Cauliflower mushroom (Sparassis crispa) is well known as a brown-rot fungi selectively degrades heartwood of wood, and that has potential anticancer and immune enhancing activity; thus its production is increasing in Korea. The mushroom is cultivated artificially using plastic pots filled with medium material, such as lignocellulosic biomass and some additives. However, most of the medium remaining after mushroom cultivation has not been utilized, except for compost. Therefore, the utilization of this waste mushroom medium could be proposed as a valuable resource, such as bioethanol and other biochemical products. In this study, we used temperature gradient for oxalic acid pretreatment to produce high fermentable sugar. We then assessed the utility of the hydrolysates for ethanol production. Solid biomass obtained after oxalic acid pretreatment was evaluated for its properties as solid fuel, in terms of biorefinery. The hydrolysates of waste mushroom medium contained high glucose and low concentrations of inhibitors. The glucose concentration in the hydrolysate particularly increased when temperature gradient was used during pretreatment, compared with that of isocratic condition. The highest increase rate of glucose was 63.16% when pretreatment was performed at 140°C for 25 min with 0.032 oxalic acid (g/g), and increased temperature to 170°C. At the same time, ethanol production from the hydrolysate was 15.72 g/l after 48h, which corresponding to an ethanol volumetric productivity of 0.33 g/l/h. Most of the lignin and some of the cellulose remained in the pretreated biomass. The calorific value in the pretreated biomass increased compared to the raw material, due to higher contents of lignin in the pretreated biomass. This study shows that waste mushroom medium has enough potential as a material for developing biorefinery. The waste mushroom medium easily converted to sugars by oxalic acid pretreatment at mild condition. In particular, the temperature gradient was a more efficient method than isocratic condition during pretreatment for production of fermentable sugar. At the same time, the hydrolysate produced high ethanol by P. stipitis without any treatment to remove inhibitors before fermentation. Additionally, pretreated waste mushroom medium could provide a suitable condition for a solid fuel, since it has been efficiently pretreated with high lignin and low ash content.

Authors: Young-Jun Seo Jae-Won Lee
A Single Step Pretreatment Process in Bioethanol Production from Sweet Sorghum Bagasse

Idan Chiyanzu,

The growing energy demands, climate changes, environmental concerns and diminishing state of fossil-based fuels has kindled the search for alternative safer fuels. Biofuels such as bioethanol is a promising alternative and is produced by fermenting sugars obtained from sugarcane juice, starch or lignocellulosic biomass. Sweet sorghum bagasse, a lignocellulose-rich agricultural residue, has significant potential as feedstock for bioethanol production due its high sugar composition. Typically, lignocellulosic biomass has to be pretreated, hydrolysed (enzymatically) and fermented to obtain bioethanol. However, the utilization of enzymes during hydrolysis potentially adds extra cost to the bioethanol production processes. The present study aims to investigate combining the two events required to get lignocellulose to fermentable sugars (pretreatment and hydrolysis) in one reaction using a domestic microwave in presence of dilute acid or base catalysts. An efficient process of sweet sorghum bagasse acid/base hydrolysis in a microwave is therefore demonstrated. The sweet sorghum bagasse was mixed with 3-7 wt% H2SO4 or 3-7 wt% Ca(OH)2 at a solid loading of 5 wt% and then pretreated at various microwave powers (180 – 300W) for 20 minutes with aliquots take every 5 minutes. The maximum yield (0.82 g/g dry biomass) of total fermentable sugars was released at 180W, 5 wt% H2SO4 concentration in 20 minutes. The highest bioethanol yield (0.5 g/g of glucose) was obtained after hydrolysate fermentation with a mixed culture of 5% (v/v) Z. mobilis and 10% (v/v) S. cerevisiae. The extent of structural disruption was evaluated using SEM and FTIR analysis. These results show microwave-assisted acid pretreatment is a powerful and effective process to obtain fermentable sugars from sweet sorghum bagasse. Keywords: Pretreatment; sweet sorghum bagasse; hydrolysis; fermentation; Bioethanol

Authors: Busiswa Ndaba, Idan Chiyanzu and Sanette Marx

Engineering Sesquiterpene-Based Biofuels in Plants

Ramesh Nair, Chromatin Inc.

Chromatin is engineering sweet sorghum to accumulate the fuel precursor farnesene, a molecule that can be readily converted to biodiesel. Sweet sorghum is naturally drought tolerant and has readily available carbohydrates to redirect to the farnesene pathway. Chromatin's proprietary technology enables the introduction of a novel biosynthetic process into the plant to produce farnesene, targeting sorghum to accumulate up to 20% of its weight as fuel. The farnesene will accumulate in the sorghum plants—similar to the way in which it currently stores sugar—and can be
extracted and converted into diesel fuel using low-cost, conventional methods. We are applying a combination of enzyme engineering and metabolic pathway engineering to increase sesquiterpene production in sorghum. Guayule, a plant that accumulates high levels of terpenoid derivatives and sugarcane that is readily transformable will be initially engineered to understand the metabolic bottlenecks of producing sesquiterpene biofuels.

Authors: Otto Folkerts, John Steffens, Katrina Cornish, Joshua Blakeslee, Craig Forsyth, Rich Burlingame and David Jessen

**Optimization the Process Variables for Fractionation of Empty Fruit Bunch By a Continuous Twin Screw-Driven reactor (CTSR) for Xylose Rich Hydrolysate**

Kyeong Keun Oh, Dankook University

CTSR process might be a viable continuous pretreatment when compared to other methods due to its unique advantages such as high shear, rapid mixing, varying residence time, moderate barrel temperature, adaptability to process modification, and above all it is a continuous operation. Considering its advantages, many researchers have explored extrusion process as one of the viable continuous biomass pretreatment methods. The reasoning attributed for the high sugar recovery in CTSR pretreatment are increase in surface area, pore size, and the decrease in cellulose crystallinity, all of these facilitate the access of enzymes to cellulose. EFB was used as a model biomass for the CTSR process with a dilute acid. This study focused on establishment of the continuous pretreatment feasibility to increase cellulose fraction in fractionated EFB and xylose concentration in hydrolyzate, and investigation of the effect of operating variables such as barrel temperature, and catalyst concentration and solid/liquid ratio, which is controlled by solid loadings and liquid flow rate. The CTSR pretreatment conditions were temperatures of 160 - 180?, liquid feeding rates of 4.5 – 13 mL/min, biomass feeding rates of 0.5 – 2.0 g/min, acid concentrations of 0.5 – 3.0% (w/v), and screw speed of 30 – 60 rpm for the optimization for sugar recovery. The enzymatic digestibility of the Fractionated EFB through CTSR processing was significantly increased over that of the untreated EFB.

Authors: Jin Young Jong, Hyun Jin Ryu

**Bioconversion of Waste Streams of the Pulp and Paper Industry into Value Added Products - Prospectives of Lignocellulosic Waste Biorefinery**

Lai Thanh Tung, Université du Québec à Trois-Rivières, Trois-Rivières, QC, Canada
In the perspective of biorefining, cellulosic wastes are available and abundant biomasses to obtain a variety of valuable products. Cellulosic wastes components can be fractionated and then transformed into valuable products, but some of these residues like pulp and paper sludge can be directly converted to such products using cellulolytic microorganisms (or enzymes) and industrial microbiology techniques. As major producer of cellulosic wastes, pulp and paper plants can seize this biorefining opportunity and relaunch or revitalize their industrial activities. Despite of having complex composition, pulp and paper sludges contain high organic material mainly in terms of carbohydrates that could be available for microbial utilization and conversion into products. Fermentation of sludge into value added products could be an economic and environmental strategy for pulp and paper plants due to: i) reduction in cost of wastewater treatment and sludge disposal; ii) avoidance of environmental impacts of landfilling disposal of sludge; iii) possibility of using on-site equipments, with minor adjustments, to produce industrial microorganisms and downstream processing their products; iv) benefit from using on-site or locally biofuels, biogas and bioproducts. Researches are conducted by our team on this subject. Primary sludges and secondary (biological) sludges were successfully used as alternative cheap media to produce various saccharolytic enzymes of Trichoderma reesei and some Bacillus sp. Moreover, secondary sludge was used as a fermentation substrate for production of polyhydroxyalkanoates based bioplastics (PHA) using Capriavidus necator. Activated sludges from wastewater biological treatment system and process waters in pulp and paper plant could also be sources of value added products. Activated sludges are known to accumulate PHA under certain conditions. Extraction of these PHA is still costly and use non environmental friendly solvents. Experiments have been conducted to extract the PHA in activated sludge with switchable solvents that can be easily recovered. Excess water from paper making process, i.e white waters, was used an alternative cheap media for production of bacterial cellulose by Gluconacetobacter xylinus as they contain residual carbohydrates. Thus, wastewater and sludges of pulp and paper can be sources of value added products or fermentation substrate for industrial microorganisms. Diversifying products from these waste streams can be a part of successful biorefinery. It offers great opportunities to relaunch some pulp and paper plants in particular regions. The salient results of our researches on this subject will be presented and discussed.

Authors: Thi-Thanh-Ha Pham, Thanh-Tung Lai, Kokou Adjalle, Daniel Montplaisir, Simon Barnabé

*Changes of Enzymes Activity During Erythritol Biosynthesis by Yarrowia Lipolytica*

Anita Rywiańska, Wroclaw University of Environment and Life Sciences; Wroclaw, Poland
Erythritol is a polyol, used as natural low calorie sweetener (0 – 0.2 kcal/g) produced in biotechnological processes, as the chemical production is not effective. The ability of its production was observed in osmophilic yeast, some fungi and bacteria. The traditional substrates used for the biosynthesis are glucose, fructose, sucrose and starch hydrolizates. The recent investigation showed the potential of Yarrowia lipolytica yeast to produce erythritol from glycerol, a by-product from biodiesel production. The key factors demonstrated to have the positive impact on the erythritol biosynthesis from glycerol by Y. lipolytica were low pH value and high osmotic pressure of the environment. The aim of the study was to compare the effect of pH and osmotic pressure on the activity of erythrose reductase, transketolase and citrate synthase. The impact of osmotic pressure, caused by the addition of NaCl (32.5 g/L), and pH value on erythritol biosynthesis by Y. lipolytica Wratislavia K1 was examined in bioreactor cultures using pure glycerol media (150 g/L). The pH values of 3.0 or 4.5 were maintained automatically by the addition of 20% (w/v) NaOH solution. In the samples osmotic pressure was measured, biomass was determined gravimetrically, whereas concentrations of glycerol, erythritol, mannitol, arabitol, ketoglutaric and citric acid were measured by HPLC method. Enzymes activity was analysed after 24h of cultivation in the sample disrupted by sonification. The erythrose reductase, transketolase and citrate synthase activities were assayed according to the method described by Lee et al. [2003], Sugimoto and Shiio [1989] and Kamzolova et al. [2008], respectively. The protein content was assayed by Lowry’s method. The addition of NaCl, followed by the increase of osmotic pressure from 2 Osm/kg (without salt) to 3 Osm/kg, as well as low pH values have positive effect on erythritol biosynthesis. In the culture broths citric acid was also presented but the relationship between its production, NaCl addition and pH was inverted, in comparison to erythritol biosynthesis. The highest erythritol production was observed in the culture conducted at pH 3.0 supplemented with NaCl, where the product concentration reached 64 g/L, corresponding to 0.42 g/g yield and productivity of 0.86 g/Lh. In the culture conducted at pH 4.5 without salt addition, yeast produced 45.9 g/L of citric acid and 32.3 g/L of erythritol. At the same pH, NaCl supplementation resulted in similar concentration of erythritol and citric acid. The NaCl addition and low pH have also positive effect on erythrose reductase activity, as the highest activity (0.230 U/mg of protein) was observed in the medium with salt and pH 3.0, and the lowest (0.115 U/mg of protein) at pH 4.5 without NaCl. The transketolase activity was the highest (3.407 • 10-3 U/mg of protein) in the culture without salt addition and pH 4.5. The activity of citrate synthase was inhibited by increased concentration of citric acid. In the culture without salt addition conducted at pH 4.5 the activity of this enzyme was the lowest and reached 0.513 U/mg of protein. Acknowledgments. This work was co-sponsored by grant No. N N312 256640 from the National Science Centre (Poland) and by the Ministry of Science and Higher Education of Poland and European Union under Project No. POIG 01.01.02-00-074/09.
Direct Thermal Conversion of Microalgae Biomass into Renewable Chemicals and Fuels

Justice Asomaning, University of Alberta

The objective of this study was to evaluate the conversion of microalgal biomass to renewable chemicals and fuels using a patented two-stage lipids-to-hydrocarbons (LTH) technology. Chlorella protothecoides (UTEX 256) was selected as model microalgae for their ability to accumulate high amounts of neutral lipids. A 10 L fed-batch bioreactor was used to heterotrophically cultivate the microalgae for 288 h with glucose and yeast extract as the main carbon and nitrogen sources respectively. Lipid accumulation in the microalgae was achieved through maintaining low nitrogen to carbon mass ratio in the culture. Algal biomass was concentrated by centrifugation and samples directly hydrolyzed in 15 mL batch stainless steel microreactors at 280 °C for 1 h with an initial pressure of 500 psi. Hydrolysis product was filtered and hydrolyzed lipids in the form of fatty acids were extracted with hexane. The extracted fatty acids were then pyrolyzed at 410 °C for 2h under N2 at atmospheric pressure in the batch microreactors. The pyrolysis product was analyzed on GC-FID and GC-MS for quantification and identification of compounds respectively. A high density culture with 40% neutral lipid accumulation was obtained. Kinetics of growth and lipid accumulation indicated that the optimum levels were achieved at 168 h. The liquid phase pyrolysis product consisted of n-alkanes, a-olefins and internal olefins from C5 to C17 that can be fractionated in fuels and platform chemicals as the main compounds. Other compounds included and fatty acids from C4 to C18 as well as C19 and higher hydrocarbons, fatty acids and carbonyl compounds. Gaseous pyrolysis product consisted of deoxygenation products (carbon dioxide and carbon monoxide) as well as light end hydrocarbons. This study demonstrates the feasibility of converting microalgae into valuable platform chemicals and fuel by using the two-stage hydrolysis-pyrolysis technology.

