Houston Brown, University of Alberta

Mixed-valent First-Row Transition Metal Clusters for Tandem Depolymerization and Refining of Lignocellulosic Biomass

While the structural composition of lignin makes it an enticing potential source of liquid fuels and bulk chemicals, this biopolymer remains the most underutilized element of lignocellulosic biomass. Only about 2 % of lignin produced annually is used to source chemical products, while the vast majority burned on-site as a low-grade fuel for pulp and paper industries. Current refining technologies upgrade lignin to high-quality liquid fuels in two distinct steps. First, hydrogenolytic-lignin depolymerisation provides a mixture of functionalized phenols known as bio-oils. Second, bio-oils are catalytically refined to a mixture of etherified liquid fuels. The latter process deserves significant research investment, since catalytic reduction of such bio-oils provides a practical strategy for the commercial production of first-generation (unfunctionalized) bulk aromatics, including benzene, toluene, xylenes (BTX), along with various alkyl benzenes: building blocks of the petrochemical industry from an underappreciated renewable resource. Research and development related to the catalytic reduction of lignocellulose has focused on the ubiquitous cobalt/nickel-promoted molybdenum hydrotreatment catalysts of the petroleum industry. The heterogeneous nature of these catalysts and the harsh reaction conditions required result in non-selective oxygen extrusion from these diversely functionalized biopolymers through competing mechanistic pathways, ultimately affording complicated mixtures of products at low conversions. Optimal catalysts for lignin refining will afford high conversions under mild conditions, thereby avoiding char formation and other competitive thermal processes. High selectivity for direct C-O bond hydrogenolysis is desired to suppress excessive hydrogen consumption as most heterogeneous catalysts hydrogenate aromatic rings prior to C-O bond scission. Moreover, the ideal biomass hydrotreatment catalyst will facilitate tandem depolymerisation and refining to convert biomass into higher value liquid fuels via non-destructive side-chain scission. Towards this goal, we have designed, synthesized, and fully-characterized discrete molecular catalysts that facilitate the hydrogenolysis of lignin-type C-O linkages under mild conditions. Our catalysts feature small clusters of cobalt centers (earth abundant, inexpensive, first-row transition metal) supported by thermally-robust anionic ligands, that can be tuned to optimize steric and electronic effects. We have shown these novel base metal hydrotreatment catalyst mediate selective C-O bond reduction under extremely mild conditions (110 oC, 1 atm H2). The catalytic hydrogenolysis of a range of aryl ethers and the ‘depolymerisation’ of lignin model substrates have been investigated. Catalytic hydrodeoxygenation of highly refractory benzo- and dibenzofurans has also been explored.

Carolina Chanis, University of British Columbia

Maximizing Sugar Recovery Via the Steam Pretreatment/Enzymatic Hydrolysis Route to Converting Wheat Straw to Fermentable Sugars

Wheat is the primary feedstock for conventional ethanol production in Western Canada. Thus wheat straw is a potential feedstock for advanced (or second generation) bio-ethanol production. In past work, we and other groups have found that acid catalyzed steam pretreatment followed by enzymatic hydrolysis of the cellulosic component is an effective way to try to maximize overall sugar recovery from several sources of biomass. However, steam pretreatment is usually a compromise whereby conditions that facilitate enzymatic hydrolysis at low enzyme loadings usually sacrifice the recovery of the hemicellulose derived sugars. Previous work has tried to determine the most severe pretreatment that could be applied during pretreatment that would preserve >70% of the hemicellulose at the expense of using relatively high (30-50 mg/g glucan) enzyme loadings. Few studies have used the total amount of sugars solubilized during the pretreatment and enzymatic hydrolysis processes to assess the effectiveness of the pretreatment conditions. Ultimately, especially during acidic pretreatments such as steam which specifically solubilize hemicellulose, it is the overall yield of fermentable soluble sugars that can be recovered after both pretreatment and enzymatic hydrolysis that is important. Therefore, in this study, the pretreatment conditions (temperature, time, catalyst loading) were varied to determine their effects on the total soluble sugar recovery after both pretreatment and hydrolysis. At a 10% solids concentration and an enzyme loading of 20 mg protein/g glucan more than75% of the original sugar could be recovered when conditions of 190°C, 8 min and 1.5% H_2SO_4 were used to pretreat the wheat straw. However, at these conditions only 52% of the original xylan could be recovered. Alternatively, a less severely treated substrate that recovered over 70% of the xylan can be treated with xylanase to obtain a 72% total soluble sugar yield. It was apparent that the total soluble sugar yields remained heavily influenced by the xylan content of the water insoluble fraction. Therefore, it may be beneficial to perform pretreatments at lower severities to maximize sugar recovery while using a combination of hemicellulases and cellulases to maximize the total soluble sugar yields after the pretreatment.

