacles to limit the economic viability of butanol fermentation is its low concentration caused by product inhibition and toxicity. So far, a number of in situ product recovery technologies have been developed to solve this problem, including gas-stripping, pervaporation (PV), liquid-liquid extraction and adsorption, etc. Among those techniques, PV is considered to be the most promising technique due to its energy-efficient, low cost, etc. Moreover, the high cost of carbon sources has been identified as a major factor affecting the economic feasibility of large-scale butanol fermentation. Cassava as a carbon source in butanol production has attracted much attention during the past three decades because it is a high-yield, non-grain crop, and can grow in dry and poor soils, avoiding land competition with other major food crops. It can be expected that butanol production cost could be decreased when the cheap carbon source cassava is used. In this work, production of acetone-butanol-ethanol (ABE) from cassava was investigated with a fermentation-pervaporation (PV) coupled process, in which a silicalite-1 filled polydimethylsiloxane (PDMS)/polyacrylonitrile (PAN) pervaporation membrane was used to in situ remove ABE products from the fermentation broth to alleviate the toxicity of solvent to the microorganism, Clostridium acetobutylicum DP217. The experimental results confirmed that in situ solvent recovery by PV could alleviate butanol toxicity and increase the yield, productivity and substrate utilization rate in ABE fermentation, thus decreasing the cost of ABE production. With the coupled process, a highly concentrated product containing more than 200 g/L total solvent was obtained, reducing energy consumption and water usage in ABE production from cassava. Moreover, the silicalite-1 filled PDMS/PAN pervaporation membrane showed very stable performance and no significant fouling was observed after running for 304 h, suggesting the membrane could be promising in ABE production by the coupled process. Keywords: butanol; cassava; coupled process; fermentation; pervaporation * This work was supported by the National High-tech R&D Program (863 Program, Grant no. 2012AA03A607) and National Natural Science Foundation of China (Grant no. 21176239).

William Bardosh

Tuesday, December 9, 2014 | 4:00pm – 5:30pm

Technical Improvements and Industrial Fermentation

Designing Separation and Purification Systems for Bio-Produced Chemicals
Donald Glatz, Koch Modular Process Systems

The Electrobiome: A Microbial Platform for the Electrosynthetic Production of Fuels & Chemicals
Harold May, Marine Biomedicine & Environmental Science Center, Medical University of South Carolina

Self-Cycling Fermentation: Bioprocessing for the -omics era
Zack Storms, University of Alberta

Successful Scale-up of Industrial Fermentations: Process Development, Engineering and Economics
Edi Eliezer, BioPrizM

Valorization of Bakery waste for Lactic Acid Fermentation
Joachim Venus, Leibniz-Institute for Agricultural Engineering Potsdam-Bornim (ATB), Dept. Bioengineering

Abstracts

Donald Glatz
Liquid-liquid extraction is an excellent first step in the recovery of valuable chemicals from broths generated via bi-processing methods such as fermentation and algae. For the past several years, Koch Modular Process Systems, LLC (KMPS) has been testing many bio-produced broths in our pilot plant facility and generating the data for accurate scale-up to commercial extraction columns and complete separation systems. Most of these broths demonstrate a high tendency to emulsify when contacted with solvents for extraction. The majority of the commercially available equipment types used for liquid-liquid extraction are not suitable for this behavior and in fact many will actually exasperate the emulsification tendency. However, KMPS has learned that the KARR® Column with a reciprocating plate stack that imparts uniform cross sectional shear can effectively handle these types of systems and provide the capacity plus efficiency required. Thus, KMPS can supply complete systems, including the KARR® Column with associated downstream distillation columns to recover and purify valuable chemicals from bio-produced broths.

Harold May
Microbes are renowned for their ability to select a niche and efficiently

catalyze the degradation or synthesis of chemicals. The Electrobiome refers to an electrosynthetic microbial community that converts electricity, carbon dioxide, and water at the cathode of an electrochemical cell into valuable fuels and chemicals. This is done without food crops or arable land, may use intermittent power, and may be centrally or remotely located. Discussed in this presentation will be a microbiome that produces kg quantities of hydrogen and organic acids per L of cathode volume per day. The productivity is sufficient to consider further development for hydrogen production as a fuel or chemical feedstock, organic acids as chemical precursors, or further advancement of the Electrobiome for the production of liquid fuels or higher value chemicals. All of these products could be generated sustainably with waste carbon dioxide and a non-carbon based, renewable source of electricity. The present capability and potential future of the Electrobiome will be discussed. The platform is being developed through a series of collaborations between the Medical University of South Carolina, Argonne National Laboratory, Synbiohm LLC, and CDM Smith Inc.