Authors: Isabel Espinosa-Gonzalez, Paolo Mussone, and David C. Bressler
FOLIUM: Tobacco as a Platform for Foliar Biosynthesis of Advanced Hydrocarbon Fuels

Orlando Chambers, Kentucky Tobacco R&D Center, University of Kentucky

FOLIUM is a DOE ARPA-E funded project that started in 2012, comprising the Lawrence Berkeley National Lab, UC Berkeley, and the Kentucky Tobacco Research and Development Center. The project entails a three-pronged effort for the production of advanced hydrocarbon fuels in green tobacco biomass: 1. Metabolic pathways for alkane and isoprenoid biosynthesis with genes from cyanobacteria, microalgae and plants are being heterologously installed in tobacco for expression in the chloroplast (alkanes and isoprenoids) or cytosol (isoprenoids). 2. Improvements in primary photosynthetic production of tobacco are pursued upon acceleration of the recovery of slow non-photochemical quenching (NPQ) components, following a transition from excess light to limiting light conditions. Efforts are also under way to minimize, or truncate the light-harvesting antenna (TLA) size of the photosystems in tobacco, thereby enhancing light penetration and utilization in high-density canopies. Independent efforts aim to insert bicarbonate transporters from cyanobacteria in the chloroplast envelope, seeking to improve CI-delivery to the tobacco chloroplasts. 3. Maximizing biomass yield from tobacco cultivation under greater canopy density conditions, greater fertilizer loads, and more frequent harvests. The Nicotiana genus contains many species with a wide range of characteristics relevant for high biomass production, strong regrowth in multiple-harvest production, distinctive morphology for identity preservation and potential strategies for genetic containment. Dramatic changes in production systems were designed to reduce the cost of production for what was previously a relatively expensive crop. This poster summarizes #1 and #2 above but mainly focuses on results from #3. Tobacco is a non-food, non-feed crop that is commonly featured as a host for plant gene expression via various technologies. However, traditional tobacco agriculture is neither economically, nor practically, ideal for a new agricultural value chain being developed from applications such as the FOLIUM project. To facilitate the continuing emergence of a tobacco-based production system, we are addressing agronomic and regulatory limitations through the development of new plant varieties and associated production practices. Results suggest tobacco biomass production over 150 T per hectare can be achieved at a commercial scale using high fertilizer rates, high-density plant populations and multiple harvesting. Additional increases can be reached by combining the optimized fertilization, spacing and harvesting regimes. Baseline economic data can be attained to estimate the cost of production for FOLIUM tobacco and provide relevant data for engaging growers and end users.

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**Fungal SMC Bioconversion: A Potential Greener Technology**

Finola Cliffe, Molecular Glycobiotechnology Group, Biochemistry, National University of Ireland, Galway, Ireland.

The Irish mushroom industry produces over 400,000 tons of spent mushroom compost (SMC) annually. Currently, this by-product is employed as a soil conditioner; however, the application for SMC is considered very limited due to associated environmental and health impacts. The relatively high carbohydrate content of SMC makes it a suitable alternative feedstock in the biofuel and biorefinery sectors. Biochemical analysis of SMC indicates its potential use as an inexpensive nutrient source for microbial enzyme production. Ligno-cellulolytic enzymes are produced by various fungi and bacteria; however, many of the xylanase- and cellulase-rich commercial enzyme preparations are derived from fungal sources. In this study, we compared the proximate, carbohydrate and metal ion compositions of SMC from different sources. We also compared the bioconversion of SMC using commercial enzyme preparations which showed conversion efficiencies of up to 70%. The study showed that the fungal enzymes can be used to generate SMC hydrolysates rich in fermentable sugars for downstream bioenergy production. Such bio-based technologies are specifically designed to target lignocellulosic feedstocks to produce not only sugar-rich mixtures for ethanol production but also value added products including proteins, amino acids, lipids, lignin and phenolic compounds. Further research will focus on the improvement of the fungal strains responsible for production of these enzymes and increased understanding of the biological mechanisms behind their synthesis.

Authors: Finola E. Cliffe, Manimaran Ayyachamy, Jessica M. Coyne and Maria G. Tuohy

**Improving Thermostability of a Bacterial Carboxylesterase by Rational Design and Application for Pitch Control in Pulp and Paper Manufacturing.**

David Charbonneau, Université du Québec à Trois-Rivières

The novel thermostable carboxylesterase EstGtA2 from G. thermodenitrificans (AEN92268) hydrolyzes a broad variety of ester molecules including p-nitrophenyl esters and triglycerides of different acyl chain length. In order to improve EstGtA2 stability and extend its viability as industrial biocatalyst, the structure of EstGtA2 was model based on the X-ray crystal structure 3RM3 (89% identity). Eight particular salt bridges, which are conserved in several closely related bacterial homologs, were identified. The contribution of these salt links to EstGtA2 stability was probed by combinatorial alanine-scanning mutagenesis and isosteric substitutions. Major impacts on stability occurred when salt bridges were broken, with changes in Tm ranging from 10-22°C for single and multiple salt bridges respectively. More importantly, results allow identifying a combination of salt bridges that are essential for protein folding and activity. An inter-loop salt bridge located in i-2 and i-4 from the catalytic Asp and His residues that are conserved among distant groups of carboxylesterases was optimized in EstGtA2 using rational side-chain pKa shift (H?R) substitution. Combined with the introduction of a new inter helix-strand disulfide bridge, a final mutant named EstGtA2 (6+) exhibits a considerable increased in
stability. This study highlights the importance of multiple salt bridges in the folding process and stability of EstGtA2 and allows for tuning stability of this enzyme for various industrial applications. The potential application of the variants in controlling extractives in wood pulp (pitch) will be analysed.

Authors: David M. Charbonneau, Fatma Meddeb-Mouelhi and Marc Beauregard

**Novel Fungal Xylanolytic Accessory Enzymes Improve Digestibility of Pretreated Lignocellulosic Biomass**

Racha Cheikh-Ibrahim, Department of Chemistry & Biochemistry, Concordia University, Montreal, QC, Canada

In addition to challenges imposed by various biomass types and pretreatment methods, inefficiency in enzymatic saccharification is widely regarded as the most critical impediment to commercializing second-generation biofuels and chemicals. This challenge is due mainly to high enzyme cost. Although research has demonstrated remarkable synergies between cellulases, hemicellulases, ligninases and non-hydrolytic cell wall enhancing proteins, commercial cellulases are yet to be optimal for direct industrial application. One way to circumvent this challenge is to exploit synergistic activities between cellulases and fungal xylanolytic accessory enzymes, namely xylanases, acetylxylan esterases, arabinofuranosidases and xylosidases. Our approach involves mining the steadily-increasing number of sequenced fungal genomes for new xylanolytic enzymes. Promising genes were cloned and expressed in native or recombinant host systems, particularly Aspergillus niger. Crude and purified protein targets were tested for enhancement of commercial cellulases at low loadings (2%) of various chemically-treated biomass types in microtitre plate format. The digestibility of various pretreated samples was estimated via BCA reducing sugar and glucose assays among other techniques. Three xylanolytic enzymes derived from Aspergillus niger, myceliophthora thermophila and Thielavia terrestris were identified as promising candidates for the enhancement of chemically-treated biomass hydrolysis by cellulases.

Authors: Reginald Storms, Justin Powlowski

**Preparation of a Culture Medium Based on Industrial Wastewaters to Produce a Rich Lipid Chlorella Sp. Consortium for Algae-Based Fuel and Energy Production**

Simon Barnabé, Pulp and Paper Specialized Center

Many industries are looking for sustainable alternatives to fossil consumption. Biomass is certainly an attractive alternative. However, among all the challenges and issues for biomass use to produce fuel and energy, securing the supply of biomass, fuel and energy remain a critical factor for success and profitability. An industry producing its own biomass can overcome this problem. In fact, many industries have CO2 stream, waste nutrients and waste energy that can be used to produce lipid-rich algae biomass for obtaining biofuel, bioenergy and coproducts. These products are marketable, but they may be also valuable for in-house uses to reduce fossil consumption in industrial plants. Such a facility co-locating approach for algae fuel,
energy and coproducts production could be profitable for the co-locating industry. A facility co-locating project is conducted in Quebec, Canada, to use industrial wastewaters from an aluminum factory for producing lipid-rich algae biomass using native lipid-rich algae consortia. In this project, a native Chlorella sp. consortium is grown under heterotrophic conditions and thus need supplement of cheap carbon sources to increase biomass productivity. Once the strain has been stabilized, carbon, nitrogen and phosphorus concentrations, which are limiting factors for the algae productivity, were studied. Preliminary experiments were conducted at C, N, P concentrations to identify the best C:N:P ratio for the system. Although the lipid cell content was the highest under N starvation condition, the biomass and lipid productivity increased with the C and N concentration. Experimental results and issues will be presented and discussed.

Authors: Nathalie Bourdeau1, Mélissa Lemire1, Frédérique Lépine1, Marc-André Déry 4, Kokou Adjallé1, Nathalie Dubois-Caléro2, Rachel Olette3, Simon Barnabé1* 1 Université du Québec à Trois-Rivières, Trois-Rivières, QC, Canada. 2 Alga-Labs, Montreal, QC, Canada 3 Centre de recherche et développement Arvida, Jonquière, QC, Canada 4 Pulp and paper specialized center, Trois-Rivières, QC, Canada.

Rate and Peak Concentrations of Emissions in Stored Wood Pellets - Sensitivities to Temperature, Relative Humidity, and Headspace Volume

Xingya Kuang, Yangpu District Central Hospital

Wood pellets emit CO, CO2, CH4 and other volatiles during storage. Increased concentration of these gases in a sealed storage causes depletion of concentration of oxygen. The storage environment becomes toxic to those who operate in and around these storages. The objective of this study was to investigate the effects of temperature, moisture and storage headspace on emissions from wood pellets in an enclosed space. Twelve 10-liter plastic containers (200 mm diameter and 320 mm long) were used to study the effects of headspace ratio (25%, 50%, and 75% of container volume) and temperatures (10-50°C). Another eight containers were set in uncontrolled storage relative humidity and temperature. Concentrations of CO2, CO and CH4 were measured by a gas chromatography (GC). The results showed off-gas emissions of CO2, CO and CH4 from stored wood pellets are most sensitive to storage temperature. Increased headspace volume ratio increased peak emissions. Increased relative humidity in the enclosed container also increased the rate of emissions and oxygen depletion. Key words: biomass, wood pellets, off-gassing emission, emission factors, storage, headspace ratio, temperature effect, moisture effect,

Authors: Xingya Kuang, Tumuluru Jaya Shankar, Xiaotao T. Bi, C. Jim Lim Shahab Sokhansanj
Reduction of By-Products in Erythritol Biosynthesis from Glycerol by Yarrowia Lipolytica

Ludwika Tomaszewska, Wroclaw University of Environmental and Life Sciences; Wroclaw; Poland

In recent years, because of the increased interest in environmental protection, especially much attention was devoted to utilization of the industrial and agro-industrial by-products by its application in biosynthesis of valuable compounds. A great example of such efforts is application of glycerol – one of the major by-products of biodiesel production – in erythritol biosynthesis by Yarrowia lipolytica yeast. Erythritol is a compound that naturally occurs in human diet (e.g. present in fruits, honey, mushrooms, seaweed). This sugar alcohol has 60-80% of sweetness in comparison to sucrose and very low caloric value (0 – 0.2 kcal/g). Moreover, it is safe for consumption, non-cariogenic, its intake did not affect blood insulin level, and it does not cause any gastric side-effects. The aim of the study was to improve the parameters and purity of erythritol production from glycerol by Y. lipolytica Wratislavia K1 strain. The erythritol biosynthesis was conducted in the bioreactor cultures, on glycerol media (pH 3.0, 2.5% NaCl) supplemented with 5 or 10 g/L of yeast extract (YE). The production process was carried out applying batch process (150 g/L of pure glycerol) and fed-batch mode (250 g/L of crude glycerol). The fed-batch processes were launched as batch cultures in which the initial glycerol concentration was about 125 g L-1. Next, pulsed additions of glycerol (about 75 g/L) were made after 24 and 48 h. After glycerol exhaustion addition of nitrogen and phosphorus source ((NH4)2SO4 + KH2PO4) was made and the process was maintained for further 48 h. In the samples from the cultures, biomass was determined gravimetrically after drying at 105°C. The concentrations of substrate, product and by-products were determined by HPLC method. The protein content of the biomass was analyzed by Kjeldahl method. In the batch culture the increase of YE concentration stimulated biomass growth. In the culture with 10 g/L of YE biomass concentration reached 28 g/L and was doubled, in comparison to the culture with 5 g/L of the nitrogen source. In the process with lower dose of YE erythritol concentration was higher and reached 78 g/L, corresponding to 0.51 g/g production yield and productivity of 1.01 g/Lh. Lower erythritol concentration (62.1 g/L) and its production yield (0.40 g/g) in the process with 10 g/L of YE was due to enhanced biomass growth observed in this culture. The protein concentration of the biomass derived after the cultures was in the range of 16.7 – 19.8%. To maximize the final product concentration and improve its purity, the fed-batch mode (250 g/L of glycerol) was applied, which allowed to obtain 145.5 g/L and 132 g/L of erythritol in the processes with 5 and 10 g/L of YE, respectively. At the end of the biosynthesis the total concentration of the by-products (mannitol, arabitol, citric and a-ketoglutaric acids) reached up to 17.6 g/L. Extension of the culture after glycerol exhaustion resulted in utilisation of by-products (final concentration of 1.2 g/L) and the increase of erythritol amount up to 165.5 g/L (5 g/L of YE). In the biomass the protein level increased up to 28%. Acknowledgments. This work was co-sponsored by grant No. N N312 256640 from the National Science Centre (Poland) and by the Ministry of Science and Higher Education of Poland and European Union under Project No. POIG 01.01.02-00-074/09.

Authors: Ludwika Tomaszewska, Anita Rywinska, Waldemar Rymowicz
SCALE-UP OF ERYTHRITOL PRODUCTION FROM CRUDE GLYCEROL BY A MUTANT OF YARROWIA LIPOLYTICA

Waldemar Rymowicz, Wrocław University of Environmental and Life Sciences, Poland

Erythritol, a four-carbon polyol, is a biological sweetener with applications in food and pharmaceutical industries. It is also used as a functional sugar substitute in special foods for people with diabetes and obesity. Erythritol is produced by microbial methods using mostly osmophilic yeasts and has been produced commercially using mutant strains of Aureobasidium sp. and glucose is used as a main carbon and energy sources. Our preliminary study showed that Yarrowia lipolytica yeasts are suitable to produce high amount of erythritol from glycerol in fed batch cultivations (up to 170 g/L). Erythritol biosynthesis is facilitated by low pH values (pH 3), and decreases with the increasing pH value of the culture media. In addition, at low pH (2.5-3.0) the synthesis of citric acid, the main by-product of the process, is not observed. The main objective of this study was scale-up and developed of erythritol production by selected strain of Y. lipolytica MK1 in big bioreactors (type 90-L MPP New Brunswick) with working capacity of 50 liters. In all investigations crude glycerol (76% ) was used as a carbon source. The investigations showed that in large scale is possible to obtain high amount of erythritol with good productivity and yield. In fed batch process with MK1 strain, erythritol productivity of 0.6 g/L/h was reached, giving 143 g/L of erythritol with a yield of 0.48 g/g. The biomass of Y. lipolytica contained about 22% of protein and 23% of lipids. The yeast protein contained essential amino acids (g/100 g of protein) at the level higher than the FAO/WHO standard for yeast. In conclusion, due to the present situation on the market - involving high costs of the traditional substrates and a very low price of easily-available glycerol, the production of erythritol from glycerol in microbiological processes using Y. lipolytica yeast, may be considered a competitive alternative.