James Coates, McGill University

Novel Plethysmography System for the Analyzing and Prediction of Radiation-Induced Lung Injury

A plethysmograph is an instrument for measuring respiratory variations and analyzing volumetric changes in a controlled volume through measurement of differential pressure or other thermodynamic variables that are physiologically relevant. In our study, we plan to measure respiratory distress to radiation exposure of the lung, which is encountered in radiotherapy treatment. Towards this goal, we introduce a novel plethysmograph design, targeted for use with small animals receiving thoracic irradiation, which utilizes a customized geometry to increase its sensitivity. The design is ultra-portable and completely non-invasive in order to allow investigators to better gauge a multitude of respiratory parameters without stressing the tested subjects. The project design utilizes state-of-the-art rapid prototyping and multi-physics simulations. The designed prototype is expected to significantly increase the efficacy of physiological measurements made via flow propagation techniques while simultaneously reducing initial investment cost and increasing precision. Comparison and benchmarking with existing systems will be conducted to ensure validity of results. Additionally, the apparatus will have the added ability to analyze nitric oxide (NO) levels as an effective method for early prediction of radiation-induced lung injury.

Jeffrey Cutler, Canadian Light Source Inc.

Investigation of the Micro- and Nano-structural Elements of Bio-composites based on Flax and Hemp Fibres

Knowledge of the nature of the chemical components of – and their location in - natural fibres (e.g. flax and hemp) is a fundamental step towards the development and performance optimisation of high-quality advanced material products, particularly bio-composites, from Canadian biomass resources. Given the current global push for the replacement of petroleum based products, this is a critical moment for the development of such bioproducts and their rational design is vital for commercial application. In the proposed project, Mid Infrared Spectromicroscopy and Soft X-ray Spectromicroscopy beamlines at the Canadian Light Source synchrotron will be used to map distribution of chemical constituents within thin sections of flax and hemp stem material. Maps and images of hitherto unachieved resolution will provide the precise anatomical location of chemical components (lipids/waxes, lignin/aromatics, pectin and cellulose) within the fibre and at the fibre/matrix interface. This information will make a unique contribution to understanding the biochemical formation and interaction and how these natural fibres perform in commercial products. Consequently this will enable the rational design of advanced materials, e.g. combinations of natural fibres and biofoams / bioresins, for specific end-use requirements

Keith Gourlay, University of British Columbia

Quantifying the Cellulose Disruption/amorphogenesis Step in a Biomass-to-fuels-and-chemicals Process

Advanced biofuels can be produced from waste lignocellulosic biomass, such as forestry and agricultural residues, via the enzymatic depolymerisation of the sugar chains within the biomass, followed by conversion of these sugars into fuels and chemicals. However, the large amount of protein/enzymes required to break down the structural carbohydrates to monomeric sugars is hindering commercialization of this sugar platform biorefinery process. It is recognised that the high enzyme dosage required is largely due to the inaccessible nature of the cellulose within the complex lignocellulosic matrix. Several microbial non-hydrolytic proteins have been shown to disrupt this matrix in a process known as amorphogenesis, thereby enhancing the accessibility of the cellulose to the depolymerizing enzymes. However, the process of amorphogenesis has proved challenging to quantify and therefore difficult to optimize. The presentation will describe the successful application of a novel quantitative technique using cellulose substructure-specific carbohydrate binding modules to assess protein-mediated amorphogenesis.

Futoshi Hara, Asahi Glass Co., Ltd.

Metabolic Regulation of L-lactate Production without Neutralization Using Fission Yeast, Schizosaccharomyces Pombe

The current main D-/L- lactic acid production method is fermentation by the lactic acid bacterium. However, neutralization was needed with lactic acid bacterium fermentation and low optics purity raise a refinement cost. Gene engineering of fission yeast Schizosaccharomyces pombe and adequately culture and ferment can produce the lactic acid that the optics purity was high with low cost. This study aimed at elucidating what kind of gene control is performed in the fermentation. Great improvement was seen in production speed and yield by letting division yeast S.pombe which changed a gene ferment by high cell density. In addition, fermentation by no neutralization was enabled by this. Transcription of the gene group that promoted for the stationary phase was seen at the repeated fermentation.

Robert Harrison, University of Washington

Nutrient Risk to Long-term Site Productivity Due to Whole-tree Harvesting in The Coastal Pacific Northwest

This study is the first step to a regional evaluation of the effects of biomass harvesting in the coastal Pacific Northwest. The growth of 68 intensively managed, mid-rotation, Douglas-fir stands in western Oregon, Washington, and British Columbia was projected to 50-55 years of age using the SMC variant of the ORGANON growth and yield simulator. From the ORGANON output, component biomass removal was estimated for stem-only harvest and a more intense whole-tree harvest. Utilizing published equations which estimate tree component N content based on biomass and the total site nitrogen from the 68 sites, nitrogen removal under the two harvest intensities was expressed as a proportion of total site nitrogen store. Based on the proportion of N removed to the total site store, the 68 sites were assigned a risk rating, and regional patterns were assessed. Based on the simulation results, 49% of the stands in the study were at risk of N depletion or site productivity loss under whole-tree harvest, while only 26% of stands were ranked higher than the lowest risk category under stem-only harvesting. The highest concentration of stands at risk of long term site productivity loss from N depletion was found on young glacial soils in Vancouver B.C and the Puget Sound region of Washington. This simulation also suggests that production Douglas-fir plantations with less than approximately 9000 and 4000 kg/ha of total site N will be at an elevated risk for long term site productivity loss under whole-tree and stem-only harvests respectively.