<u>Zack Storms</u>
While our understanding of genetic and metabolic networks within microorganisms has increased by leaps and bounds over the past two decades, our approach to large-scale fermentation has seen little change. Combining the metabolic insight developed through the years with the novel tools of synthetic biology now allows for targeted engineering and evolution of organisms to over-express desired molecules or achieve specific traits. Recent work has shown that traditional fermentation systems may not fully capture the production potential of microorganisms. This is mostly due to the distribution of metabolic states within the culture, a factor too often brushed over. Although synchronizing a culture such that physiological and metabolic traits of each of its individuals are uniform throughout the production period is often challenging or thought to be unfeasible at large scales, recent studies have shown this to be not only feasible but also to impart marked advantages over traditional batch or fed-batch cultures in different production settings. This talk will highlight some of the recent developments in synchronization technology and its application to industrially-relevant fermentation processes. Self-Cycling Fermentation (SCF) is an easily scalable method to synchronize microbial populations in bioreactors. SCF is a cyclical, semi-continuous, automated process that maintains the bacterial culture in logarithmic growth. By design, the SCF process aligns the cycling time with the cell doubling time such that cells double once per cycle, leading to a significant level of synchronization. For example, when applying SCF to populations of Escherichia coli, cell synchrony indices of up to 0.74 were obtained — compared to 0 for a batch culture and 1 for an ideally synchronized population. Synchronized growth magnifies cellular events by temporally aligning metabolic activity of the whole population. Ideally, the behavior of a

synchronized culture directly reflects that of an individual cell, enabling optimization of product formation according to cell metabolic function during the doubling time of the cell. Although the technology was developed over 25 years ago, it received little attention from industry due to niche applications and a system arrangement that did not lend itself to scalability. Recent improvements in design and control schemes have solved these initial problems and SCF has been recently applied to various production processes with increased yield and productivity over batch systems. SCF boosted recombinant protein productivity of an Escherichia coli culture by 50% over a batch culture grown under similar conditions. Analysis of an E. coli-Phage T4 production system revealed that intracellular resource availability is the most probable explanation of increased productivities achieved in SCF. Bacteriophage production was found to be strongly correlated to the cell-cycle, peaking immediately prior to binary fission when cells were largest and intracellular RNA concentrations were highest. These two systems – recombinant protein production and bacteriophage production – will be presented in detail. Ongoing studies on the suitability of SCF for diverse applications such as methanotrophic fermentation, aromatic compounds production in yeast, and directed evolution will also be highlighted.

Edi Eliezer
Industrial fermentations developed for biobased chemicals & biofuels, require six major critical parameters to be considered for successful scale-up, new facility engineering and cost effective commercial product. The first critical parameter is the selection and optimization of the biocatalyst or culture type. The second one is the raw material or substrate (liquid, gas) selection as a crude or refined one. The third one is the process technology: batch, fed-batch, or continuous processes and efficient bioreactor designs that can significantly impact costs. The fourth one is the product type: low or high purity, sensitivity to physical and bio/chemical challenges, low or high-value added (cost sensitivity). The fifth one is the scale of operations (scale up and engineering challenges), production capacities (commercial operations aspects). The sixth one is the economics of processes and products at given production scales (capital & operating costs). The author, a bioindustry expert of many years, including leadership at DuPont, Abbott and Fluor, addressed above subjects in many projects where he developed the most appropriate design solutions. The presentation will illustrate how a good understanding, techno-economic modeling and industrial experience in above parameters will result in the selection of optimal & economical solutions.

Joachim Venus
Besides increasingly important issues with regard to quantity and availability of raw materials together with their properties and quality the feedstock costs are very important for the production of bulk chemicals. Especially for biotechnological processes, in which the carbon of

various substrates should be converted into microbial products (e.g. lactic acid), there is an increasing interest in the use of biogenic residues and waste materials. Carbohydrates like starch, which is the main constituent of the bread dry weight, are preferably used as substrates/nutrients for several biotechnological (fermentation) processes. The environment benefits because bioprocesses are efficient users of (partly residual) raw materials, creating little end-of-pipe waste, which itself can be often used as input into a further bioconversion process. At the same time, moving from chemical to biological processes can lead to significant reductions in carbon dioxide emissions, energy consumption, and water use. Biotechnology offers new ways to improve the environmental performance of industrial processes in various sectors. It can contribute to reducing energy consumption and waste and to achieve sustainable industrial and societal development. For the here addressed specific residual bakery waste some examples of industrial application (enzyme production, aroma compounds, bioethanol, baker's yeast, succinic acid) already exist but only very little information according to the lactic acid fermentation itself are available. On the other hand it seems to be a good combination between a bread-based, starch containing substrate and the lactic acid bacteria due to their ability (at least for specific strains) of starch utilization. At the ATB's lactic acid fermentation group there are long-term experiences with several feedstocks and bacterial strains, respectively. According to the difficulties mentioned in the mobilization of fermentable sugars a range of other, easy accessible substrates are suitable for subsequent fermentation processes (such as residues from fruit and vegetable processing, by-products from starch and sugar factories or from the baking industry). During a Life+ EU demonstration project (BREAD4PLA) has been shown at the European level how residual material from the agri-food sectors (especially the baking industry) can be supplied for innovative, consumer-friendly and environmentally compatible recycling. The project consortium partners were working on several parameters, in order to show a new solution for the closed circuit of the residual waste collection through processing to bioplastics: organic waste from the bakery industry, fermentative production of lactic acid from remaining bread, polymerization of lactic acid to polylactic acid, PLA modification by compounding and film production as well as final testing and evaluation of these films for packaging. The main objective of BREAD4PLA was to show the production of polylactic acid from residual bread in a pre-industrial continuous process. In the sense of a closed loop, the 100% biodegradable PLA film can subsequently be used for the packaging of fresh baking goods. Selected results about the potential of waste bread as an alternative substrate source for lactic acid fermentation are presented and discussed.