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Authors: Waldemar Rymowicz, Anita Rywinska, Aleksandra Mironczuk, Piotr Juszczyk

Second Generation Ethanol Production from Sugarcane Bagasse Hemicellulosic Hydrolysate by S. Stipitis Immobilized Cells at Different Agitation Levels

Silvio Silva, School of Engineering of Lorena

In the microbial fermentation processes for bioethanol production, immobilized cells have shown several advantages including the easy separation of bio-products, recovery of the biocatalyst and the use of high cell densities. Among the xylose-fermenting yeasts, Scheffersomyces stipitis NRRL Y-7124 has shown its potential for bioethanol production from pentose and hexose sugars present in lignocellulose hydrolysates in addition to considerable tolerance to inhibitors and ethanol. In this context, the present study was aimed to evaluate the production of ethanol from sugarcane bagasse hemicellulosic hydrolysate by calcium alginate immobilized cells of S. stipitis at different level of agitation. The cell suspension was added to a solution of sodium alginate (2%) to obtain the final cells concentration of 10.0 g/L.
Spheres of gel were produced by dripping it into CaCl2 (0.1 M) solution under stirring conditions. Fermentation tests were carried out in 125 mL Erlenmeyer flasks containing 50 ml of hemicellulosic hydrolysate supplemented with media ingredients and incubated at 100 or 300 rpm at 30°C for 72 hours. Maximum ethanol production (8.9 g/L, Yp/s 0.33 g/g) was observed after 48 hours at 100 rpm. On the other hand, fermentation at 300 rpm showed comparatively low ethanol production (4.8 g/L, Yp/s 0.15 g/g). At 300 rpm, it was observed a higher free biomass growth (6.8 g/L) than in assay using 100 rpm (2.8 g/L). This result clearly reveal that high oxygen availability due to high agitation leads to high production of cell mass due to the shifting of pyruvate in citric acid cycle instead of ethanol formation. Therefore, agitation is an important factor for ethanol production influencing oxygen yields in the process and metabolism direction between respiration and ethanol production.

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Authors: Thais S. S. Milessi, Felipe A. F. Antunes, Anuj K. Chandel, Silvio S. Silva


Archana Parashar, University of Alberta

Liquid lactose is a by-product of cheese manufacturing and whey processing in the dairy industry. The current market, as a livestock feed additive, is disappearing and so there is an urgent need to find a new viable alternative market for this excess liquid lactose to prevent a negative economic impact on the dairy industry. Our aim is to develop a technology to integrate liquid lactose into the existing ethanol industry as a cost-effective co-fermentation substrate with grain. The specific objectives are to optimize the inclusion of the liquid lactose substrate in regards to concentration and timing, reduce process water requirement, and investigate the impact of lactic acid bacteria present in liquid lactose on the co-fermentation process. Liquid lactose was procured from a large dairy processing facility and hydrolyzed under optimized conditions to fermentable sugars using lactozyme; >95% hydrolysis efficiency was achieved as was confirmed by high performance liquid chromatography (HPLC). Benchmark fermentations were then carried out on the established prairie cereal grain, wheat, and 100% liquid lactose (24% solids) under current industrial protocols for commercial ethanol production. Briefly, wheat mash was prepared by liberating starch via dry-milling and hydration, followed by two enzymatic treatments to reduce viscosity, increase starch accessibility and supply additional free amino nitrogen. Ensuring all known variables remain constant, liquid lactose was incorporated as a partial replacement of wheat and process water to assess different grain-lactose co-blending formulations. Cold starch hydrolysis (Stargen)-based fermentations were carried out in triplicate with an initial pre-saccharification step; the simultaneous saccharification and fermentation using Saccharomyces cerevisiae (QuickStartTM) was monitored for 72 hours. The progress and efficiency of fermentations was monitored by HPLC to assess sugar consumption and gas chromatography to assess increase in ethanol content over time. Co-fermentations of liquid lactose and wheat resulted in increased ethanol production and reduction in process water requirement. Co-fermentations with reduced wheat concentrations along with corresponding liquid lactose supplementation showed that
a portion of the fermentable carbon from wheat can be replaced with that from liquid lactose without affecting ethanol production. Also, results suggest that an optimal combination of initial fermentable sugar concentration and total fermentable carbon is critical for achieving increased ethanol production in the co-fermentations. Potential contamination concerns and organic acid build up during fermentation was investigated using HPLC. In all of the different co-fermentation trials, the amount of lactic and acetic acids detected was low suggesting that the presence of lactic acid bacteria in the liquid lactose does not pose a threat to the co-fermentation process and more importantly does not negatively affect ethanol production. Overall, this work has provided proof-of-concept and validated that co-fermentation of liquid lactose is feasible with wheat. Efforts to further optimize the co-blending and scale-up the co-fermentation process expanding to a commercial scale are currently in progress. This would improve economics of grain-to-ethanol fermentation, and create a value-added market and reduce potential waste costs for the dairy industry.

Authors: Archana Parashar, Yiqiong Jin, David C. Bressler.

**Use of Agricultural Residues for Screening and Identification of Cellulolytic Bacterial Strains with Potential Application in Bioethanol Production**

François Laframboise, Université du Québec à Trois-rivières

Bioethanol is seen by many as a complement or a substitute for oil in the future years, mainly, because it uses plants as its basic source of carbon. There are multiple types of bioethanol, and the “second generation” bioethanol which uses either agricultural residues or forestry residues is currently the most viable option. Most of such cellulosic residues must be converted into cheap sugars source first which can be transformed into bioethanol. In this project we screened microbial organisms that would produce enzymes required for agricultural residues degradation. Twelve cellulolytic bacterial strains were isolated from various agricultural residues found on a local farm. Phylogeny based on 16S rRNA gene sequences revealed the presence of five different Bacillus strains. Comparison of 16S rDNA sequences and biochemical characterization allowed identification of species as well as relationships to closest known related strains. Isolates were related to Bacillus licheniformis (93-99%), Bacillus cereus (99%), Bacillus thuringiensis (99%), Bacillus aryabhattai (99%) and Bacillus pseudomycoides (99%). All the strains screened from residues produced extracellular cellulolytic enzymes with a high affinity for extruded maize crops residue. To our knowledge, the ability of these strains, B. pseudomycoides and B. aryabhattai, to degrade cellulosic substrate is reported here for the first time.

Authors: François Laframboise, Fatma Meddeb-Mouelhi, Kokou Adjallé, Marc Beauregard and Simon Barnabé
Utilization of Whey Lactose for Ethanol Production by Simultaneous Lactose Hydrolysis and Fermentation

Yiqiong Jin, University of Alberta

Whey, a by-product of the cheese industry, has been produced in millions of tons annually. Lactose is a major component of whey and deproteinized whey called whey permeate. Current whey/whey permeate utilization consumes only a small portion of whey lactose generated. The large amount of whey surplus has made whey lactose an interesting and promising substrate for industrial chemical and fuel production. The bio-ethanol industry, which has significantly expanded worldwide over the last few decades, is a potential down-stream market for whey lactose consumption as a co-substrate in addition to grain starch. Thus, our goal is to effectively and efficiently utilize whey lactose in commercial wheat-to-ethanol production by Saccharomyces cerevisiae. Whey permeate was co-blended with wheat to prepare fermentation media for ethanol production. A corresponding amount of wheat was reduced in order to maintain the total fermentable sugar concentration at the same level as the benchmark fermentation which used wheat alone. Simultaneous lactose hydrolysis and fermentation was conducted to eliminate lactose pre-hydrolysis step by adding β-galactosidase at the onset of fermentation. The effectiveness of β-galactosidases from Kluyveromyces lactis and Aspergillus oryzae on lactose hydrolysis during fermentation was investigated and the optimal enzyme was selected. In addition, the dosage of β-galactosidase was optimized to obtain maximum lactose utilization efficiency. The fermentation with β-galactosidase from A. oryzae had significantly higher lactose hydrolysis efficiency than that using β-galactosidase from K. lactis. Increasing the dosage of β-galactosidase from K. lactis did not improve the lactose hydrolysis efficiency. A dosage of 20 U/g lactose for β-galactosidase from A. oryzae resulted in up to 90% lactose hydrolysis. Based on these results, we concluded that simultaneous lactose hydrolysis and fermentation for ethanol production is feasible by using β-galactosidase from A. oryzae.

Authors: Yiqiong Jin, Archana Parashar, David Bressler*

Studies and Commercialization of Non-Food Feedstock Biofuels and Chemicals in China.

Xue-Ming ZHAO, School of Chemical Engineering & Technology Tianjin University

As the biggest developing country, China faces a serious challenge in satisfying its need for huge amounts of energy resources and chemicals. The Chinese government has recently started the non-food feedstock biofuels and chemicals projects. In this presentation, I will introduce the work done by our school: laboratory studies on biofuels and chemicals production by systems biology and synthetic biology[1-5] and commercialization of non-food feedstock (cassava or corn cob) bioethanol and chemicals(200000 t/a bioethanol, 50000 t/a bioethene and 100000 t/a bioethylen acetate)[7-8]. References 1. Jing-Jing Liu et al., Improving ethanol fermentation performance of Saccharomyces cerevisiae in very high-gravity fermentation through chemical mutagenesis and meiotic recombination,Appl Microbiol Biotechnol, 2011, 91:1239–1246 2. Jinmei Xia et al. Comparative Lipidomic Profiling of Xylose-Metabolizing S. cerevisiae and Its Parental Strain in Different Media Reveals
3. Ming-Zhu Ding et al., Comparative metabolic profiling of parental and inhibitors-tolerant yeasts during lignocellulosic ethanol fermentation, Metabolomics, 2012, 8:232–243
7. 200000 t/a bioethanol non-food feedstock (cassava) bioethanol http://www.bioindustry.cn/info/view/13170
8. 50000 t/a bioethene and 100000 t/a bioethylen acetate http://www.bioindustry.cn/info/view/18389

Authors: Xue-Ming Zhao

**Evaluations of Chemical Properties of Lignocellulosic Feedstocks for Renewable Fuels**

Sonil Nanda, Lassonde School of Engineering

The climate change and diminishing fossil fuel supplies are issues of acute concern for most countries today. These have led to the use of lignocellulosic biomass as a renewable energy source. Lignocellulose, produced by vascular plants, is the most abundant renewable resource for production of biofuels, especially bioethanol and biobutanol. Plant cell walls comprise of three major biopolymers, namely cellulose (30-50 wt%), hemicellulose (15-35 wt%) and lignin (10-30 wt%). Since, lignocellulosic biomass is a heterogeneous mixture of these key structural organic components (cellulose, hemicellulose and lignin) along with accessory organic and inorganic composites, it is essential to understand its basic composition and properties for finding its utility for fuel. Hence, the overall characterization of a feedstock is indispensable not only to predict its energy output but also to identify the challenges, logistics and economics related to its processing in a biorefinery. In this study, we characterize lignocellulosic biomass of different origin such as agricultural (wheat straw), forestry (pinewood) and perennial grass systems (timothy grass) for their usage towards next generation biofuels. The biomasses were investigated physiochemically, biochemically and morphologically to understand their structural and compositional characteristics. Various analytical techniques involved in the study were carbon-hydrogen-nitrogen-sulfur (CHNS), inductively coupled plasma-mass spectrometry (ICP-MS), Fourier transform infrared (FTIR) spectroscopy, Raman spectroscopy, thermogravimetric and differential thermogravimetric (TG/DTG) analysis, X-ray diffraction (XRD), high pressure liquid chromatography (HPLC), scanning electron microscopy (SEM) and atomic force microscopy (AFM). Morphologically, pinewood was found to be more fibrous and recalcitrant than timothy grass and wheat straw as studied through SEM and AFM. The recalcitrance and thermal stability of pinewood was also identified through TG/DTG analysis, which explained its maximum weight loss at ~380°C than ~350°C.
as in case of timothy grass and wheat straw. The XRD analysis of ashes from the three feedstocks showed the presence of various species of inorganic components such as carbonates, silicates, sulfates along with traces of chlorides. Various crystallographic phases in the XRD of ashes were due to minerals commonly Na, Mg, Al, Ca, Fe and Mn. XRD patterns of the feedstocks also demonstrated the presence of cellulosic polymorphs and hemicellulose at 15.5°, 21.7° and 34.5° 2-theta positions, respectively. Relatively high amount of alkaline earth metals (Na, Mg, P, K, Ca) were found in timothy grass and wheat straw because of their fast-growing nature. Chemically, hemicelluloses were high in timothy grass (30.1 wt%) than in wheat straw (24.1 wt%) and pinewood (23.6 wt%). On the other hand, the tightly bound fibrous structure of pinewood contributed to its ligneous nature (20.4 wt% lignin). Wheat straw was highly cellulosic in nature (39.1 wt% cellulose) than pinewood (38.8 wt%) and timothy grass (34.2 wt%). FTIR and Raman spectroscopy revealed the presence of waxes, fatty acids, aldehydes, alcohols, ethers, carboxylic acids and esters in the feedstocks at varying intensities. With these physiochemical and biochemical findings, the advantages and challenges of using these woody and herbaceous feedstocks in a biorefinery will be discussed.