Jens Asmus Iversen, Aalborg University, Denmark

Monitoring Yeast Fermentation Real-time using Raman Spectroscopy with Sapphire Ball Probe

Raman measurements have the advantage of only minimal interference from water in analysis of aqueous samples compared to traditional IR methods. This study demonstrates in-situ monitoring of a Saccharomyces cerevisia fermentation process using Raman spectroscopy instrument equipped with sapphire probe ball. The probe is designed to minimize scattering interference from particulates and withstand harsh process environment as present during sterilization. Spectral analysis of single fermentation components enabled real-time monitoring and quantification of glucose consumption and ethanol production. Attenuation of Raman signal due to light scattering from yeast cells complicates quantification. However, scattering correction was achieved by correlating yeast cell concentration with the amount of light extinction from scattering, thus significantly improving quantification and also enabling Raman measurement of yeast cell concentration during fermentation. The method is intended to support development of process control technologies for 1st and 2nd generation bioethanol industry.

Nadim Jessani, Genedata, Inc.

Genedata Selector™: Software for Optimization of Feed and Food Stock Crops in the Biofuel Industry

The global demand for sustainable feed and food stock crops to produce biofuels and biobased chemicals is constantly growing, and maximizing biomass is a primary focus of the agricultural and biofuel industry. However, it is predicted that climate change will decrease yields of most important crops dramatically through an increase in arid land area.

Breeding new feedstock crops resistant to environmental stress (such as drought) requires integration of diverse biological data including genotypes, phenotypes and expression measurements. Triggered by advancements in cost efficient next generation sequencing (NGS), plant genome sequencing has become readily accessible, and become an integral part of marker-assisted breeding (MAB) programs. Genedata Selector™ supports MAB through extensive data integration and management of a virtually unlimited number of genome sequences.

We present Genedata Selector™, a comprehensive data management platform for the biofuel industry used for optimization of biomass. Here we demonstrate how Genedata Selector™ has been successfully used to compare crop variants that have enhanced and improved resistance to climate related stress factors. We show how Genedata Selector™ is used to annotate new sequenced genomes and refine gene models. Mutations are identified via related information from different biological sources, including experimental transcriptomic, proteomic and metabolomic data, and viewed in different biological contexts, for example regulatory networks and metabolic pathways. We focus on variant transcription factors which regulate stress related target proteins in feedstock crops such as corn, maximizing crop yields under abiotic stress conditions including drought. Additionally, target genes of interest are linked to phenotypes and assay data, as well as patents e.g. to ascertain IP positions and freedom to operate.

Il Lae Jung, Korea Atomic Energy Research Institute

Electrochemical Reducing Power can Trigger Ethanol Production in Genetically Modified Ralstonia Eutropha

In our previous studies, the electrochemical bioreactor has been developed to generate biochemical reducing power by catalysis of neutral red immobilized in graphite felt cathode. Facultative anaerobic bacterium Ralstonia eutropha containing alcohol dehydrogenase (adh) and pyruvate dehydrogenase (pdc) genes originated from Zymomonas mobilis autotrophically grew when carbon dioxide (CO2) was supplied in the electrochemical bioreactor. The genetically modified strain also produced bioethanol when glucose was supplied in the electrochemical bioreactor, but not in the conventional bioreactor that biochemical reducing power was not supplied. These findings suggest that electrochemical reducing power can trigger ethanol production from glucose in genetically modified Ralstonia eutropha. When raidoactive 13CO2 instead of 12CO2 was supplied to the reactor, 13C/12C ratio in the biomass was greatly increased, suggesting that the autotrophically assimilated CO2 may be building blocks for biomass synthesis and biochemical reducing power let cells to produce bioethanol from glucose.

Hyun-Woo Kang, Changhae Institute of cassava & ethanol research, Changhae Ethanol Co., Ltd.

Bioethanol Production by Temperature Shift - Simultaneous Saccharification and Fermentation from Miscanthus

Lignocellulose from biomass such as Miscanthus could make bioethanol more competitive with fossil fuels while avoiding the ethical concerns associated with using potential food resources. And, simultaneous saccharification and fermentation (SSF) has been suggested as the favorable strategy to reduce the process cost in cellulosic bioethanol production. In the present study, the potential of temperature-shift process as a tool for SSF optimization for bioethanol production from Miscanthus was examined. In the TS-SSF, thermostable yeast was used to overcome the difference in appropriate temperature for saccharification and fermentation. The overall mass balance of the process was calculated to evaluate the bioethanol production from Miscanthus by the TS-SSF process.