Authors: Sonil Nanda, Janusz A. Kozinski, Ajay K. Dalai
Effect of Organic Loading Rate on Bio-H2 Production Using a Steam Exploded Corn Cob Liquor Fed to Mixed Anaerobic Cultures in Upflow Anaerobic Sludge Blanket Reactors

Sathyanarayanan Veeravalli, University of Windsor

Effect of organic loading rate on bio-H2 production using a steam exploded corn cob liquor fed to mixed anaerobic cultures in upflow anaerobic sludge blanket reactors

Authors: Subba Rao Chaganti and Jerald A. Lalman

Effect of pH and Linoleic Acid on Mesophilic Hydrogen Consumption by Mixed Anaerobic Cultures

Saravanan Shanmugam, University of Windsor

Biohydrogen (bio-H2) is considered a promising alternative to fossil fuels as a sustainable energy source. The main advantages of using H2 include a high energy content and upon combustion water is the only by-product. Hydrogen can be derived from a wide variety of renewable energy resources. Dark fermentation using mixed anaerobic cultures is the most preferred route to produce H2 because sterile operating conditions are not required and the process can utilize a wide variety of organic substrates. However, a major problem in employing mixed cultures for H2 production is related to the presence of H2 producers (acidogens and acetogens) and H2 consumers (homoacetogens and hydrogenotrophic methanogens). Successful H2 production via dark fermentation can be achieved by selective inhibition of H2 consumers by treatment methods such as heat, acid, alkali, aeration and chemical. Among the various chemical treatment methods, linoleic acid ((LA), C18:2), a long chain fatty acid (LCFA) has been reported as an effective inhibitor of H2 consumers. In this study, the effect of LA at pH 4.5 and pH 7.5 on H2 consumption was investigated using cultures maintained at 37°C. In the LA untreated cultures (controls with no LA added), all the injected H2 was consumed at both pH 4.5 and 7.5. In comparison, in LA treated cultures, only approximately 10-15% of the injected H2 was consumed within 96 h. Methane was not detected in the LA treated cultures; however, acetate was observed in the LA treated culture at pH 7.5. Acetate production was not observed at pH 4.5 in the LA treated culture. A principal component analysis of the fermentation metabolites revealed that the LA treated cultures were grouped separately in comparison to the controls. Microbial community analysis using terminal fragment length polymorphism (T-RFLP) revealed that Clostridium sp, Kosmotoga olearia, Thermoanaerobacter sp., were present in all the cultures. However, the abundance of Clostridium novyi was higher in the LA treated cultures at pH 4.5 when compared to other cultures. Methanogens utilizing H2/CO2 (Methanomicrobiales) and acetate (Methanosarcinales) were present in the control cultures at pH 7.5. Methanocalculus halotolerans, a major LCFA degrading organism was detected in the LA treated cultures at pH 7.5. Methanococoides alaskense was present only in the control cultures at pH 7.5 indicating that either the addition of LA or adjusting the pH to 4.5 inhibited these organisms. These results suggest that the addition of LA and lowering the pH could synergistically inhibit several H2 consuming populations in mixed anaerobic communities. Keywords: Homoacetogens, hydrogenotrophic methanogens, linoleic acid, mixed anaerobic cultures, T-RFLP
**Enhanced Hydrogen Production by Inhibited Mixed Anaerobic Communities**

Wudneh Shewa, University of Windsor

Several studies have shown evidence of the inhibitory effect imposed by C18 long chain fatty acids (LCFAs) such as linoleic acid on hydrogen (H2) consumers and H2 producers. This work is intended to demonstrate using an alternative, economical and sustainable feedstock for enhanced H2 production as opposed to costly individual LCFAs. Vegetable oils, which are present in spent frying oils and waste streams from fried food manufacturing, could serve as inhibitors to H2 consumers. Decoupling the syntrophic relationship between H2 consumers and H2 producers by inhibiting the H2 consuming population will lead to increasing the H2 yield. Fed-batch studies were carried out to evaluate the effect of safflower and/or olive oil on H2 production from glucose. A mesosphilic anaerobic mixed culture was fed 5,000 mg/L glucose plus 2000 mg/L emulsified oils at pH 5.5 and 37oC. Data from the study showed that in comparison to control cultures (fed only glucose), the H2 yields increased by 12%, 7% and 11% in cultures fed safflower oil, olive oil and a 1:1 mixture of safflower oil and olive oil. In the glucose controls, the H2 yield reached 2.58 mol H2 per mol of glucose. Although methane production was inhibited in cultures receiving safflower and/or olive oil, the electron flux was not directed to H2 production but instead a metabolic shift to reduced by-products was observed. In the presence of both oils (individually or combined), a larger fraction of electron equivalents was directed towards H2 production. The maximum yield observed in this study was 2.89 mol H2 per mol glucose in reactors fed emulsified safflower oil. An electron mass balance was conducted by comparing the quantity of electrons in the substrate and fermentation products. In the control samples, the major byproducts detected included acetate, propionate and butyrate. A metabolic pathway analysis demonstrated an inhibitory effect of safflower and olive oils on the methanogenic and acetogenic populations. The inhibition on the different populations was attributed to the presence of LCFAs. The T-RFLP results confirmed inhibition of methanogens and acetogens and an enhancement of the H2 producing population (Clostridium sp and Bacillus sp). Microbial community variations were detected in cultures fed with both oils. This study demonstrates the possibility of using waste vegetable oils to inhibit selected microbial populations and subsequently increase the H2 yield.

Authors: Subba Rao Chaganti, Jerald A. Lalman, Daniel Heath

**Bio-Conditioners for Enhancing Biosludge Dewaterability**

Sofia Bonilla, University of Toronto

Biosludge dewatering is a challenge in municipal and industrial wastewater treatment plants. Thus, there is great value in maximizing dewatering of biosludge, either to enhance energy recovery and/or reduce the volume of it for disposal or other uses. Addition of active enzymatic supplements has been previously reported to enhance sludge dewaterability. However, it is not fully understood how these additives
Lysozyme, a commercially available enzyme, was found to have potential as it increased sludge dewaterability by approximately 50% measured with capillary suction time (CST). This enzyme was the first selected for a further characterization of its effect. Biosludge chemical, physical and dewatering properties were analyzed before and after treatment, and our results suggest that the effect of lysozyme on dewaterability might not be directly associated with its enzymatic activity since native and thermo-inactive preparations showed similar results. Additionally, polymer demand was reduced from 11% to 6% when lysozyme was added and there is evidence that lysozyme aids the settlement of particles. The effect of temperature and mixing rate on the treatment did not seem to be significant in ranges from 23°-50 °C and 0-200 rpm, respectively. Additionally, site-directed mutagenesis was used to evaluate the effect of enzymatic activity on the treatment. Further work will be focused on screening new biopolymers and to determine the biosludge properties that change during the dewaterability enhancement.

Authors: Sofia Bonilla, D. Grant Allen

**Step-Up to Greater Production Efficiency and Profit through Enhanced Grain Utilization**

Suzanne Clark, Novozymes

The corn to ethanol industry in the United States is operating with minimal profit when market prices of major costs such as corn, energy, and depreciation are compared to the value of ethanol and DDGS [1]. To address this, Novozymes has embarked on an intensive, multi-year research program designed to understand the role which enzymes play in enhancing the utilization of corn components for improving process efficiencies, rates, and yields. The key findings of this research, which will be illustrated during this presentation, revealed multiple avenues whereby enzymes could unlock lost potential, thereby allowing for the more rapid conversion of this potential into additional ethanol yield and improved backend efficiency. A specific liquefaction enzyme, with the commercial name Avantec, was developed and up-scaled based on this research and launched on October 30th 2012. The process entry point for Avantec is similar to existing alpha amylase enzymes, i.e., before the high temperature liquefaction stage. Full-scale plant evaluations confirmed the substantial benefits observed during product development regarding enhanced yields and smoother, more efficient processing with reduced energy usage. These results have allowed for increased profits to ethanol producers. An overview of these data will be presented together with an expected economic benefit model. Additional information can be found on www.Bioenergy/novozymes.com 1Center for Agricultural and Rural Development, Iowa State University, www.card.iastate.edu

Authors: S.E. Clark, R. Deinhammer, G. Coward-Kelly, B. R. Knudsen, A. G. Hjulmand

**AgBioproducts Refinery Feasibility Study for Northwest Ohio**

Richaard "Max" Maksimoski, Ohio Bioproducts Innovation Center
The goal of the study is to provide a community planning group in Northwest Ohio with the information needed to understand and take advantage of the opportunities that may avail themselves with the building of a biorefinery. Commercialization of biobased chemicals is evolving rapidly and is poised to transform the production of industrial chemicals. Ohio has a unique opportunity to be an emerging leader in developing a bioeconomy base because Ohio’s leading agriculture and polymers industries provide an enviable infrastructure that positions the State to rapidly and successfully leverage bioproduct production. This will revitalize the chemicals and polymer industries while creating opportunities for new business growth, particularly in rural areas, through the development of biomass supply systems. Such an outcome is expected to create a significant number of new jobs, new companies, and bring added economic security to rural sectors. Northwest Ohio has significant competitive advantages as a biorefinery location. It is in close proximity to biomass from agriculture, food production, and municipal solid wastes. Superior transportation logistics provide an edge in the transport of diverse feedstocks and delivery of products to market. It has a highly skilled workforce in many key areas of the value chain, and is supported by research and education from world-class universities. The study recommendation is to continue defining options for a biorefinery hub located at the Port of Toledo. The early vision is of a bio-demo facility operating alongside a commercial-scale, ‘anchor’ biorefinery. The infrastructure needed to support the anchor biorefinery would be leveraged by small to mid-size technology companies operating within the bio-demo facility to accelerate their scale up. As these companies reach scale, it is believed that they would consider the Port as a prime location for a full scale commercial enterprise. A bio-demo facility would not only support scale-up but should be broad enough in its mission to accelerate the coordinated development and optimization of the entire bioproduct value chain – leading to substantial growth in jobs and economic development in the region.

Authors: Richard "Max" Maksimoski

*Fungal SMC Bioconversion: A Potential Greener Technology*

Finola Cliffe, National University of Ireland, Galway

The Irish mushroom industry produces over 400,000 tons of spent mushroom compost (SMC) annually. Currently, this by-product is employed as a soil conditioner; however, the application for SMC is considered very limited due to associated environmental and health impacts. The relatively high carbohydrate content of SMC makes it a suitable alternative feedstock in the biofuel and biorefinery sectors. Biochemical analysis of SMC indicates its potential use as an inexpensive nutrient source for microbial enzyme production. Ligno-cellulolytic enzymes are produced by various fungi and bacteria; however, many of the xylanase- and cellulase-rich commercial enzyme preparations are derived from fungal sources. In this study, we compared the proximate, carbohydrate and metal ion compositions of SMC from different sources. We also compared the bioconversion of SMC using commercial enzyme preparations which showed conversion efficiencies of up to 70%. The study showed that the fungal enzymes can be used to generate SMC hydrolysates rich in fermentable sugars for downstream bioenergy production. Such bio-based technologies are specifically designed to target lignocellulosic feedstocks to produce not only sugar-rich mixtures for ethanol production but also
value added products including proteins, amino acids, lipids, lignin and phenolic compounds. Further research will focus on the improvement of the fungal strains responsible for production of these enzymes and increased understanding of the biological mechanisms behind their synthesis.

Authors: Finola Cliffe
Feedstock Crops and Biomass Supply

Comparison of the Energy Balances of Five Cellulosic Ethanol Production Processes

Judi Bahl, International Institute for Sustainable Development

Lignocellulosic biomass contains carbon primarily in the form of lignin, hemicellulose, and cellulose, as well as starches, lipids and ash. These constituents can be processed, often into gases and sugars which are used as building blocks for higher value chemicals. Prominent among the portfolio of emerging bioproducts is cellulosic ethanol, and commercial scale cellulosic ethanol facilities are now entering production. Lignocellulosic ethanol made from agricultural waste products or purpose grown crops has the potential to reduce reliance on non-renewable fossil fuels and avoids the use of food crops for fuel production. Production of lignocellulosic ethanol can also reduce greenhouse gas emissions and can protect water quality from nutrient leaching. Several production methods for making ethanol from lignocellulosic material are under study. This paper examines the energy balance of five prominent production methods in detail, encompassing pre-processing, pretreatment, hydrolysis, fermentation, and distillation stages. The five methods are: 1) dilute acid pretreatment followed by four enzyme hydrolysis with E. coli fermentation; 2) lime pretreatment, three enzyme hydrolysis and E. coli fermentation; 3) ammonia pretreatment with two enzyme hydrolysis and E. coli fermentation; 4) AFEX (ammonia fibre expansion) pretreatment, two enzyme hydrolysis and E. coli fermentation; and 5) steam explosion pretreatment followed by four enzyme hydrolysis and E. coli fermentation. For each production method, the process energy balance was calculated under three scenarios: 1) where lignin is used as an energy source and waste heat from certain processing steps is recycled internally, 2) where the lignin removed in pretreatment is used as an energy source for the process, 3) no use of lignin as an energy source or heat recycling. The energy balance (ratio of ethanol energy output to total energy inputs) for scenario 1) from 2.55 for the AFEX process to 6.04 for the steam explosion process. For scenario 2 the results range from 0.67 for dilute acid to 1.06 for AFEX. For scenario 3 the results range from 0.35 for dilute acid to 0.43 for AFEX.

Authors: Matthew McCandless1,2, Xi Chen1, David Levin2, Henry David Venema1, Nazim Cicek2 1 International Institute for Sustainable Development, Winnipeg MB 2 University of Manitoba, Department of Biosystems Engineering

Decentralized Fractionation of Sweet Sorghum Bagasse for Centralized Biorefinery Operations

JIBY KURIAN, McGill University

Decentralized pretreatment of lignocellulosic biomass would address the issues associated with the handling and transportation of feedstocks for biorefinery operations. In the context of utilizing lignocellulosic biomass for biofuels and biomaterials there should be more involvement and participation by the biomass producers in the processing and trading of the feedstocks produced by them. Most of
the biomass pretreatment processes developed so far are energy and capital intensive and they can be used at sophisticated industrial facilities only. The overall objective of this research work is to optimize lime treatment process for the decentralized and onsite pre-treatment of lignocellulosic biomass for the centralized bio-refinery operations. Pretreatment parameters like lime concentration, substrate concentration, temperature, retention time, and post-pretreatment processes would be optimized. The techno-economic and ecological studies will also be conducted. This work envisages developing a market pathway where the farmers or their co-op would treat the available biomass at their own facilities to isolate the component polymers like cellulose, hemicellulose, lignin and minerals in its pure and compact form. The fractionated materials would be collected by the industries in their purer and dense form which would reduce the cost of processing and also ensure continuous supply of the raw materials. Direct involvement of the biomass producers in the biomass processing would create more job opportunities and thereby facilitating the rural development and youth employment.

Authors: Vijaya Raghavan

**Development of a Dedicated Crop for Oil-Based Biofuels Production Based on Camelina Sativa**

Danny Schnell,

The overall objective of this project is to develop Camelina sativa as a dedicated, non-food, oilseed crop with the capacity to produce oils and terpenes for biofuels production that exceed the current the yields of ethanol from corn grain. To achieve this overall objective, we will introduce highly efficient mechanisms of photosynthetic carbon fixation from photosynthetic bacteria increase Camelina productivity. Our goal is to double the efficiency of carbon capture by the ribulose-1,5-bisphosphate carboxylase/oxygenase (rubisco), the rate limiting enzyme in photosynthesis. We will engineer Camelina via a combination of nuclear and plastid transformation with two carbon concentrating mechanisms (CCMs) to increase rubisco efficiency. In parallel, we will modify the metabolic pathways of lipid synthesis in Camelina to redistribute the increased fixed carbon into oils and terpenes. By increasing the flux of increased fixed carbon into seed oils, we anticipate an approximate 50% increase in seed oil content. Our strategy involves 1) increase oilseed production by the seed-specific over-expression of enzymes that limit the accumulation of the seed triacylglycerols, the feedstock for biofuels production, and 2) define and manipulate the heterologous and endogenous terpene synthesis pathways in Camelina to increase the yield and accumulation terpenes in both vegetative tissues and seeds. The potential to increase plant terpenes with properties similar to gasoline provides an attractive alternative biofuel additive and a value added product for other biotechnological applications.

Authors: Danny Schnell, Jeffrey Blanchard, Michelle DaCosta, Amit Dhingra, Cheryl Kerfeld, Jennifer Normanly,

**Winter Cover Crops In A Corn-Forage Sorghum Crop Rotation System**

OLIVER FREEMAN,
Cover Crops have proven to be beneficial to the production of field crops. In the Central Plains, cover crops can contribute by increasing nitrogen and soil organic matter, and the establishment of soil cover. This study focuses on using cover crops (winter wheat and Austrian winter pea) in different rotations of corn and forage sorghum in Kansas (Manhattan, Tribune) to establish a ground cover, and to potentially work as a ‘catch’ crop to help reduce nitrogen loss through leaching and to potentially add nitrogen to the soil for use by corn and sorghum in the rotation(s). Corn and forage sorghum were planted into plots, each with a randomly assigned 101 kg ha-1 and 0 kg ha-1 nitrogen treatment, and harvested for total biomass (grain and stover) at the end of the growing season. Cover crops were then planted in the fall along with a fallow treatment into each of the plots, and then harvested for dry matter and terminated at corn and forage sorghum planting time(s) of the following year. Results indicated that the most significant effect came from the nitrogen treatment under all rotations for each crop. In 2010, in Manhattan, the 101 kg ha-1 nitrogen treatment produced as much as 42687 kg ha-1 total forage sorghum biomass while the 0 kg ha-1 nitrogen treatment produced as much as 36764 kg ha-1. In 2011, in Manhattan, forage sorghum total biomass peaked at 47976 kg ha-1 with the 101 kg ha-1 treatment. With the 0 kg ha-1 treatment, yields reached up to 38039 kg ha-1. Corn yields for the 101 kg ha-1 treatment were up to 50742 kg ha-1. Under the 0 kg ha-1 treatment, yields reached 39846 kg ha-1. In Tribune, with the 101 kg ha-1 treatment, yields got up to 70259 kg ha-1 for forage sorghum, while the 0 kg ha-1 treatment yields made it up to 66938 kg ha-1. With Corn, yields reached 51027 kg ha-1 under the 101 kg ha-1 treatment. Where the 0 kg ha-1 treatment was applied, yields were up to 42802 kg ha-1.

Authors: Scott A. Staggenborg, Department of Agronomy, Kansas State University, Manhattan, KS, USA Mary B. Kirkham, Department of Agronomy, Kansas State University, Manhattan, KS, USA

**Changes in Enzymatic Activity, Biomass Components and Conversion to Ethanol Yields of Sorghum Biomass after Post-Harvest Storage**

Anne Rigdon, Grain Science and Industry, Kansas State University

With increased mandates for biofuel production in the US, ethanol production from lignocellulosic substrates is burgeoning, highlighting the need for thorough examination of the biofuel production supply chain. This research focused on the impact storage has on biomass, particularly photoperiod-sensitive sorghum biomass. Biomass was stored under four different storage treatments for a total of six months, with sampling every two months. Biomass quality parameters were monitored and included the biomass components, cellulose, hemicellulose and lignin, along with extra-cellular enzymatic activity (EEA) responsible for cellulose and hemicellulose degradation and conversion to ethanol yields. Analyses revealed dramatic decreases in uncovered treatments, specifically reduced dry matter content from 88% to 59.9%, cellulose content from 35.3% to 25%, hemicellulose content from 23.7% to 16.0% and ethanol production of 0.20 g L-1 to 0.02 g L-1 after 6 months storage along with almost double EEA activities. In contrast, biomass components, EEA and ethanol yields remained relatively stable in covered treatments, indicating covering
of biomass during storage is essential for optimal substrate retention and ethanol yields.

Authors: Anne R. Rigdon, Ari Jumpponen, Praveen Vadlani, Dirk Maier

Conquering Barley and Other Agribusiness Data Challenges Using an Enterprise Database and Analysis Platform

Ben Adamczyk

Modern plant breeding and genetic engineering approaches for crop improvement place an increasing need for scientists to gather and interpret genome sequence information. While the advantages are clear, researchers are faced with large sample sizes, and complex genomes that vary across many different strains or may exist in different states of assembly. To manage this data and enable interrogations based on sequences, genes, and phenotypes, we developed a platform for genome management and analysis with agribusiness goals in mind. Genedata Selector™ is an enterprise system that is highly scalable, includes interactive visualization tools, and can be used to guide the discovery of genotype-phenotype relationships. In this study, we take advantage of this platform to investigate barley, a highly repetitive 5.1Gb genome, and discover genes that could be targeted for beer making. As a first step, RNA-seq data was used to refine gene model predictions of the recently published barley genome. We then used the nucleotide sequence data to identify amylase homologs which could have a role in the malting process. The homologous genes were then annotated in the system, so that the genomic ranges can be quantified for downstream gene expression experiments. Next, we compared the amylase sequences to those in rice, bacteria, and fungi, to learn about conserved proteins that could be targeted to alter malting. At each step in the analysis, data are stored, tracked, and annotated through a central database, and all information is accessible and searchable via a friendly and feature-rich interface. Through this example we demonstrate the scalability of the system for large data volumes and the capability to integrate public and proprietary data for sequence and pathway-based analyses.

Authors: Nadim Jessani1, Asa Oudes1, Niko Bausch2, Sebastien Ribrioux2, Thomas Hartsch1, Ludwig Macko2, Julia Retey2 & Tim Zeppenfeld2 Genedata, Inc., San Francisco, USA1; Genedata AG, Basel, Switzerland2

The Feasibility of Utilizing Conservation Reserve Program Land in Kansas for Producing Cellulosic Ethanol Feedstock

Michael Lindbloom, Kansas State University

Problem Statement The cellulosic ethanol mandates set by the 2007 renewable fuel standards require that 16 billion gallons of cellulosic biofuel be produced annually by the year 2022. Various studies have identified CRP land as a potential resource for cultivating and harvesting biomass for cellulosic biofuel and according to the USDA and Farm Service Agency (FSA), in October 2012 there were about 2.3 million acres of land enrolled in CRP in Kansas. This constitutes close to 9% of total CRP acres in the U.S., making Kansas an important location to consider when determining where
biomass for cellulosic ethanol production can be obtained. Objectives The objective of this study is to create and analyze a hypothetical market and government support program for the utilization of Conservation Reserve Program (CRP) land in Eastern Kansas for growing cellulosic ethanol feedstock. Data and Methods Using data from Kansas State University (KSU) Research and Extension, biomass yields from CRP land for the periods 2008-2012 will be obtained and used as a benchmark for feedstock availability. Combining this yield data with farm level cost data from the USDA and KSU Research and Extension, cost structures for feedstock producers will be created. Then using GIS software, cost estimates from the ethanol industry, and nonlinear optimization methods, break-even cost structures will be determined for the cellulosic ethanol producers. We assume that the production facility is a co-located facility, which means it will be constructed adjacent to the site of an already existing ethanol facility. Ethanol producers have determined that there are cost reducing benefits to co-locating cellulosic and starch ethanol facilities (which primarily include the combustion of non-fermentable solids from the cellulosic process to provide steam energy for the starch and cellulosic ethanol production process, as well as shared use of transportation infrastructure). Finally, using USDA data on past and projected CRP payment structures, hypothetical government support systems for harvesting feedstock grown on CRP land will be developed and used for a sensitivity analysis. Implications One advantage of this method is that most of the environmental benefits of the CRP program will be maintained by utilizing perennial crops, whose roots will remain in the ground after harvesting. Another benefit is that by using CRP land, land currently used for row crop production will not be infringed upon eliminating the food vs. fuel debate. In addition, the USDA has reported that over 600,000 CRP contracts in Kansas will be expiring over the next five years. Harvesting cellulosic ethanol biomass from these fields could therefore be a conservation-centric solution to re-enrollment concerns.

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**Giant Reed (Arundo donax L.): Desirable Traits for a Sustainable Energy Crop for 2nd Generation Ethanol.**

Sergio Miele, Consortium INSTM

Giant reed (Adx) is the leading candidate among ligno-cellulosic feedstocks for 2nd generation ethanol under warm temperate climates, for its high yield of ethanol-per-hectare and low ecological demands. Although it is retained a noxious plant for its invasivity in some areas, Adx has some desirable traits for an energy plant. It is a highly salt tolerant plant (halophyte) and can act as an interceptor crop to remove certain potential pollutants such as heavy metals, nitrogen and potassium from wastewaters and produce high yields, independently of the nutrient regimes. These aspects makes Adx particularly interesting to avoid competition with food crops for best soils, as it can exploit marginal lands and wastewaters. Presently, we are investigating some agronomic and physiological traits of Adx which contribute to its adaptation to harsh cropping conditions: anoxia, salinity, heat and cold stress tolerance. Preliminary results indicate that Adx has a DL50 (lethal dose causing 50% plant death) salinity threshold of 20 dS/m, and maintains good yields and quality at 5-6 dS/m.

Authors: Sergio Miele

**Is Your Bioenergy Project Looking for a FRIEND?**

Tim Hughes, Kentuckey Division of Biofuels

While there are a number of critical elements needed to site a successful bioenergy project, the terms that can be derived from the acronym – FRIEND, can be instrumental in helping a project get started on a firm foundation. • The first priority is Feedstock availability. Kentucky is a forage rich state with an abundance of productive woodlands. A strong agricultural heritage and infrastructure would enable the coordinated production of a variety of sustainable feedstocks with a multitude of processes. • Kentucky’s universities are already providing leadership in a number of Research initiatives. Research collaboration between the private and public sector will be critical to advance technologies, equip an effective workforce, and document stewardship of limited financial resources. o University of Louisville – License agreement with AliphaJet on technology related to biobased jet fuels o University of Kentucky – Using algae to sequester carbon from coal fired electric plant emissions at Duke Energy. Biomass to cellulosic fuel research and logistics with Case-New Holland. o Eastern Kentucky University – Department of Defense funding related to algal based fuels with General Atomics o Murray State University – On farm demonstrations related to a variety of dedicated energy crops with Ceres and Betaseeds. o These are just a few examples, but we have a number of other key projects. • Infrastructure for the efficient transportation of raw resources and finished bioenergy products will be essential to ensure competitiveness. Kentucky is well positioned relative to the majority of the US population and our interstate system, river resources, and rail lines will enable firms to fill these growing markets. • Enthusiastic support from our Governor and Legislature for bioenergy development is another important asset to assist with the organizational and regulatory hurdles encountered by new ventures. o Governor’s Biomass Task Force –
http://energy.ky.gov/resources/Pages/btf.aspx o 2010 and 2011 Interim Joint Committee on Agriculture focus sessions on bioenergy • Networks of key bioenergy stakeholders are important to ensure continued support for all of these tools. We have a number of organizations such as the Kentucky Clean Fuels Coalition, Kentucky Agricultural Council, Kentucky Renewable Energy Consortium, etc. that are creating dialogues, providing educational opportunities, and assisting our bioenergy industries grow. • While we are all dealing with austere budgets, the strategic allocation of Dollars to these potential projects continues to be a coveted carrot among these companies as they evaluate homes for their plants. o The Kentucky Cabinet for Economic Development offers a variety of financial incentives with several targeted to energy projects – http://www.thinkkentucky.com/KYEDC/kybizince.aspx o The Kentucky Science and Technology Corporation also offers grant and equity investment infusions – http://www.kstc.com/index.php?option=com_content&view=article&idid=133&Itemid =283 o The Kentucky Department for Energy Development and Independence has provided funding for a variety of energy initiatives (pages 50-51) - http://energy.ky.gov/resources/Annual%20Summaries/annual%20summary%20with%20calendar%202-23-12.pdf o The Governor’s Office of Agricultural Policy has provided funding for an ethanol and biodiesel facility and has supported other efforts to create new bioenergy opportunities for Kentucky farmers.

Authors: Tim Hughes

Moving Towards Commercialization of Lignocellulosic Biomass to Fuels and Chemicals. How to Deal with Heterogeneous Biomass.

Renata Bura, University of Washington

Improving the efficiency of lignocellulosic biofuel and biochemicals production is of the utmost importance if cellulosic bioproducts are to be competitive with fossil fuels and first generation bioethanol from starch and sucrose. Improvements in individual processes (pretreatment, saccharification, fermentation) have been ongoing, but few researchers have considered the effect that the incoming raw biomass can have on the process. Even within the same species, the lignocellulosic biomass is physically and chemically very heterogeneous due to the agronomy practices for stand establishment, water and nutrients management, weed control, harvest and storage, growing seasonal precipitation requirements, seasonal changes, and age. Rather than designing a biorefinery around an ideal source of a given feedstock, it is preferable to understand how we can process heterogeneous feedstock. How can we alter the heterogeneous biomass to provide the maximum yield of hydrolysable and fermentable sugars from whatever is available? In this paper we discuss how by improving the uniformity of heterogeneous feedstocks in terms of moisture content and particle size we could improve the overall products yields. Another means of dealing with heterogeneous biomass is to improve overall process control by increasing the level of data collection for each process. Particularly in the case of a biorefinery producing biofuels and biochemicals, monitoring each process can provide early detection of feedstock heterogeneity, process upsets, contamination, and any deviation from normal and thereby increase yields and efficiency by maintaining a steady state environment. In this paper we discuss how, Raman spectroscopy in particular, could be used to monitor reactions, leading to increased real-time
awareness and enabling instant control over process parameters. Finally, when processing heterogeneous biomass overall results of the techno-economic analysis have to be incorporated into life cycle assessment work to estimate life cycle greenhouse gas emissions from mixed lignocellulosic.

Authors: Renata Bura

Renewable Chemical Platforms and Biobased Material

BIOSURFACTANTS PRODUCED BY INDIGENOUS PSEUDOMONAS AERUGINOSA GROWN ON CANE MOLASSES

Djaber TAZDAIT, Mouloud Mammeri University of Tizi-Ouzou

A bacterium, Pseudomonas aeruginosa was previously isolated from Hassi Messaoud soil, Algeria, contaminated with crude oil and was examined for its ability to produce biosurfactants. In this study, we are trying to use sugar cane molasses as a carbon and energy source and urea and ammonium chloride as nitrogen source to produce biosurfactant. Results showed that the growth of bacteria on medium; called M1; containing glucose gives an emulsification index of 56% after 96 hours culture. Whereas, the growth of bacteria on optimized medium; called M3; containing 31 g/l cane molasses, permitted an improvement of emulsification index value. Urea seemed to be a good nitrogen source used by Pseudomonas aeruginosa strain selected. The kinetic of biosurfactant production on fermentor showed that production reached its maximum rate (E24=71%) after 26 hours culture. The thin layer chromatography analysis showed that Pseudomonas aeruginosa strain tested in this study produced glycolipids in media.

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Blueberry Leaves: A Rich Source of Useful Phytochemicals

Winny Routray,

Blueberry is a popular North American fruit. In past years a lot of studies have been conducted on these berries to observe their different compositional characteristics and health beneficial effects. However the blueberry leaves have become a subject of interest recently. The leaves of these plants are also a rich source of different phytochemicals which also include polyphenolic compounds. Application of advanced extraction technologies such as microwave assisted extraction can potentially
increase the extraction efficiency. Hence during the current study microwave assisted extraction was applied to extract different polyphenolic compounds from highbush blueberry leaves using eco-friendly solvents, which can be used in other applications.