Tae Hyeon Koo, Korea Food and Drug Administration

Comparative Evaluation of Three Different Extraction Methods for Rice and Rice-Containing Processed Food

DNA extraction is one of the most technically demanding and labour intensive procedures. Most common DNA extraction method is manual sample preparation, which is time consuming and susceptible to contamination and handling errors. The other is automated DNA extraction systems, which is efficient recovery, lack of cross contamination and ease of performance. However, in the case of GMO sample, plant or processed food, it is not easy to apply the automated DNA extraction system due to plant cell wall, various food matrices and DNA degradation during food processing steps. Therefore, it is ideal to assess and compare it with the existing methods before being applied to use. In this study, we compared extracting DNA from rice and various rice-containing processed food by three different extraction methods; manual Qiagen Plant mini kit, Promega`s automated Maxwell 16 systems and Biomerieux`s automated Easymag system. And both DNA purity and quality was evaluated by several methods; UV spectrophotometry assay, fluorescence assay, PCR and real-time PCR. Although three different extraction methods did not yield remarkable results, the Qiagen Plant mini kit produced a good-quality DNA from rice grain and the Maxwell 16 gave the high levels of DNA in most of samples. In the case of Easymag, it showed best results from highly processed foods. Therefore, this study suggested automated systems, Maxwell 16 and Easymag, could be replaced with manual Qiagen Plant mini kit in various rice-containing processed foods and allowed to extract DNA from relatively high throughput samples simultaneously.

Larry Koskan, Global Green Products LLC

What's Aspartic Acid Got to Do with It?

The poster will feature new applications for a renewable polymer based on aspartic acid for oil and unconventional gas production. Benefits of use of these products include being a. readily/inherently biodegradable, b. non-toxic, c. non-hazardous, d. completely stable in storage, application, and operating conditions - particularly at higher temperatures, e.easily handled and applied due to low viscosity and water solubility such that metal corrosion and scale inhibition properties are competitively positioned to be advantaged when compared to the traditional chemical candidates.

Jae-hyunf Jo, Department of Bioscience and biotechnology, Hankuk University of Foreign Studies

Enhanced Production of AK by Disruption of Genes in Corynebacterium Glutamicum

In order to develop a-ketoglutarate (a-KG) overproducing strain with Corynebacterium glutamicum that overproduces of L-glutamate, disruption of three genes involved in the a-KG biosynthetic pathway were conducted. The genes aceA (encoding isocitrate lyase, ICL), gdh (encoding glutamate dehydrogenase, L-gluDH), and gltB (encoding glutamate synthase or glutamate-2-oxoglutarate aminotransferase, GOGAT) were blocked by knocking out for the biosynthesis of a-KG. The recombinant strain with aceA, gltB, and gdh disrupted showed reduced ICL activity and no activities of GOGAT and L-gluDH. In the flask culture, the recombinant strain produced up to 16-fold more a-KG than the control strain, mainly due to the disruption of gdh. In addition, blocking glyoxylate pathway resulted in the ICL inactivation and leads the carbon flow to a-KG. But the disruption of gltB did not affect the biosynthesis of a-KG. These results can be applied in the industrial production of a-KG by using C. glutamicum as producer.

Colleen Lerro, Biotechnology Industry Organization

Food vs. Renewable Chemicals: The Biotechnology Industry Organization's New Industry Position

Chunzhao Liu, Institute of Process Engineering, Chinese Academy of Sciences

Immobilization of Laccase on Magnetic Mesoporous Silica Nanoparticles for Enhancing Biocatalysis

Large-pore magnetic mesoporous silica nanoparticles (MMSNPs) with wormhole framework structures were synthesized by using tetraethyl orthosilicate as the silica source and amine-terminated Jeffamine surfactants as template. Iminodiacerate was attached on these MMSNPs through a silane-coupling agent and chelated with Cu2+. The Cu2+-chelated MMSNPs (MMSNPs-CPTS-IDA-Cu2+) showed higher adsorption capacity of 98.1 mg g-1-particles and activity recovery of 92.5% for laccase via metal affinity adsorption in comparison with MMSNPs via physical adsorption. Storage stability and temperature endurance of the adsorbed laccase on MMSNPs-CPTS-IDA-Cu2+ increased significantly, and the adsorbed laccase retained 86.6 % of its initial activity after 10 successive batch reactions operated with magnetic separation. The immobilized laccase on the magnetic mesoporous silica nanoparticles has been developed for efficient phenol degradation. The degradation rate of phenol by the immobilized laccase was 2-fold higher than that of the free laccase, and the immobilized laccase retained 71.3 % of its initial degradation ability after 10 successive batch treatments of coking wastewater. The phenol degradation in the coking wastewater was enhanced in a continuous treatment process by the immobilized laccase in a magnetically stabilized fluidized bed because of good mixing and mass transfer.