Authors: Winny Routray and Valerie Orsat

**Use of Fe3O4 Nanoparticles-Chitosan Hydrogel for Magnetic Separation of Bovine Serum Albumin**

Hossein Salehizadeh,

Background: Magnetic nanoparticles are nanomaterials with a diameter less than 100 nm and suitable for separation of cells and intracellular components. Magnetic nanoparticles are interesting and useful due to their bioconjugating ability, catalytic surface, and their potential for a variety of biological applications. Chitosan as a biocompatible polymer has many favourable properties including: low toxicity and high biocompatibility. It has been widely used in many fields, such as biomedical applications as a drug carrier, therapy for repairing spinal damage. Additionally, magnetite based chitosan nanocomposites have been attracted much attention over the recent years and can be used for downstream bioprocessing such as purification and bioseparation of biomolecules especially proteins. Results: In this research, magnetic seeds of Fe3O4 with average size of 10 nm were synthesized by co-precipitation method at pH = 10 and under N2 protection gas. The nanoparticles stabilized with chitosan crosslinked by formaldehyde. Then, the synthesized hydrogel was used for recovery of bovine serum albumin (BSA) from solution. The separation efficiency of BSA by magnetic Fe3O4 nanoparticles-chitosan hydrogel has been investigated. The BSA recovery efficiency was improved up to 70% using magnetic Fe3O4 nanoparticles-chitosan hydrogel rather than 48% by Chitosan hydrogel. Conclusions: Briefly, Fe3O4 magnetic nanoparticles based chitosan hydrogel was prepared using a mild and rapid method. Fe3O4-chitosan network was used for separation bovine serum albumin (BSA). Fe3O4-chitosan network exhibits greater potential for proteins bioseparation of protein from large volume solution in future.

Authors: Hossein Salehizadeh, Meisam Sadeghi

**Synthetic Biology and Microbial Genomics**

**Metagenome Analysis and Biogas Production Optimization Using the Genedata Selector™ Platform**

Nadim Jessani

Biologically derived methane (biogas) is a renewable energy source that plays an increasingly important role in satisfying worldwide energy demand. For industrial biogas production, microbes can be used to break down plant material and animal waste into fermentable sugars. A primary goal for research in this field is to increase
the amount of biogas that can be extracted from biomass, which can be accomplished by optimizing the microbe populations that catalyze this process. To meet this goal, scientists need to interpret large volumes of complex data representing diverse microbial genomes and strains. Here we present Genedata Selector™, a collaborative platform to help collate, analyze, and compare genome sequence data for metagenomics analyses. In addition to acting as a central database for proprietary sequence data, the system includes bioinformatics tools for next generation sequencing analyses, taxonomic profiling of microbial communities, and de novo discovery of genes that are involved in biomass degradation. It is a scalable enterprise-ready platform which is also designed to incorporate gene expression and metabolic pathway information to identify rate-limiting steps in the biogas production process. With this aim, Genedata Selector™ is used by GOBI, an interdisciplinary research consortium with a focus on improving the overall efficiency of biogas production. We demonstrate how Selector™ can be deployed within a consortium or industrial biotechnology organization to manage, analyze, and integrate such complex and diverse data.

Authors: Thomas Hartsch1,2, Niko Bausch1, Sebastien Ribrioux1, Ludwig Macko1, Julia Retey1,2, Tim Zeppenfeld1,2, Nadim Jessani3 & Asa Oudes3 Genedata AG, Basel, Switzerland1; Genedata GmbH, Munich, Germany2, Genedata, Inc., San Francisco, USA3
Dissolution of Lignocellulosic Materials with Ionic Liquids and Its Effects On the Physical Properties of Composite Films

Alinaghi Karimi Mazraehshahi,

In this study, an imidazole-based ionic liquid (ILs), 1-butyl-3-methyl-1-imidazolium chloride ([BMIM]Cl), was used to dissolve ball-milled poplar wood (PW), chemimechanical pulp (CMP) and cotton linter (CEL). A set of comparative experiments was carried out and the physical properties of composite films made from the three different raw materials were determined, namely; optical transparency (OT), water absorption (WA), thickness swelling (TS), water vapor permeability (WVP). The overall evaluation indicates that dissolution using [BMIM]Cl was able to dissolve all lignocellulosic materials by destroying inter and intramolecular hydrogen bonds between lignocelluloses. The OT, WA and TS of regenerated CEL films were much higher than those of CMP and PW films. In addition, CEL films showed the lowest WVP compared to PW and CMP composite films. This study reveals a promising method for the preparation of biodegradable green cellulose composite films.

Authors: Ebrahim Hojjati, Ali Abdolkhani, Samaneh Karimi

Metagenome Analysis and Biogas Production Optimization Using the Genedata Selector™ Platform

Thomas Hartsch,

Biologically derived methane (biogas) is a renewable energy source that plays an increasingly important role in satisfying worldwide energy demand. For industrial biogas production, microbes can be used to break down plant material and animal waste into fermentable sugars. A primary goal for research in this field is to increase the amount of biogas that can be extracted from biomass, which can be accomplished by optimizing the microbe populations that catalyze this process. To meet this goal, scientists need to interpret large volumes of complex data representing diverse microbial genomes and strains. Here we present Genedata Selector™, a collaborative platform to help collate, analyze, and compare genome sequence data for metagenomics analyses. In addition to acting as a central database for proprietary sequence data, the system includes bioinformatics tools for next generation sequencing analyses, taxonomic profiling of microbial communities, and de novo discovery of genes that are involved in biomass degradation. It is a scalable enterprise-ready platform which is also designed to incorporate gene expression and metabolic pathway information to identify rate-limiting steps in the biogas production process. With this aim, Genedata Selector™ is used by GOBI, an interdisciplinary research consortium with a focus on improving the overall efficiency of biogas production. We demonstrate how Selector™ can be deployed within a consortium or industrial biotechnology organization to manage, analyze, and integrate such complex and diverse data.

Authors: Thomas Hartsch1,2, Niko Bausch1, Sebastien Ribrioux1, Ludwig Macko1, Julia Retey1,2, Tim Zeppenfeld1,2, Nadim Jessani3, Ben Adamczyk3 & Asa Oudes3 Genedata AG, Basel, Switzerland1; Genedata GmbH, Munich, Germany2, Genedata, Inc., San Francisco, USA3
Novel Biopolyester Synthesis From Crude Glycerol and Succinic Acid for Value Added Applications

Manju Misra, University of Guelph

Crude glycerol is the main co-product of biodiesel industry, one of the most readily available biofuel, with an approximate generation of 1 kg of crude glycerol for each 10 kg of biodiesel produced. The rapid expansion of biodiesel industry led by environmental and economic issues has increased crude glycerol production drastically and lowered its cost to approximately $0.1 USD/kg, making it an abundant and cheap feedstock for value added products. This feedstock is a complex mixture of glycerol, mono, di and triglycerides, free fatty acids, fatty acid methyl esters (FAMEs, i.e., biodiesel), soap, methanol, water and alkaline catalysts, and its composition is variable according to the feedstock and the process employed for biodiesel production, which makes its conversion to value added products challenging. One alternative for utilization of crude glycerol is the synthesis of biopolymers through esterification of glycerol with mono or diacids. In particular, reacting crude glycerol with succinic acid yields aliphatic polyesters, whose structural, chemical and thermal properties depend on the properties of crude glycerol and synthesis conditions. In our research, we have characterized two different crude glycerol samples, coming from biodiesel factories in Canada, in terms of its free glycerol content and employed a standard polycondensation procedure for producing polyesters from these samples and succinic acid. We have found that direct polycondensation of crude glycerol with succinic acid is producing polyesters with different physical, chemical and thermal properties according to heterogeneous nature of crude glycerol, as well as a liquid oil phase mainly comprised of free fatty acids and glycerides. Specifically, we observed that mixtures with stoichiometric ratio of free glycerol to succinic acid (2:3 free glycerol to succinic acid) are producing soft, hydroxyl functionalized polymers, whereas mixtures with an excess of succinic acid are producing brittle polymers with no presence of hydroxyl functionalities in it. This research is financially supported by the Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA)/U of Guelph Bioeconomy Industrial Uses Research Program and Ontario research fund, research excellence, round-4 (ORF RE04) from Ontario Ministry of Economic Development and Innovation (MEDI).

Authors: Oscar Valerio, Amar Mohanty and Manjusri Misra

Oxidation of Plant-Polysaccharide Through Engineered Oligosaccharide Oxidase

Maryam Foumani Alhaeri, University of Toronto

The production of bio-based chemicals and materials from residual plant biomass is gaining interest given the renewability of corresponding products, and the relevance of high-value bioproducts to the economic sustainability of emerging bio-refineries. One route to synthesizing high value bioproducts begins with the selective oxidation of plant polysaccharides, which can facilitate the subsequent grafting of different functional groups to polymer surfaces. In particular, enzymatic oxidation is an advantageous approach given the specificity of enzyme catalyzed reactions, and mild
reaction requirements that minimize loss in the degree of polymerization and crystallinity of the starting substrate. Accordingly, the present work describes engineering of a glucooligosaccharide oxidase from Acremonium strictum (GOOX) to increase enzyme efficiency on polymeric substrates. For this purpose, various carbohydrate binding modules (CBMs) were fused to GOOX. Selected CBMs included CBM3, CBM11, and CBM44, which demonstrate affinity towards crystalline cellulose, amorphous cellulose, and glucomannan, respectively. In addition the impact of N-terminal versus C-terminal fusions, the impact of the linker between the catalytic and the CBM modules, codon optimization of the CBM sequence for P. pastoris, and optimal conditions for expression and purification of the chimeric proteins was assessed. To date, six CBM-containing enzymes have been expressed and purified using affinity chromatography. The binding assay result shows a significant enhancement in binding of CBM chimeras to polymeric substrate compared to that of the wild-type GOOX. The activity result also shows a slight gain of activity by CBM-fused GOOXs towards regenerated amorphous cellulose and konjac glucomannan. These data, along with initial biochemical characterizations and application trials will be presented.

Authors: Maryam Foumani, Emma R. Master

Production and Immobilization of a New 1,4-β-D-Glucosidase From a Recombinant Escherichia Coli.

In Taek Hwang, Korea Research Institute of Chemical Technology

The function of β-1,4-D-glucosidase gene (PGDB4) isolated from Paenibacillus sp. strain HPL-001 (KCTC11365BP), has been cloned and expressed in Escherichia coli. The molecular weight of PGDB4 was 79 kDa upon SDS-PAGE analysis. The PGDB4 gene consists of 2,160 nucleotides, encoding a polypeptide of 719 amino acid residues. PGDB4 exhibited 68% identity (DNA) to the previously reported glycoside hydrolase family 3 domain protein (GB: ZP_07902992.1) belonging to the glyco-hydro-3 superfamily, glyco-hydro-3-C superfamily and BglX multi-domain. The purified enzyme showed maximum specific activity of 57 and 15 units to p-nitrophenyl-β-D-glucopyranoside (pNPG) and p-nitrophenyl(N)-β-D-cellobioside, respectively, at a temperature of 40 °C and pH 6.0. The Km value of 0.893 mg pNPG/ml was estimated against pNPG by means of Hanes-Woolf equation. However, the enzyme was not effective for the substrates, such as p-N-a-D-glucopyranoside, p-N-β-D-xylopyranoside, p-N-a-L-arabinopyranoside, p-N-β-L-arabinopyranoside, p-N-β-D-mannopyranoside, and p-N-β-D-galactopyranoside. Most common ions, such as Na+, Li+, K+, NH4+, Ca2+, Mg2+, Mn2+, Zn2+, and Fe3+, did not affect the enzyme activity. In the presence of ethylenediaminetetraacetic acid, 2-mercaptoethanol, dithiolthreitol and phenylmethanesulphonylfluoride, the activity was also not changed. However, it is interesting to note that the decrease in the activity was observed by 40%, when in the presence of 1 mM of Cu2+ ion. The purified enzyme hydrolysis of the substrate cellobiose to glucose was confirmed by HPLC analysis. The enzyme was then immobilized on aldehyde-, amine-, SH-, epoxy-functionalized mesostructured cellular foam with mesopores. Among them, immobilized enzyme on aldehyde-functionalized MCF retained about 90% of the initial enzyme activity even after 10 consecutive recycles.
Authors: Dal Rye Kim, Ha Young Song, Jeong Ho Ko, Hee Kyung Lim, No-Joong Park, Young Kyu Hwang, Jin-Soo Hwang, Kee-In Lee, and In-Taek Hwang

Protein-Surfactant Synergists: From Basic Science to Industrial Applications

Michael Goldfeld, Advanced BioCatalytics Corporation

The core of the ABC’s technology is a newly discovered effect of synergistic enhancement of the efficiency of synthetic, or bio-derived surfactants by forming tight complexes with low molecular weight exo-proteins from a modified fermentation of baker’s yeast. Using a broad arsenal of surface science techniques, it has been shown that these protein-surfactant complexes (PSC) form aqueous solutions displaying significantly lower surface tension interfacial oil/water tension (IFT), and critical micelle concentration as compared to the same surfactants taken alone, in the absence of proteins. Record low IFT (ca. 10^-4 mN/m) were achieved with some formulations designed for the enhanced oil recovery. PSCs also provide better wetting, penetration and uptake by hydrophobic surfaces, as documented using contact angle measurements. PSCs are remarkably stable in a broad range of pH and temperatures, and are compatible with certain oxidants, such as hydrogen peroxide, bleach and iodine. PSCs were found to activate lipase, and such an activation effect can possibly be applicable to other essentially interfacial and industrially important enzymes, such as cellulase, lignase and alike. Besides these immediate functionalities, PSCs were found to produce a longer-term effect of activating the bio-oxidation of organic contaminants in water, waste water, and soil, contaminated by petroleum oil and other organic pollutants. In the aerobic waste water treatment (WWT) process, enhanced bio-oxidation occurs with a concurrent reduction in the accumulation of biomass. Through field observations and bench studies, it has been proven that the activation is due to an uncoupling of the electron transfer from energy consuming biosynthetic processes necessary for bacterial proliferation. Through the tests with model artificial membranes, it was shown that the uncoupling is related to a PSC-facilitated leak of protons across bacterial membrane, in accordance with chemo-osmotic model of oxidative phosphorylation. Formulations have been developed on these grounds, for applications in industrial cleaning, petroleum oil production, WWT, remediation of natural waters in the aftermath or petroleum oil spills through oil herding, dispersion and bioremediation, removal and prevention of biofilms, odor control and alike. Additional advantage of PSC-based products is their ability to convert grease such as in sewer pipes, into surface active, soap-like materials by their combined action with naturally present microflora. This effect essentially converts sewer lines into a pre-processing bioreactor, improving the quality of waste water released into a municipal WWT plant. Applications of PSCs as wetting-enhancement agents and bioactive adjuvants in agricultural applications are under development, as are fire-fighting formulations and personal care applications. Only non-toxic synthetic surfactants, accepted in food industry, according to FDA regulations, are used in the ABC’s products which received certification from the US National Sanitary Foundation (for potable water), and International Maritime Organization (for chemical cargo tank cleaning), and are included into the US EPA National Contingency Plan list (as dispersant and surface washing agents). ABC’s intellectual property includes 6 patents issued and 7 more pending.
Use of yeast extract to promote growth and spore formation by Bacillus thuringiensis and other related species

Alain Sourabie, PhD, Bio Springer

Use of yeast extract to promote growth and spore formation by Bacillus thuringiensis and other related species Bacillus thuringiensis (Bt) is a gram-positive, rod-shaped and spore-forming, bacteria naturally found in various environments such as soil, water and grain dust. This bacterium is the most common representative of a group of bacteria (most from the genus Bacillus) that produce bioinsecticides. Bt produces a natural insecticide which is a diamond-shaped crystal originating from its crystal proteins (Cry proteins). This microorganism has been used for decades to control crop-eating insects and worm parasites of both humans and animals. Bt has been recommended by the World Health Organization to biologically destruct the disease-bearing insects. The use of Bt increased when insects became gradually more resistant to the synthetic insecticides and due to the movement towards an environmentally conscious culture. It is of great importance nowadays to think about the danger of chemicals in products and how they affect the environment. Bt based and the other biopesticides have the great advantage to be organic, to exhibit a wide spectrum and to target only specific insects without persisting in the environment. There are currently hundreds of strains of Bt and other biopesticides producing micro-organisms such us Bacillus firmus, Bacillus pumilus or Pasteuria penetrans. Although bacteria of the Bacillus group have a recognized potential for use as ecological control agents against phytopathogenic organisms, their utilization in commercial agriculture depends on the availability of reliable methods for their large-scale production. Biopesticides producing micro-organisms can be cultivated in liquid, solid and semi-solid media. Most of their commercial production is currently performed through batch fermentation with a mixture of complex media containing a carbohydrate source, a nitrogen source (yeast extract or soy), or diverse industrial by-products such as corn steep liquor. However, yeast extract contains the optimal mix of nitrogen and growth factors to support bacterial growth. In an attempt to develop cost-effective processes for bioinsecticide production by B. thuringiensis, many cheap protein sources were first considered. However, many studies concluded that yeast extract was more suitable for promoting growth and spore formation in Bt and related species. In particular, free amino acids, small peptides and trace elements contained in yeast extract were shown to maximize both spore and toxin yield and productivity. When fed to Bt and other related species growing cells, yeast extracts enhanced bacterial biomass during the first stage and subsequently spores and crystal protein yield during the second phase. This presentation describes the varying range of yeast extracts used to produce bacteria used in crop protection.
**Value-Added Natural Monomers and Polymers from Renewable Sources**

Zhi Yuan Wang, Carleton University

At present, a vast majority of synthetic polymers are based on fossil resources, particularly petrol and natural gas, which are likely to start dwindling within one or two generations. Like minerals, petrochemicals are non-renewable resources without a sustainable yield. In contrast, the natural products and agriculture-based feedstock are sustainable and renewable sources and are being intensively explored for many applications. Natural polymers such as cellulose, chitin, and starch are either used directly or modified for use. Natural monomers are also available in nature and a number of natural monomers have been as a source for various resins, such as terpenes and rosin. The second-generation of natural monomers are made available from natural products, such as furan derivatives and lactic acid from sugars and as well their natural oligomers and polymers, polyols from vegetable oils, succinic acid and butanediol from agriculture products. The third-generation of natural monomers refers to the derivatives from the natural products through a few steps of chemical modification. These monomers and the corresponding synthetic polymers will have the value-added properties for niche applications. We are interested in exploring the chemistry, process and applications of the second- and third-generations of monomer derivatives from natural renewable sources. We will represent our recent results and future R&D direction.

Authors: Di Zhang, Jane Gao, Zhi Yuan Wang

**Wood Products Biotechnology Developed at FPInnovations**

Dian-Qing Yang, FP Innovations

Wood is a renewable resource and plays an important role in the world economy. Biotechnology brings together some of fastest-growing technologies, and it offers tremendous economic opportunities on transformative forest products, especially for improving the quality and durability of traditional wood products and developing new wood/fiber materials. FPInnovations has made big achievement on application of wood products biotechnology on the development of new wood/fiber bio-products and bio-processing during the past years; such as on development of bio-control products against wood infection and deformation, bio-incising to harden wood, bio-conversion of lignin and bio-modification of wood strands to reduce panel resin use. This presentation will give a brief review on some of such bio-products and bio-processes developed at FPInnovations and demonstrate some potential trends for future research on application of wood products biotechnology in wood/fiber industry.

Authors: Dian-Qing Yang

**Progress on Biochemical Platform**

Brandon & Frank Emme & Haagensen, Novozymes
This panel will provide the audience with updates on the latest progress on technical and commercial aspects for production and conversion of a bio-based sugars within the Biochemical Platform. The panel will include status updates from three key technology providers that are all focused on bringing biochemical processes to the center stage of renewable fuels, chemicals and materials. The panel will feature presentations by Chemtex, Genomatica, and Novozymes. These companies will provide updates on current status of three essential aspects of biobased chemicals production; including processes, enzymes, and microorganisms. Flexibility in Feedstock and in Sugar Streams for Optimal Production of Biochemicals Speaker: Kevin Gray, Chemtex Every biochemical production process has a different ‘sweet spot’ for the composition of the sugar streams that will make for optimal efficiency – not just in production, but to reduce subsequent distillation and purification needs. Likewise, the most economic feedstock near different production plants around the world will vary. Kevin will discuss how Beta Renewables’ PROESA process technology can act as a platform to support the flexibility needed for multiple bioproducts. The technology roadmap for commercializing biomass to chemicals Speaker: Nelson Barton, Genomatica Lots of companies have slides that make it look easy to go from biomass to chemicals – but the devil is in the details. Nelson will highlight the differences in making biomass cost-effective as compared to using conventional sugars and the types of fermentation and process changes needed to make integrated processes really work. Techno-economic analysis of enzymatic biomass to sugar platforms for biochemicals Speaker: Brandon Emme, Novozymes With commercialization of lignocellulosic sugars to ethanol on the near horizon, the advanced biotech industry has increased their call for biomass sugars for biochemical production. Brandon will provide an update on enzymatic biomass conversion for cellulosic sugars and will further introduce a flexible biomass-to-sugar cost model as a powerful tool for process and technology developers.

Authors: Kevin Gray, Chemtex Nelson Barton, Genomatica Brandon Emme, Novozymes

Specialty Chemicals, Pharma Intermediates, Food Ingredients

Antitumor Activity of Chemically Prepared Shrimp Chitin, Chitosan and Low Molecular Weight Chitin

Rym SALAH-TAZDAIT, Mouloud Mammeri University of Tizi-Ouzou

Cytotoxic drugs continue to play a major role in cancer therapy. However, cytotoxic drugs produce side effects, especially the destruction of lymphoid and bone marrow cells. Therefore, strategic improvements in cancer therapy are needed to ameliorate efficiency while decreasing side effects. Chitin is a linear polysaccharide joined by β-(1,4)-linked N-acetylglicosamine (GlcNAc) units. It is the second most abundant natural polymer after cellulose. Their unique properties, biodegradability, biocompatibility and non-toxicity, make them useful for a wide range of applications. Although chitin has very strong functional properties in many areas, the water-insoluble property of a-chitin is disadvantageous for its wide application. In the
research field of chitin, functional property has been developed for pharmaceutical and new drug candidate. In the present study, chitin was extracted from shrimp shells obtained from a seafood restaurant. It was confirmed that all shells were from a single species of shrimp Parapenaeus longirostris (Lucas, 1846). The obtained chitin was deacetylated to prepare chitosan. In other hand, chitin was also depolymerized to prepare low molecular weight chitin. Then, chitin, chitosan and low molecular weight chitin were characterized by FT-IR. Further, anticancer activities of chitin, chitosan and low molecular weight chitin were evaluated using a human tumor cell line, THP-1. The cytotoxic effects of chitin, chitosan and low molecular weight chitin were also evaluated using a normal human fetal lung fibroblastic cell line, MRC-5. The results indicated that chitin, chitosan and low molecular weight chitin exhibited no cytotoxic effects at concentrations inferior or equal to 2000µg/ml. The influence of chitin, chitosan and low molecular weight chitin on the growth of THP-1 cancer cell line was determined using noncytotoxic concentrations (= 2000µg/ml) on normal human lung fibroblasts, MRC-5. The antitumor effects of chitin, chitosan and low molecular weight chitin were established. In fact, the results indicated that low molecular weight chitin have the potential to suppress 100% of the growth of THP-1 tumor cells at concentrations equal or superior to 250µg/ml. However, chitin and chitosan have the potential to suppress 100% of the growth of THP-1 tumor cells at concentrations equal or superior to 1500µg/ml. Thus, chitin, chitosan and low molecular weight chitin has promising roles in natural cancer prevention and treatment. The higher order structures of active chitin, chitosan and low molecular weight chitin and the details of its mechanisms of action in the host are now under investigation, especially to clarify the entity intervening between polysaccharides and tumors.

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Bioconversion of Curcumin, a Major Component of Turmeric

Azam Hassaninasab, Graduate School of Life and Environmental Sciences, The University of Tsukuba, Japan

Curcumin is a chemical of the polyphenol family derived from the rhizome of spice turmeric (Curcuma longa Linn) that belongs to the ginger (Zingiberaceae) family. Curcumin is a super compound with the far-reaching medical properties including antitumor, anticancer, anti-inflammatory, antioxidant, and analgesic uses. Despite the impressive applications of curcumin particularly in medicine prospective, it exhibits poor bioavailability both in vivo and in vitro. Therefore there is a need to investigate modified curcumin or curcumin-derived compounds for improved the
pharmacokinetics properties of this nature’s miracle substance. Microorganisms with the curcumin-degrading potency have been isolated from different sources. To isolate the microorganisms with curcumin-converting ability, samples were inoculated in a test tube containing liquid media with the addition of curcumin. Cultivation was performed with shaking at 28°C for 1 week. Once a week, 1 ml of the culture was inoculated into 10 ml of fresh medium. After 4 weeks of acclimatization culture, the culture solution was spread on agar plates and growing microorganisms were isolated. They were liked to be different according to their colony morphology. These microorganisms were identified based on biochemical tests and 16srRNA sequence analysis. The ability of curcumin degradation by the isolated microorganisms was investigated by means of resting cell and cell-free extract reactions with curcumin as the substrate. The curcumin degradation was measured by HPLC equipped with a reverse-phase column. The peak related to curcumin decreased and new peaks for feasible products derived from curcumin degradation were detected at different wavelengths. Moreover, the enzyme involved in curcumin degradation was purified.

Authors:

**Enzymatic Technology for Low-Cost Mitigation of CO2 Emissions**

Jonathan Carley, CO2 Soulutions

Carbon Capture, Sequestration and Utilization (CCSU) is widely viewed as an essential tool for the large-scale reduction of carbon dioxide (CO2) emissions as part of overall global efforts to mitigate climate change. However, current post-combustion CO2 capture approaches using solvents are largely uneconomic for commercial deployment. As a solution to this cost challenge, CO2 Solution of Québec City, Québec, Canada is commercializing a patented biotechnological capture approach using the powerful enzyme catalyst carbonic anhydrase (CA). In the process, CA is employed with certain aqueous amines, carbonates and amino acids which have low energies of regeneration, but which are kinetically limited. CA dramatically accelerates the rate of capture of CO2 in these solvents, increasing their kinetics to the level of a conventional monoethanolamine (MEA) process. The result is lower energy consumption combined with suitable absorber equipment sizing and the industrial enablement of more environmentally benign solvents. Results to date have demonstrated that the technology has strong potential as a low-cost carbon capture approach which can be retrofitted into existing gas scrubbing process technology. The presentation will discuss the basis of the technology, development status, pilot testing results achieved for coal-fired power generation, and the Canadian application of the process to reduce the environmental footprint of Alberta oil sands production.

Authors: Azam Hassaninasab, Yoshiteru Hashimoto and Michihiko Kobayashi
A Strain Development and Optimization Platform Designed to Leverage High-Throughput Genotype and Phenotype Information

Asa Oudes

The engineering of microbial strains to efficiently produce commercially useful compounds is a key objective within the industrial biotechnology community. One success in the field has been the development Corynebacteria glutamicum strains, which are estimated to generate a staggering 900000 tons of L-Lysine annually. To optimize the production capability of such workhorse microbes, researchers collect genotype data and phenotypic characterizations for a large number of strains. For many, managing and exploiting this information is a challenge, but is absolutely essential for reaching their goal. Here, we present Genedata Selector™, a platform tailored for the industrial biotechnology community, which enables researchers to manage and analyze all of this data for the purpose of strain optimization. The platform is an enterprise solution which integrates and organizes data from various technologies and sources, and is highly scalable for a large number of genomes. We demonstrate how this system was used to evaluate L-Lysine production for thousands of C. glutamicum strains by making comparisons with key phenotype information: production yield, strain stability, and growth rate on various media. In addition, complete genome sequences for 80 C. glutamicum strains were loaded into the Selector™ platform, and analyses were performed to identify and characterize mutations that are optimal for L-Lysine production. The use case presented here showcases the power of this system for both management and analysis of data across a large number of strains. The Genedata Selector™ platform was developed in part for the Integrated Phenotype-Genotype (IPG) project, a research consortium including Evonik Degussa, GATC, and Bielefeld University.

Authors: Isis Trenchard and Christina D. Smolke

Activation of Secondary Metabolic Pathways by Chitin in Streptomyces Coelicolor A3(2) Grown in Soil

Behnam Nazari, National Institute for Agro-Environmental Sciences-Japan

Streptomycetes have an important ecological role in biodegradation of insoluble biomaterials using their broad range of extracellular hydrolytic enzymes such as amylases, cellulases, and chitinases. According to the environmental conditions, these bacteria exhibit morphological differentiation accompanied by the formation of spores and the production of a wide range of valuable bioactive secondary metabolites such as antibiotics. Recent studies have been shown that these bacteria harbor a large reservoir of gene clusters, which have the potential to produce novel secondary metabolites. Streptomycetes have traditionally been isolated from soil, which contains of a highly variable physical and chemical matrix and thousands of different strains of bacteria. Our method based on cultivation of streptomycetes in soil containing culture provides an alternative approach to investigate the activation of these gene clusters. In this study, genome-wide microarray analysis was performed using RNA extracted from soil cultures of Streptomyces coelicolor A3(2) with or without chitin, the most abundant insoluble nitrogen-containing polysaccharide on earth. The vast majority of genes for the chitin and amino sugar
metabolism as well as for carbon, nitrogen, and sulfur metabolism were differentially expressed in response to chitin. Moreover, the expression of eight gene clusters for secondary metabolites was also significantly up-regulated in chitin-amended soil. In particular, under existence of chitin, prominent upregulation of the cryptic type I polyketide synthase gene cluster and a putative nonribosomal peptide synthetase (NRPS) gene cluster was observed in S. coelicolor A3(2) grown in soil. This study provides new information about the relationship between chitin and antibiotic production in streptomycetes grown in soil and new conditions for activation of cryptic gene clusters in streptomycetes.