Mel Maizirwan, Faculty of Engineering, International Islamic University, Malaysia

Optimization Study of the Ultrasonic Oil Extraction and Insitu Transesterification of Microalgae for Biodiesel Production

The objectives of this research are to identify the dominant factors in the extraction and in situ transesterification processes; and to determine the optimum state of particular combination of various factors using the ultrasonic method. This is an experimental laboratory study that was run using ultrasonic homogenizer Omni Ruptor 4000 for examining the effect of solvent type, solvent concentration, alga-solvent ratio, ultrasonic power, ultrasonic time, ultrasonic pulse and mixing toward yield. Box-Behnken Design of Response Surface Methodology by a quadratic model was used to evaluate the correlation of the parameters for analysing certain factors and combination of factors. As a result obtained in this study, it shows that power, time and pulse are the most significant factors that influence the yield. In the extraction process, the combinations of pulse-time give better result than power-pulse combination. Meanwhile, in the insitu transesterification, the power-time combinations give better result than power-pulse combination. Even though the optimum point has not been reached yet, in general the combination of power-time is categorized as the most influential combination to increase the yield. The experimental values are also shown that the coefficient of correlation (R2) is 0.97977 (for extraction) and 0.98743 (for in situ). The density of Nannochloropsis sp is 0.924 g/ml, saponication number is 114, 269 KOH/1 g oil. The percentage of FFA is 19.67% consisting of monounsaturated and polyunsaturated Octadecenoic acid (C18:1) 43.49%, Dedecanoic acid (C12) 16.30%, Hexadecanic acid (C16:0) 12.51%, Tetradecanoic acid (C14) 11.43%, Octadecadinoic acid (C18:2) 5.85% and Octadecanoic acid (C18:0) 5.62%.

Zivko Nikolov, Texas A&M University

Harvesting Marine Algae via Electrolyte Flocculation

Harvesting dilute microalgal cultures could be costly on a large scale. Several different methods have been tested for harvesting microalgae biomass including electrolyte flocculation. This work focuses on understanding coagulation/flocculation variables that affect harvesting of Nannochloris oculata and Nannochloropsis salina. Initial cell density, ionic strength, coagulant dosage, and media pH were analyzed for their significance on algae removal efficiency. Initial cell density and coagulant dosage had a significant effect on the removal efficiency, but not the ionic strength. Acidification of N. oculata and N. salina cultures before Al-flocculation was helpful for reducing coagulant dosage. N. salina slurries required significantly less acid to reach the optimal flocculation pH than N. oculata. Flocculation of N. salina with five cationic polymers without previous culture acidification resulted in similar recovery efficiencies as $AlCl_3$ but at significantly lower concentrations.The effect of extracellular organic matter (EOM) on flocculation efficiency, dosage, and cost will also be reported and discussed.

Asa Oudes, Genedata, Inc.

Genedata Selector™: Software for Optimization of Biocatalysts in the Biofuel Industry

Worldwide increases in energy consumption, competition for food stocks and growing fossil fuel prices are driving research into sustainable energy resources. Biofuel producers use a variety of organisms, including microbes, fungi, algae, and plants to produce biomass or biocatalysts for synthesis of bio-based chemicals. To better characterize production organisms, new emerging NGS and omics technologies are used in the R&D process. Due to the large amounts of data being generated, storage, management and analysis are a growing challenge to be addressed, and necessary for efficient IP protection of proprietary crops or microbes. Genedata Selector™ is a comprehensive data management system which generates a new level of information by connecting a broad range of data types, including genomes, metabolic pathways, phenotypes and patents. It has been used successfully to select optimal strains or genes for biofuel production. Genedata Selector™ for Biofuels integrates public and user's proprietary genomes from next generation sequencing technologies, and integrates them in a single system to develop novel, high-value strains for renewable chemical synthesis. We present how Genedata Selector™ for Biofuels can be used as a species-independent platform for improving biocatalysts. We cover two workflows in fungi, from genome analyses to the engineered end product. The first study demonstrates the rational design by metabolic engineering of Trichoderma reesei, a cellolytic fungus, to optimize it for ethanol production. The aim is to enable it to convert cellulose to glucose through introduction of the cbh1 (cellobiohydrolase) gene from different donor genomes. The second study illustrates the metabolic engineering to generate feedstock flexible yeast which can convert C5 and C6 sugars to ethanol, through the introduction of bacterial and fungal Xyla (xylose isomerase) genes. In both studies comparative genomics and sequence based searches were used to identify candidate donor genes from different species, which could then be cloned. The newly engineered production strains were loaded into the Genedata Selector™ database, and their metabolic capacity characterized on the transcriptome level by deep RNA sequencing, and on the pathway level by mass spectrometry based metabolite measurements. Additionally, Selector™ can leverage metagenomics data to identify novel or improved means to efficiently produce a broad spectrum of bio-based chemicals, including biofuels. The studies show how the Selector platform integrates data from nucleotide to patent across various species and from different research groups, thereby increasing knowledge sharing and operational efficiency.

Aaron Philippsen, University of Victoria

TBD

Dr. Murali Reddy, University of Guelph

Life Cycle Analysis of Biocomposites versus Traditional Composites

Life cycle analysis helps us in understanding the various aspects of materials production including synthesis of raw material, usage of the finished product and waste management. The biggest challenge in designing the structurally and functionally stable sustainable biocomposites is to devise an environmental benign synthesis and modification process with appropriate disposal methods or waste management methods.In our present work, life cycle assessment of biocomposites produced with perennial switchgrass with recycled plastic matrix is conducted and the results are compared with the similar traditional plastic. These biocomposites were deemed to replacement for virgin plastic materials for durable consumer applications such as bolt-bins. Recycled polypropylene and polyethylene, switchgrass, and additives were used as raw materials in designing these materials. Various compositions were of biocomposites were considered and compared with virgin plastic platforms. The use of all raw materials, energy and resources, in addition to the emissions to the environment of each process -from recovery of secondary materials to product manufacturing- were identified and analyzed. The analyzed environmental impact categories include fossil fuel energy resources depletion, acidification, global warming, and eutrophication. Results were analyzed using SimaPro software and IMPACT 2002+ (combination of IMPACT 2002, Eco-indicator 99, CML and IPCC) method was used for calculating impact categories. This research is financially supported by the OMAFRA – 2009 New Directions & Alternative Renewable Fuels ‘Plus’ Research Program-SR9223 and Hannam Soybean Utilization Fund (HSUF)-2008 and Ministry of Economic Development and Innovation (MEDI), Ontario Research Fund - Research Excellence Round 4 program.

Maya P. Piddocke, University of British Columbia

High Gravity and High Cell Density Enhances the Fermentation of Hexose Sugars Present in Softwood Hydrolysates

Biomass-based ethanol has the potential to provide substantial environmental, energy security and geopolitical benefits in comparison to fossil based transportation fuels. However, fermentation of softwood derived sugars to ethanol is usually problematic due to the generally low sugar concentrations that can be supplied and due to the naturally occurring and process derived inhibitors that are typically present. In the present study, the hexose rich, water soluble fraction obtained after steam treatment of Douglas fir chips was further supplemented with glucose up to 22% (v/v) to simulate high solids, un-detoxified substrate, to see if a high gravity/high cell consistency approach could better cope with known inhibitory materials. Several yeast strains were assessed and Tembec T1, T2 and Lallemand LYCC 6469 proved to be the most promising in terms of ethanol productivity and yield. It was apparent that a high cell density approach was required for efficient ethanol production by all of the evaluated yeast strains. When strains LYCC 6469, T1, and T2 were grown in the Douglas-fir water soluble fraction a faster reduction in the furan content was observed. The addition of supplemental glucose enabled the faster and quantitatively higher removal of hydroxymethylfurfural (HMF) and this observed boosting effect was more pronounced with the three superior strains. It appears that high cell density can provide effective fermentation at high sugar concentrations while enhancing inhibitor reduction. A 77% ethanol yield could be achieved with strain LYCC 6469 after 48 h at high cell density with some nutritional supplementation

Amadeus Pribowo, University of British Columbia

Enzyme Recycling (By better Understanding Specific Enzyme Adsorption Profiles to Biomass) as a Means of Reducing the Cost of the Hydrolysis Step of a Biomass-to-Ethanol Process

The ongoing challenge in making the production of sugars from lignocellulosic biomass and the emerging biorefinery platform more of an economic reality is to further decrease hydrolysis costs while maintaining, or even improving, enzyme performance across a broad range of substrates. To improve hydrolysis efficiency and overcome feedstock heterogeneity, we have tried to optimize biomass pretreatment while trying to better define what constitutes an effective enzyme mixture and the possibility of reutilizing the key enzymes in the “cellulase” mixture.

When comparing the enzymatic hydrolysis of corn (starch) versus cellulosic biomass, the production of sugars from cellulose requires 5-10x more enzymes than required for starch hydrolysis. This large amount of enzymes translates into enzymatic hydrolysis costs that are 5-15 times higher than the cost to hydrolyze starch. Due to the large amount of protein (enzyme) required, it is anticipated that, despite the probable gains in hydrolysis efficiency resulting from better enzyme mixtures and improved activities, a further decrease in hydrolysis costs for cellulosic biomass will still likely require the incorporation of an enzyme recycling strategy. Over the past two decades, many enzyme recycle strategies have been evaluated to recover enzymes from the hydrolysates, the solid residues, or both. It is evident that the efficiency of any of these recycle strategies depends primarily on the ability to recover key enzymes for the next round of hydrolysis. Thus, it is important to better understand the nature of interaction of individual enzymes in these increasingly complex cellulase enzyme mixtures during hydrolysis of various (ligno)cellulosic substrates.

The presentation will describe the use of a combination of techniques such as zymograms, mass-spectrometry and protein electrophoresis to monitor the behavior of specific enzymes as well as the development of a novel antibody-based assay to quantify the adsorption of individual enzymes during hydrolysis. By monitoring the adsorption profiles of the individual enzymes and the assessing the influence of the various substrate characteristics on the overall hydrolysis performance, this work has provided some insights on how to recover key enzymes and improve the efficiency of the overall enzyme recycle strategy.

Noberto Quinones

Habitat and Biodiversity Mapping, for the Determination of Algal Biomass Aquaculture Sites in the Costal Areas of Puerto Rico

The increasing demand for fossil fuels and the energy crisis caused by the massive exploitation of these nonrenewable resources have pushed for the development of new technologies that will aid in the transition towards a sustainable and clean system of energy production. Here we analyze and map coastal habitats and biodiversity for the optimum production of algal biomass for the production of biofuels in Puerto Rico. The study evaluates the territorial water of Puerto Rico using several factors: benthic habitats, water depth, critical habitat distribution, vessel concentration and route, taking into consideration current laws and regulations for Puerto Rico. Two models were developed to analyze possible aquaculture sites. The first model evaluates conflict areas and the second model incorporates optimal areas for aquaculture sites. The union of both models represents 1,463.45 km2 on the island that can be developed for algae-based bioenergy systems in the territorial waters.

Darryn Rackemann, Queensland University of Technology

Production of Organic Acids and Furanics from Sugarcane Bagasse

Sugarcane waste (i.e. bagasse) represents an abundant and relatively low cost carbon resource that can be utilized to produce chemical intermediates such as levulinic acid and furanics. These chemicals can be easily upgraded to commodity and specialty chemicals and biofuels by high yielding and well established technologies. Integration of value-adding opportunities for bagasse while utilising the existing infrastructure and utilities of sugar factories would turn the factory into a biorefinery allowing greater utilisation of the sugarcane resource. To overcome some of the challenges associated with mineral acid catalysts and hydrolysis processes using biomass, research was undertaken using homogeneous green acids and lignin solvents such as glycols. The poster examines the yields and processing conditions for producing levulinic acid and furanics from bagasse using selective solvents and green catalysts with comparison to conventional technologies highlighting the benefits of solvent and catalyst selection.

Sean Sleight, Department of Bioengineering, University of Washington

Randomized BioBrick Assembly: A novel DNA assembly method for randomizing and optimizing multi-gene circuits and metabolic pathways

Synthetic biology requires DNA synthesis or the assembly of genetic parts into functional genetic circuits and metabolic pathways. The optimization of circuits and pathways often requires constructing various iterations of the same construct, or directed evolution to achieve the desired function. Alternatively, a method that randomizes individual parts in the same assembly reaction could be used for optimization by allowing for the ability to screen large numbers of individual clones expressing randomized circuits or pathways for optimal function. Here we describe a new assembly method to randomize genetic circuits and metabolic pathways from modular DNA fragments derived from PCR-amplified BioBricks. Each fragment of a particular part type (e.g. promoters, coding sequences, and transcriptional terminators) has the same standardized overlap on either side of the functional DNA, allowing for independent assembly with other fragments having the complementary overlap. When multiple fragments of a particular type are used in the same assembly reaction, there is competition between fragments, allowing for randomized assembly. As a proof-of-principle for this method, we first assembled eCFP, maxRFP, and eYFP gene expression cassettes with independently randomized promoters, ribosome binding sites, transcriptional terminators, and all parts randomized at once. These randomized expression cassettes were then combined to make fully functional and randomized three-gene circuits that express CFP, RFP, and YFP, producing colors close to Cyan, Magenta, and Yellow (CMY) under UV light. Sequencing results from 12 CMY circuits with nine randomized terminators show that 9/12 circuits are distinct and at least one circuit contains each of the nine terminators. When all parts are shuffled at once, 11/12 circuits are distinct and expression ranges from about 2 to 160-fold above background levels depending on the fluorescent protein. We then adapted this method to randomize the same promoters, ribosome binding sites, and terminators with the enzyme coding sequences (crtE, crtB, and crtI) from the lycopene biosynthesis pathway instead of fluorescent proteins, designed to allow each enzyme in the pathway to be independentally controlled from a different promoter. Sequencing results show that 7/8 pathways with all parts shuffled at once were distinct and lycopene production can be controlled with different inducer concentrations. Although the sample size is low, the randomized lycopene pathways have a strikingly different frequency of promoters and RBSs relative to the CMY circuit. These results demonstrate the ability to generate multiple unique three-gene circuits and pathways in the same assembly reaction, allowing for the construction of large libraries that can be subjected to high-throughput selections and screens. We expect that this randomization method will be useful for

increasing DNA assembly efficiency, optimizing metabolic flux to maximize products of interest (e.g. biofuels), and is likely adaptable to other circuits and pathways.

Vekalet Tek, Applied Research Center, Florida International University

Screening of Native Floridian Algae For Biofuel Production

Increasing energy demand and concerns about climate change require advances in manufacturing transportation fuels from sustainable resources. Microalgae are a promising source of biofuels, such as biodiesel, due to their potentially high fuel yield per unit area of cultivation. Biodiesel and other fuels from microalgae have the potential to displace fossil transportation fuels with minimal impact on the environment, since algae can be cultivated on marginal land using brackish (or salty) water and absorbing CO2 from the atmosphere or from emission sources.

The goal of this project was to identify promising algae from a collection of 31 strains of native Floridian freshwater green algae in terms of growth rate and lipid accumulation. The growth conditions of the selected strains will be optimized to maximize lipid (and hence biodiesel/biofuel) production. The lipid content of the strains was quantified via the Nile Red method by FIU student Priyanka Narender and a few strains were identified as the most promising ones. Those strains and a control strain were growth and cultivated in 3-liter flasks under same conditions to compare their growth rates and the lipid contents.

Vekalet Tek, Applied Research Center, Florida International University

Photobiological Hydrogen Research

The goal of this R&D project is to identify the structural and active site maturation genes of an O2-tolerant NiFe-hydrogenase, which are critical to optimal expression of the enzyme in E. coli. The hydrogenase was derived from the non-sulfur purple photosynthetic bacterium Rubrivivax gelatinosus CBS developed by NREL. This work will contribute to the development of a more efficient and robust system for photosynthetic hydrogen production in E. coli, a robust industrial microorganism. Expression in E. coli will also facilitate eventual expression of the hydrogenase in cyanobacteria. CBS hydrogenase is a heterodimeric protein without a C-terminal extension and is assumed to share maturation process characteristics with E. coli hydrogenase 3.

So far, we have cloned six hydrogenase assembly genes (hypA through hypF), previously identified by NREL scientists, on Duet expression vectors or TOPO cloning vectors. CooH encodes the large subunit of hydrogenase that carries the active metal center, whereas CooL encodes its small subunit. We have also cloned the structural genes CooK, CooU, CooX, and the subunit genes CooH (with and without strep-II tag) and CooL on Duet expression vectors under T7 promoters. Cloning of CooM, the largest gene encoding the membrane-anchoring protein of hydrogenase, is underway. CooK and CooH (with no tag) genes were cloned on TOPO vector for DNA sequencing of the PCR products and then transferred onto Duet vectors directly. Co-transformation of CooH with the structural and maturation genes of the CBS O2-tolerant NiFe-hydrogenase into zero background E. coli cells prepared by NREL is also pending.

Sonia Wallman, NBC2 - Montgomery County Community College

Educating Technicians for Biofuels Production and Analysis/Career Pathways for Biofuels Production and Analysis

The NBC2 is a National Science Foundation Advanced Technological Education center focused on developing local infrastructures to support biomanufacturing technician and professional education and training and the biomanufacturing workforce, locally and nationally.

The NBC2 has produced a textbook, Biofuels Production and Analysis, and a Biofuels Production and Analysis Laboratory Manual containing hands-on curricular materials to support education and training in seven specific aspects of biofuels production and analysis. The modules were developed by partners of the NBC2 including Dr. Elmar Schmid, MiraCosta College, Oceanside, CA; James Hewlett, Finger Lakes Community College, Canandaigua, NY; and Michelle Gilbert and Damon Tighe, Bio-Rad, Hercules, CA.

The poster will overview the content of the hands-on biofuels production and analysis modules for use in college courses in biofuels, alternative energy and engineering and in high school biology and chemistry classes to motivate students to consider career pathways in biofuels production and analysis.

Swati Yewalkar, University of British Columbia

FDA and PI staining for monitoring algal growth –Applicability and Limitations

A method for differentiation of live and dead algal cells in photobioreactors by fluorescein diacetate (FDA) and propidium iodide (PI) fluorescence staining has been developed using simple fluorometry. FDA stains fluorescent green to the living cells while PI stains to the dead cells, allowing the discrimination of live and dead cells. The method was evaluated using two green algae and two strains of cyanobacteria. The algae growth in shake flask and the continuously stirred photobioreactors was monitored with FDA-PI staining. The method was found applicable for Chlorella pyrenoidosa and Synechococcus 7002 but was not applicable for the cultures of Scenedesmus dimorphus and Synechococcus elongatus 7942. We conclude that FDA is a good stain for estimating live algal cells in photobioreactors but its applicability for individual species of algae must be evaluated before the FDA staining is applied for monitoring the viability.