Authors:

**Bacterial RNAs as Determinants of Human Disease.**

Amir Shmaryahu, Fundación Ciencia para la Vida

RNA silencing in animal cells is carried out by micro RNAs (miRNAs) and small interfering RNAs (siRNAs) of around 22 nucleotides, which specifically hybridize with target RNAs to inhibit their expression. Perfect sequence complementarity between siRNAs and their target sequences results in the cleavage of target mRNAs by the RNA-induced silencing complex (RISC), whereas imperfect matches, as typically observed between miRNAs and their targets, result in repression of translation. Recent studies now show that vertebrate viruses encode products that interfere with the RNA silencing machinery, suggesting that RNA silencing may indeed be important for antiviral responses in vertebrates. RNA silencing in response to virus infection could be due to miRNAs encoded by either the virus or the host. We have developed a bioinformatic pipeline for possible miRNAs discovery generated from bacterial RNAs that may have potential to regulate gene expression of the host human cell, in case of infection. Given their versatile roles in transcriptional and translational control of gene expression and in quality control of macromolecular products, it is suggested that the study of these predicted miRNAs will yield important clues in the future as to how the host human fine tune cell processes possible human diseases like as cancer in response to changing bacterial environments. Acknowledgements: Work supported by Conicyt Basal CCTE PFB-16 and Fondecyt 3110015.

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**Determination of Survival and Resistance to Acidity of Infant and Calves Faecal Bifidobacteria**

asmaa abdelmalek,

Bifidobacteria are an important part of the normal faecal flora and may provide health-promoting benefits to the host. High bifidobacterial counts are especially important in newborns early in infancy (human and animal). The aim of this study is to determine the level of bifidobacteria in infant faeces and calf faeces, and compare
the resistance of common Bifidobacterium spp. to acidity. Forty samples from twenty breast-fed infants in the age between 5 to 180 days and twenty five samples from ten calves between 5 to 60 days were investigated. Bifidobacteria and other bacterial groups were determined by cultivation. Faecal samples were examined for the activity of fructoso-6-phosphate phosphoketolase (F6PPK) and for other enzymatic reactions using the API-ZYM kit. Nine infants had high numbers of bifidobacteria (usually higher than 9 log CFU/g) in their faeces. Five infants did not contain detectable amounts of bifidobacteria in their faecal samples. The remaining six individuals had low counts of bifidobacteria (3–6 log CFU/g). Most negative infants possessed major amounts of clostridia in their faecal flora. All calves contain high numbers of Bifidobacteria in their faecal samples. Detection of F6PPK, agalactosidase and a-glucosidase activities could routinely be used for the rapid and simple detection of bifidobacteria in all faecal samples. Acid tolerance was determined by introducing Bifidobacterium to pH-adjusted skimmed milk and enumerating during storage at 4_C. The viability of the organism decreased during storage. Bifidobacterium spp. isolated from calf faeces showed superior survival abilities compared with their isolates from infant faeces.

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Xylanase Super-Producer: Genome of a Compost-Loving South African Soil Thermophilic Fungus, Thermomyces Lanuginosus SSBP

Nokuthula Peace Mchunu, Durban Univeristy of Techno

Thermomyces lanuginosus is a thermophilic fungus that can be found from most composting environments. Many strains of T. lanuginosus have been reported to be hyper producers of xylanase and other enzymes that have industrial application. Here we report a high quality draft genome sequence of T. lanuginosus. The genome approximate size is 23.3 Mb contained in 30 scaffolds with 5,105 candidates as protein-coding genes. The total number of carbohydrate-active enzyme (CAZy) genes for biomass degrading and related proteins identified in T. lanuginosus genome was 224. This was relatively fewer compared to other filamentous fungi with most species containing over 200 CAZy family related proteins. We identified 8 candidates that could be cellulases or have cellulase activity. This is surprising because this fungus has been generally described as a non-cellulolytic organism. Only one gene was identified as encoding for a β-xylanase enzyme in spite of this strain being a xylanase-hyperproducer. Numerous genes encoding for ubiquinones/ menaquinones and phosphoethanolamine methyltransferases were identified which may promote survival of T. lanuginosus in its composting environment by providing efficient energy production and balanced membrane fluidity. Interestingly 73 proteins were identified that have biological function related to adaptation to high temperature. Further analysis of these genes revealed that T. lanuginosus contained almost double the number of heat shock proteins compared to other filamentous fungi species. The genome sequencing of this fungus is a major step towards understanding the biological interactions in thermophilic fungi.
Technical Presentations

Molecular Calculation of Picloram Interaction with Anti-Picloram Binding Fragments

Mohidus Samad Khan,

Picloram (4-amino-3,5,6-trichloropyridine-2-carboxylic acid), a herbicide used to kill unwanted plants, enters the fresh water supply and cause Health and Environmental problems. Picloram has a very long life-time and is considered mildly toxic to birds and mammals and moderately toxic to aquatic species. The Environmental Working Group (EWG) reports that people are consuming water, contaminated with Picloram and other Herbicides, in Western Countries. Paper based Sensors can be used to detect, and filters to remove Picloram from Contaminated Water. Researchers have developed a filtration system by fusing an Anti-Picloram Antibody Fragment to Pulp Fibre to remove Picloram. In this Poster the Picloram interaction with the specific antibody is Modelled using 3D Homology Model and Docking followed by Quantum Chemistry. A combination of Molecular Mechanics and Quantum Mechanics is used to model Antigen Binding Sites and Binding Locations. Different geometrical properties and electronic properties of modelled molecules are calculated and analyzed to elucidate the binding characteristics of Picloram to the 3D structures of Picloram Antibody at different pH and solvation conditions. These calculations will illuminate the morphology of Antigen-Antibody interactions on paper and will help to develop bioactive filter papers and paper sensors to assist in health and environmental problems.

Authors: ABDELMALEK, ASMAA . DALI YAHIA, RADIA and BENSOLTANE, AHMED

Nanocellulose Conductive Films for Flexible Electronic Applications

Manju Misra,

In recent years, there has been an increasing interest in the preparation of thin conductive films due to their lightweight and flexibility for applications in a wide range of electronic devices, packages, and connections. Current environmental concerns require industries, particularly the electronics industry, to find alternative methods and materials for the fabrication of various essential components. In addition, for commercial viability, the alternative materials must be inexpensive and the methods of fabrication must be simple and cost effective. In our work, nanocellulose, a renewable and green material has been chosen as the base material for the films due to its strength and availability. In order to fabricate conductive films, the cellulose nanofibres were mixed with nanosized carbon-black particles. Both components were dispersed in water and made into films via a film casting method. For best results, the carbon particles should not just be dispersed but coated onto the cellulose fibres. The high conductive films and pure cellulose films can be fabricated layer by layer to generate a variable sensitive capacitor which can harvest energy from the environment. The morphology, conductivity, and durability
of the films is being optimized and tested using scanning electron microscopy (SEM), transmission electron microscopy (TEM), dynamic mechanical analysis (DMA), thermogravimetric analysis (TGA). And four probe measurement is used to measure the film conductivity with Keithley 2701 multimeter. This research is financially supported by the Ontario Ministry of Agriculture, Food, and Rural Affairs (OMAFRA)-University of Guelph- (Bioeconomy for Industrial Uses Program); OMAFRA-New Directions Research Program 2009; the Ontario Research Fund (ORF) Research Excellence (RE) Round-4 from the Ontario Ministry of Economic Development and Innovations (MEDI).

Authors:
A Bench Top Photobioreactor for Scale Down Studies of Photosynthetic Microorganisms

Stephen Hawley,

Problem Photosynthetic biocatalysts have great potential in the bioenergy field but face novel challenges in developing commercial processes. Data is needed on how the reactor environment changes catalyst performance early in the design process to inform both catalyst and reactor design. R&D costs and time to market are both increased without this data. The reactor design presented is well suited to these experiments. Photosynthetic Biocatalysts Industrial agriculture is a highly tuned system for converting solar energy into commodities. The system produces impressive quantities of material, but plant photosynthesis has low yields relative to photosynthetic microorganisms. Furthermore, process intensification at the photosynthesis stage reduces costs by eliminating transportation and material handling costs from biomass. Photobioreactor Design A design for a bench scale photobioreactor is presented. This design enables low cost pre-pilot testing of photosynthetic biocatalysts. Notable features include configurable mixing regimes, thermal sterilizability, and internal illumination. Mixing regimes Productivity limitations have been observed in large scale reactors due to limited availability of carbon dioxide in solution. Active mixing is essential for commercial operation. Biocatalysts need to be tested under these conditions to find the optimal balance between mixing and cell death to maximize productivity. The reactor can easily be configured for three different mixing regimes: stirred tank, bubble column, and internal loop airlift. Thermally sterilizable Sterilization of culture vessels is an essential step taken to ensure the integrity of the results of the experiment. Thermal sterilization is inexpensive and routinely performed in biotechnology laboratories. Designing the reactor to be compatible with these routine procedures reduces R&D costs. Internal illumination Internally illuminated photobioreactors have been investigated for their ability to fill a volume with light while decoupling the illuminated surface area from the vessel surface area. As a result, they can achieve higher volumes of illuminated culture in the same footprint. At the bench scale, the principal benefit of internal illumination is to simplify the measurement of light absorbed by the culture and the calculation of photosynthetic yield of product. It does this by eliminating the need for transparent materials for the reactor vessel, preventing light directed into the reactor from escaping through the vessel walls. This also makes the vessel easier to fabricate and more durable. A light pipe is used to distribute light within the reactor, rather than using an internal source such as a bulb or LED. This simplifies design of the vessel and has two specific advantages: 1) it is heat stable (enabling thermally sterilizing the reactor), and 2) it requires only one point through which light can enter or exit the reactor (simplifying measurements for photosynthetic yield calculations).

Authors: Stephen D. Hawley

3D Molecular Modelling of Antigen-Antibody Interactions

Mohidus Khan, McGill University
Antibodies have a wide range of applications that include immunochemistry, medical diagnostics, antibody based therapeutics, antibody based electrochemical sensors, paper filters and sensors to detect and deactivate pathogens and unwanted chemicals in water and bio-fluids. Antibody active paper filters and sensors that detect blood groups, and the presence of herbicides and pesticides in water have been reported. However, the biotechnology industry has a limited understanding of the antibody-antigen interactions at different pH conditions on cellulosic materials which are important in medicine and pharmaceutical industries. Computational methods offer access to molecular details that are inaccessible by experiment and predict chemical properties cheaply and quickly in silico, unlike expensive, difficult and time-consuming experiments. X-ray crystallography and nuclear magnetic resonance are robust experimental techniques to determine Antibody structures, but are laborious, expensive and time consuming. High-resolution 3D homology modelling is the state of the art in structure-based protein engineering applications, especially when a crystallographic or nuclear magnetic resonance (NMR) spectroscopic structure is unavailable. Antibody 3D homology modelling and antigen-antibody docking calculation followed by Quantum Mechanical calculation can be highly useful to understand antibody structure and binding mechanism. Picloram (4-Amino-4 3,5,6-trichloropyridine-2-carboxylic Acid) and anti-Picloram binding fragments (Fab) were studied in this way. The 3D Molecular Modelling of Picloram and anti-Picloram Fab interactions allowed the structural, electronic properties and energies of Picloram interaction with possible binding sites at different pH to be calculated. The electronic properties and energies show that strong interaction occurs at pH 7 and not when acidic. This information can be useful in developing and regenerating antibody based sensors and filtration systems. Besides antibody based filtration technique, molecular study of antibody-antigen interactions at different pH can be useful in detoxification of drug or toxic chemicals from the human body.

Authors: Mohidus Samad Khan, M.A. Whitehead, and Theo G.M. van de Ven

**Psychrophilic Anaerobic Digestion of Lignocellulosic Biomass: Characterization Study**

Noori Saady, Dairy and Swine Research and Development Centre

**Introduction**

Psychrophilic anaerobic digestion (PAD) substantially reduces the energy input required for heating the bioreactor. PAD could offer a solution to recover green energy from farm wastes such as manure in the form of biogas in cold climate regions. Dairy manure contains undigested lignocellulosic biomass plus bedding materials such as straw. Manure fibers contain (as % of dry matter) cellulose (25%), hemicellulose (12-20%), and lignin (15%). The objective of this study is to assess the extent of degradation of cellulose, hemicellulose (xylan), cellulose and xylan mixture, wheat straw, and cow feces in batch PAD reactors.

**Materials and methods**

Cellulose, hemicellulose (xylan), cellulose and xylan mixture, wheat straw, and cow feces have been anaerobically digested in duplicate 1 L batch bioreactors under psychrophilic conditions (20 °C). The batch reactors have been intermittently mixed for 1 min once a week just before mixed liquor sampling. Biogas production has been
monitored daily while biogas composition has been analyzed weekly using a gas chromatograph (GC). Mixed liquor samples have been analyzed weekly for total chemical oxygen demand (TCOD), volatile fatty acids, pH, alkalinity, and volatile and total solids (VS and TS, respectively) according to the standard methods. VFAs have been measured using a liquid chromatograph. All chemicals (substrates and standards) have been purchased from Sigma Aldrich. All batch reactors received an organic loading rate of 3 g TCOD L⁻¹. All the results are normalized to the control samples (only culture with no substrate).

The anaerobic culture has been obtained from anaerobic bioreactor (TS = 27%) treating dairy manure and wheat straw. A dilute suspended culture (TS = 0.96%) has been prepared by liquefying and sieving (250 μm) the culture to remove the fibers and solid material.

**Results and Discussion**

Within 118 days of incubation the specific methane yields (SMY; expressed in mL CH₄ kg⁻¹ VS) were as follows: 376.4±14.0 (cellulose), 353.0±36.5 (cellulose:xylan mixture), 349.6±1.87 (xylan), 278.1±16.3 (cow feces), and 273.3±18.4 (wheat straw). The COD recovery percents were: cellulose (81.5±3.0%) = xylan (77.8±0.4%) = cellulose:xylan mixture (77.8±7.2%) > cow feces (62±2.4%) > wheat straw (54±2.2%). The SMYs from xylan, cellulose, and cellulose:xylan mixture were statistically the same. The COD recovery from xylan, cellulose, and cellulose:xylan mixture were statistically the same, but they were greater and significantly different from that obtained from cow feces and wheat straw. COD recovery from wheat straw was lower and significantly different from that of other substrates tested.

**Implication**

The results showed that the methane yields and production rates from pure substrates (cellulose and xylan or their mixture) were higher than that from complex substrates (cow feces and wheat straw); therefore, pretreatments which delignify the complex substrates and release the cellulose and hemicellulose components would increase the yield and the rate of methane production at psychrophilic conditions (20 °C) given that a well-adapted inoculum is used.

Authors: Noori M. Cata Saady and Daniel I. Masse

**Bioplastic Production From Pulp and Paper Secondary Sludge**

Lalit Kumar,

Authors: