Production of Biodiesel from Algae Oil by Supercritical Transesterication Using Continuous Reactor
Aline Scotelari de Sousa, University of Campinas

Biodiesel is advised for use as an alternative fuel for conventional petroleum-based diesel because it is a renewable, with an environmentally friendly emission profile and is readily biodegradable. The optimum conditions of supercritical transesterification process for biodiesel production from algae oil were studied. To obtain a high quality biodiesel fuel that comply the specification of standard method (ASTM D 6751 & EN 14214), some important variables such as temperature, oil to ethanol molar ratio, time reaction were selected. The highest approximately 95% biodiesel yield acquired under optimum conditions of 1:25 (v/v) oil-to-methanol ratio at 250 ºC reaction temperature and 10 minutes of reaction time, these operation conditions were based on the experimental results and response surface methodology (RSM) analysis. Result showed that the biodiesel production from algae oil presented high yields. The research demonstrated that biodiesel obtained under optimum conditions from algae oi was of good quality and could be used as a diesel fuel which considered as renewable energy and the recovering ethanol environmental process.

Product Optimization and Characterization of New DNase Enzyme from Soil Bacteria
Amin Alborzian, Imam Khomeini International University

Bacterial source materials are so important and interestingly cheap and accessible. Soil bacteria are very different. We identify many genus of bacteria in our work. The physiological tests that used were oxidize, Geram and etc. For better identification the molecular tests were done. Five of genus showed DNase enzyme. The production condition was optimized for maximum amount, pH and temperature were the parameters that optimized for that aim. After optimization of amounts, the enzymes were sequenced. We compared the sequenced proteins and achieve the phylogenetic tree.

Assessment of the Potential Suitability of the Enzymes Produced by an Environmental Isolate for the Pretreatment of Lignocellulose for Bioethanol Production
Angela Boyce, University of Limerick

Assessment of the potential suitability of the enzymes produced by an environmental isolate for the pretreatment of lignocellulose for bioethanol production (Angela Boyce and Gary Walsh) The development of biofuels has gained increasing interest in recent years due to an urgent global need to reduce greenhouse gas emissions from the transportation sector and reduce dependency on fossil fuels. Second-generation biofuels, produced from lignocellulosic biomass feedstocks (for example, crop residues, agricultural processing byproducts, forest and sawmill residues and energy crops) can reduce greenhouse gas emissions without competing with food production. Second-generation bioethanol can be produced from lignocellulosic feedstocks by a biochemical process involving enzymatic hydrolysis of the lignocellulosic material with subsequent fermentation of the resulting sugars to ethanol. Prior to enzymatic hydrolysis, a pretreatment step is undertaken to modify the recalcitrant structure of lignocellulose and improve enzyme accessibility. One of the most commonly used pre-treatment methods is dilute-acid pre-treatment which involves treatment of the lignocellulosic material with acid (typically 0.1-5%) at temperatures of 140-220°C. The present study focuses on the potential application of enzymes in this pre-treatment step. The incorporation of appropriate thermoacidophilic lignocellulosic enzymes in dilute-acid pre-treatment could potentially reduce the acid concentration and temperature required for pre-treatment thereby reducing the severity of the process. The usefulness of currently available commercial enzymes for this application is likely to be limited due to the extreme pH and temperature conditions involved. Likely advantages of an enzymatic/dilute-acid pre-treatment

approach include reduced production of degradation products which represent a loss of sugar and have a negative effect on subsequent process steps, reduced energy and acid requirements, reduced requirements for neutralisation prior to the hydrolysis step, reduced corrosion of process equipment and improved safety. Microbial screening studies resulted in the identification of an Aspergillus strain producing enzymes of potential interest for this application. During solid-state fermentation on a wheat bran based media the strain produced endo-1,4-ß-glucanase, endo-1,4-ß-xylanase, ß-D-glucosidase, 1,4-ß-D-xylosidase, a-L-arabinofuranosidase and acetyl xylan esterase activities, all of which were active at 70°C and pH 3. Maximum cellulase activity was observed at 50-70°C and 63%, 39% and 20% of original cellulase activity was retained after heating for 1 hour at 70°C, 80°C and 90°C, respectively. Preliminary lab-scale lignocellulosic pretreatment studies were undertaken by incubation of the crude enzyme mixture with straw in sulphuric acid at 70°C with subsequent quantification of the amount of reducing sugars released. Inclusion of a low level of crude enzyme (16.55 units) in an acid/straw mixture containing 10% (w/v) straw in 0.15% (w/v) H2SO4 resulted in the release of approximately 3 times the amount of reducing sugars relative to that released by acid only after 30 min. The results indicate that the crude enzymes produced by this strain are capable of hydrolysing straw at high temperature in the presence of acid and are of potential interest for the development of an enzymatic/dilute-acid pretreatment method.

Fractionation of Sugarcane Bagasse with Aqueous Ammonia Fiber Expansion (AAFEX) for Ethanol Production

Anuj Chandel, University of Sao Paulo

Fractionation of sugarcane bagasse with aqueous ammonia fiber expansion (AAFEX) for ethanol production Anuj K. Chandel*, Victor F. Marino, Anna C. Teles and Silvio Silvério da Silva Department of Biotechnology, Engineering School of Lorena, University of São Paulo, Lorena- 12.602.810, Brazil. E-mail: anuj.kumar.chandel@gmail.com; silvio@debiq.eel.usp.br Abstract Fuel ethanol production from sustainable and largely abundant agro-residues such as sugarcane bagasse (SB) provides a long term, geopolitical and strategic benefits. Pretreatment of SB for improved cellulase-mediated hydrolysis is an inevitable process. In recent times, aqueous ammonia based pretreatment process so called aqueous ammonia fiber expansion (AAFEX) got a significant importance towards the development of an effective and economic pretreatment strategy. In this study, we statistically designed an experiment considering responsive surface methodology (Taguchi method, L8 orthogonal array) to optimize the maximum delignification and finally optimum enzymatic saccharification of AAFEX bagasse. Three independent variables (ammonia concentration, temperature and time) were considered at two levels with center point. Recovery of sugars after enzymatic hydrolysis of ammonia pretreated SB was response variable. A total of 9 experimental runs were performed with center points in duplicates. The substrate to liquid ratio was kept constant (1: 10) in each set of experiment. SB was soaked with hydrated ammonia for 2 h prior to pretreatment. The ammonia pretreated bagasse samples were washed and enzymatically hydrolysed by commercial enzymes (Celluclast 1.5 L and Novozym 188) using 15 FPU/g and 17.5 Beta-glucosidase/g at 50 0C, 150 rpm for 96 h. A maximum of 28.50 g/L reducing sugars was obtained in experimental run 7 (20% ammonia, 70 0C, 24 h) after 96 hrs of enzymatic hydrolysis. Among the tested parameters, temperature and pretreatment time contributed maximum influence (p-value, 0.061013, 0.053282) and ammonia concentration least influence on sugars recovery. Pretreatment time and ammonia concentration exhibited maximum interaction effect on sugars yield. Application of Taguchi approach demonstrated in easy process optimization and higher sugars yield. The changes in the ultra structure of native, ammonia pretreated and enzymatic hydrolysis SB were confirmed by Scanning electron microscope (SEM) and X-ray diffraction (XRD). SEM analysis clearly reveals the cell wall disruption leading loosening the grip of lignin on carbohydrate fraction in SB after ammonia

pretreatment and enzymatic hydrolysis compared to native SB. XRD analysis showed that enzymatically digested SB has maximum crystallinity (70.2%) followed by ammonia pretreated (63.5%) and native SB (61.9%). The enzymatic hydrolysates of ammonia pretreated SB were submitted to ethanol fermentation by Scheffermyces (Pichia) stipitis NRRL Y-7124 under batch cultivations. Ethanol production profile will be discussed in detail during the poster presentation. The findings presented here will give significant contributions for new potential applicable methods for efficient biomass pretreatment aiming bioethanol production. Acknowledgement: Financial support from BIOEN/FAPESP and CNPq is gratefully acknowledged. Keywords: Sugarcane bagasse, Taguchi design, Aqueous ammonia fiber expansion, Scheffermyces (Pichia) stipitis, Bioethanol

Genedata Selector™ for Biofuels R&D
Asa Oudes, Genedata, Inc., San Francisco

Increased demand for sustainable energy resources has led to the rapid growth in the number of R&D projects with a focus on delivering novel fermentation-based production processes of biofuels beyond ethanol. A variety of organisms, including microbes, fungi, algae and plants are investigated as potential biofuel producers. We report how Genedata Selector, an innovative computational system has successfully been used to select the optimal strain of a specific organism for biofuel production. In particular, we show how Genedata Selector can analyse an unlimited number of genomes and predict their capabilities to efficiently generate biofuels. A special focus is on how Genedata Selector's data analysis workflow can be applied to process next-generation sequencing data in the context of producing alkanes and butanol from microbes and fungi. The integration of next generation sequencing (NGS) based genotype and profiling data for transcriptomics together with mass-spectrometry based proteomics and metabolomics data improves and streamlines development cycles. Genedata Selector systematically processes these data and annotates the different organisms and derived strains with high quality. The system also automatically identifies and categorizes point mutations in their genetic and biological context. This process is assisted by tailored viewers that drill down to sequencing data, and by Genedata Selector's algorithms that predict the mutations' influence on gene products (e.g. modifying an enzyme's active site) or on gene regulation (e.g., altering a transcription factor's DNA binding site) down to the metabolic pathway level. We will also demonstrate analysis tools useful for fermentation-based production of new enzymes for cellulose degradation. Authors: Asa Oudes1, Sebastien Ribrioux2, Nadim Jessani1, Thomas Hartsch2, Niko Bausch2, Julia Retey2, Tim Zeppenfeld2 & Hans-Peter Fischer2 Genedata, Inc., San Francisco, USA 1, Genedata AG, Basel, Switzerland 2

Conceptual Design of Sustainable Biorefineries - Metabolites and Bioenergy Prduction in the Amazon
Carlos Ariel Cardona, Universidad Nacional de Colombia

The amazon is considered the best example of earth equilibrium consumptions of carbon dioxide. Deforestation, urban development in the Amazon and a not controlled exploitation of resources cause the reduction of this important rainforest. Additionally, the high biodiversity of this region has created a growing billion dollars market for native metabolites used today in medical and food applications. However, Amazon communities had not received any contribution or advantages form this market. Moreover, different cities in the Amazon not connected to the grid and far away from the fuel sources suffer the problems of energy supply. One alternative is to use the land that has been deforested for bioenergy crops and the ancestral knowledge in using and growing native metabolites to increase the level of life and to ensure the energy security in these cities.In this work a biorefinery proposal is developed to produce different metabolites from 2 native fruits (copoazu, naiku) integrated to ear elephant and palm crops as a source of starch and oils for ethanol and biodiesel production. Finally the

lignocellulosic residues from fruits, palm and elephant ear are also included for ethanol production. The overall performance of the synthetized biorefinery shows a sustainable alternative for cities in the hearth of the Amazon.

Characterization of Monoacylglycerols from Chemical and Enzymatic Glycerolyses of Canola Oil
Cibelem Iribarrem, University of Campinas

Monoacylglycerols (MAG) have been used in cosmetics, toothpastes, hair care additive and medicine. In addition, MAG represent about 70% of all synthetic emulsifiers used industrially. The substitution of natural products by artificial ingredients has gained worldwide attention in the food, pharmaceutical and other industries. Glycerolysis of fats and oils employing alkaline catalyst under high temperature is the process currently used in industry for the large-scale synthesis of MAG. The enzymatic glycerolysis occurs at lower temperature, however with increased of reaction time and cost. In this work, calcium hydroxide (Ca(OH)2) and Candida antarctica B (Novozym 435) were employed as catalyst in the chemical and enzymatic glycerolyses, respectively. In both cases, the product obtained was a mixture of acylglycerols containing MAG, di- and triacylglycerols (DAG and TAG), which may contain glycerol and free fatty acids. Therefore, the aim of this study was characterize the canola oil and the mixture produced by chemical and enzymatic glycerolyses. The samples were analyzed through fatty acid composition, iodine index, saponification number, free fatty acids, moisture content, peroxide index, acylglycerols content, determination of mass per unit volume (liter weight) and differential scanning calorimetry (DSC) to evaluate the product composition and quality. According to the results, the chemical glycerolysis produces a mixture with higher levels of MAG than enzymatic glycerolysis. The results obtained through characterization of the canola oil and the mixture were analyzed and compared with oil standards, especially unsaturated fatty acids levels, which remained constant after the reaction. Comparing both processes, besides MAG content, should be considered other physicochemical characteristics of the mixtures. Despite the chemical glycerolysis to produce a higher MAG content, the process has severe operational conditions. On the other hand, the enzymatic glycerolysis is a process less economically viable, but with a product of higher nutritional quality (due to enzyme specificity). Generally, MAG from canola oil are natural emulsifiers with high content of unsaturated fatty acids, adding value to the product and bringing benefits to consumers.

Enzymatic Kinetics Predictability Model for Biopolymer Damage Removal in Oil Wells.
Debayan Ghosh, Epygen Labs FZ LLC

In an effort to clean-up fossil fuel production process, Enzymatic technology offers environmentally clean solutions tailored to remove Biopolymer based Filter Cake damage from the wellbore surface. Water based drilling fluid Biopolymer linkage specific enzymes offer a safe and effective alternative to conventional clean-up methods replacing harsh chemicals like bleach and acid. However, down-hole physical and chemical conditions are often stressful and multi-component enzymatic hydrolysis mechanism being quite complex, renders the effectiveness of an enzyme unpredictable in such situation. Purpose of this study is to propose a predictability model by evaluating the ‘Kinetic Rate Constant’ of this filter cake hydrolysis, derived from reducing sugar released as hydrolysis product of the Long chain biopolymers, by a range of customized Enzyme-Buffer systems, using Starch-Xanthan Biopolymer based Drilling Fluid as substrate. This index is designed to predict how an enzyme system will perform in Hydrolyzing substrate biopolymer of a particular Biopolymer Cake in specific down-hole environment, to match various operational needs of breakage time and intensity. An initial substrate concentration was determined by hydrolyzing the Starch Biopolymer drilling fluid with Maximum Concentration of Enzyme, at ultra boil temperatures for a definite time. Subsequently, enzyme treated

samples were incubated at time intervals. Residual Biopolymer (substrate) after the enzymatic Hydrolysis was hence calculated by [St] = [So] – [RS], where [St] is the theoretical Biopolymer residue (mgs/ml) at time 't' of enzymolysis, [So] the initial Biopolymer concentration and [RS] being the reducing sugar concentration (mgs/ml) in the supernatant of samples measured by Dinitrosalicylic Acid Solution method, OD measured at 540 nm using UV spectrophotometer. The net Hydrolyzed amount of Biopolymer was determined by: [Y] = [So] - [St] and the relation between residual Biopolymer and treatment time [Y] / [So] = 1- e-kt,. Finally 'K' value was determined by plotting ln(100 X [St]) / [S0]) for each enzyme system against time and calculating the slope. The predictability of 'K' value hence attained, was crosschecked by High Pressure High temperature filtration experiments and was found to be suitable.

Optimization of Enzymatic Hydrolysis from Pretreated Sugarcane Bagasse Using Commercial Cellulases
Ellen Giese, Sao Paulo University

Second generation ethanol is receiving increasing attention as an alternative renewable energy source instead of fossil fuels. The sustainable reuse of agroindustrial residues as a Brazilian sugarcane bagasse to cellulosic bioethanol production regards a clean and safe environment. Therefore, the enzymatic hydrolysis of biomass polysaccharides to fermentable sugars by yeast remains a major obstacle that must be overcome. In this aspect, our project aims at the fractioning of sugarcane bagasse in its main components (cellulose, hemicellulose and lignin) for their use in the production of ethanol in order to achieve a cost-effective process and establish technologies to extend the competitiveness of second generation ethanol. In this study, commercial preparations of cellulolytic enzymes were evaluated in the optimization of pre-treated cellulosic bagasse hydrolysis using a statistical experimental design. Sugarcane bagasse was pretreated under acid and alkaline hydrolysis pre-optimized conditions. The resulting cellulosic pulp was used as substrate in the enzymatic hydrolysis experiments, which were conducted in 125 mL Erlenmeyer flasks containing cellulosic bagasse, 25 mL citrate buffer (50 mM, pH 5.0) and commercial preparations of Cellulase (DYADIC) and cellobiase (SIGMA) as well as addition of surfactant Tween-80 according experimental design. The reaction was conducted at 50 °C under constant agitation at 100 rpm during 48h. Hydrolysis products were monitored as reducing sugars using a cuproarsenate method of Somogyi & Nelson; and residual glucose using HPLC. A factorial design using the response surface method with four replicates at the central-point summarizing 20 experimental runs was planned with the four main variables influencing enzymatic hydrolysis of cellulosic bagasse: x1, Cellulase (DYADIC) (150 - 300 U/mL); x2, Cellobiase (SIGMA) (40 - 80 U/mL); x3, Tween 80 (0 - 0.15 g/g bagasse) and x4, cellulosic bagasse (5 - 10% w/v). Cellulase activity was the variable most influencing delignified bagasse enzymatic hydrolysis following those interactions among the variables x1 and x2. The effect terms of the variable x4 were discarded as being non-significant. According to the results, an intercept was significant indicating that the central-points were correctly chosen. Maximum bagasse saccharification value was obtained using 300 U/mL cellulase, 80 U/mL cellobiase, 0,15 g/g Tween-80 and 5% w/w cellulosic bagasse, which resulted in ˜ 68 g/L of reducing sugars that corresponded to 34 g/L of glucose. The R-squared value implies that almost 97% of the variability in the observed response values can be explained by the model, or by experimental factors and their interactions. The response surface methodology demonstrated that enzymatic hydrolysis of delignified sugarcane bagasse can be useful to development of alternatives of bioconversion to second generation ethanol. Supported by FAPESP and FAPEMIG.

Greener Production: Reducing the Phosphorus Content of the Fermentation Media of Escherichia Coli in Recombinant Protein Production
Gary Walsh, University of Limerick

Greener production: reducing the phosphorus content of the fermentation media of Escherichia coli in recombinant protein production Witt, M., O'Dwyer, T. and Walsh, G Little consideration is traditionally given within the biotechnology sector to the potential environmental impacts of media ingredients unutilized during microbial fermentation. The main goal is the production of high cell (and hence product) yields. As media ingredients are usually relatively inexpensive they are often added in excess to actual cell growth requirements. This study investigated the scope for reducing the quantities of phosphorus present in both a complex (TB) and semi-defined (M9/YE) fermentation media used to culture a model E. coli strain constructed to produce a recombinant protein (ß-galactosidase). Reductions of up to 70% of exogenously added phosphorus salts did not adversely affect biomass yields attained. Further reductions lead to a drop in mean dry cell weight recorded. This was particularly evident in the case of the semi-defined media, most likely due to reduced phosphate-mediated media buffering capacity. Removal of all exogenous phosphorus from TB media had little effect upon total recombinant protein expression levels achieved. Reductions greater than 70% of exogenously added phosphorus negatively affected product expression levels in the case of M9/YE media. Protein functionality, assessed by the kinetic parameters Km and Vmax, was not influenced by the type of media nor the phosphorus concentration present. Overall the results indicate that the phosphorus salts added to both types of fermentation media can be reduced by a minimum of 70% without adversely affecting the biomass yield, the recombinant protein yield or protein functionality. Such reductions would lead to significant phosphorus savings in the large-scale production of biopharmaceuticals and other proteins produced by genetic engineering in E. coli. Lower phosphorus levels would reduce the extent of fermentation waste stream treatment required and reduce the pollutive potential of the spent media while simultaneously decreasing the production costs and the rate of industrial utilisation of a finite natural resource.

Biométhodes: the optimized bio-refinery concept
Gilles Amsallem

Biométhodes is based in France and the US with labs near Paris, and a US subsidiary, OPTAFUEL US, located in Southwest Virginia.
Unlike current bio-refineries, Biométhodes aims at developing and commercializing a proprietary technology specially designed to convert non-food ligno-cellulosic feedstock into high value renewable chemicals and biomaterials. The proprietary Process is designed to extract the value of each and every component of ligno-cellulose, and to optimize value and capital investments. The technology can be potentially used by first generation bio-refineries and by existing pulp and paper plants to produce high valued chemical fibres, fertilizers and biomaterials markets.

Technology
From a technical standpoint, the process successfully integrates two crucial steps in the processing of ligno-cellulosic materials, namely chemical pre-treatment and enzymatic hydrolysis.

— **Delignification and decrystallization process**

The technology is a novel low temperature delignification method based on conjunction of phosphoric acid and solvents, which enables extraction of high-purity lignin and produces an amorphous material that reduces the hydrolysis and increases the fermentability of the cellulose.
Pre-treatment of ligno-cellulose influences all subsequent process steps, and especially future biological steps. Enzymatic hydrolysis is crucially impacted by the degree of crystallinity of the extracted cellulose,

making this step one of the main cost and technical hurdles in all known processes. Further down process, fermentation of hexoses is hindered by the complex mixture produced, composed of degraded lignin, C5 and C6, and the presence of numerous toxic contaminants, such as furfural, or sulphates.

— **Enzymatic platform**
Biométhodes owns a patented platform to improve the activity of the enzymes required to hydrolyse the amorphous cellulosic material. Novel enzymes produced via these techniques are fully proprietary.
— **Lactic acid production**

The production of amorphous cellulose via the process has also opened the way towards one-step production of lactic acid, and other high-value materials, from biomass via the potential use of recombinant cellulolytic bacilli. Biométhodes has engaged exploratory work in this area also.

Advantages of Biomethodes technology:	**OPTALYSIS**	
Classic 2nd generation		
Temperature	180 to 190 °C	50 °C
Cellulose	Resistant - CRYSTALLINE	Not-resistant - AMORPHOUS
Lignin	Denatured or burned	Valued as high value chemicals
Hemi-cellulose	Denatured or converted in low value bioethanol	Valued as high valued chemicals
Fermentation inhibitors	YES (Sulphates et Furfural)	NO (Phosphates)
Enzyme hydrolysis	Very long (72 h)	Quick (24 h)
7 enzymes	2-3 enzymes	
1USD/gal of EtOH	0.50USD/	

BIOMETHODES TECHNOLOGY
Comparison of OPTALYSIS process with classic 2nd generation process
Intellectual Property
Regarding Intellectual Property, Biométhodes owns four key patent families, two pertaining to delignification and decrystallization of cellulose exclusively licensed from Virginia Tech, and two pertaining to its enzyme optimisation platform.

Effect of Extracting Solvents on Total Phenolic Content and Antioxidant Activity of Chlorella Vulgaris Extracts
Giuliano Dragone, University of Minho

Authors: Giuliano Dragone, Mariana Anjos, Silvia Martins, Solange Mussatto, António A. Vicente, José A. Teixeira
Algal Sphere Bacteria Enhance the Growth of Eukaryotic Microalga, Chlorella Vulgaris
Hee-Sik Kim, KRIBB

Microalgae generally grow in the presence of bacteria, which could be designated as algal sphere bacteria (ASB). The algal sphere, like rhizosphere, the zone of soil that is directly influenced by root secretions and associated soil microorganisms, is the narrow region between the algal outer membrane and the aqueous zone in which microalgae and their associated microorganisms mutually affect. We

compared the culture properties of Chlorella vulgaris (KCTC AG10032) between axenic and xenic state. While xenic state means the microalgal cell culture is associated with ASB, axenic strain is the only microalgal cells without bacteria. The diversity of ASB in the xenic culture was analyzed with 16S rRNA gene by pyrosequencing. We obtained the axenic strain of Chlorella by sequential subculture using antibiotic containing plate. We confirmed the axenic strain by microscopic observation of cells, culturing on the agar plate, and DGGE. The growth rate, cell mass and flocculation of xenic strain were significantly higher than axenic strain. From these results, we found out that ASB significantly enhanced the growth and flocculation of microalga, Chlorella vulgaris. Therefore, a direct application of these ASB could be inoculants for large-scale microalgal cultures. They could optimize biomass production by enhancing growth, particularly in the microalgae that have low growth rate.

Integrated Analysis Method for the Production of Volatile Isoprene in Microbial Fermentation
HongWeon Lee, Korea Research Institute of Biosience and Biotechnology

Isoprene (2-methyl-1,3-butadiene) is one of the commodity chemicals used in a wide range of industrial application, and is naturally made from dimethylally diphosphate (DMAPP) by isoprene synthase (ispS) through methyl-erythritol 4-phosphate pathway (MEP pathway) in the plants. Microbial isoprene production was recently attempted by using recombinant Escherichia coli containing codon-optimized ispS originated from Populus trichocarpa. Despite successful development of microbial strain producing isoprene, it is difficult to analyze and optimize the fermentation process due to its highly volatile property. In this study, on-line monitoring system of isoprene was developed by using on-line gas chromatography or quadruple mass-spectrometer. Also, various culture conditions were tested for the optimization of microbial isoprene fermentation process. In the nitrogen source-limited fermentation using glycerol as a carbon source, 17.2 g/L of isoprene was obtained at 56 hrs.

Potential Use of Apple Processing Co-products as a Feedstock for Manufacturing Bio-fuel and Organic Acids
H.P. Vasantha Rupasinghe, Nova Scotia Agricultural College (NSAC)

Bio-conversion of agricultural wastes provides a viable solution to multiple environmental problems as well as production of natural products. Apple processing for manufacturing juice, pies and sauce results in significant volumes of under-utilized co-products. This study aims to optimize the method for producing fermentable sugars from apple processing by-products. A pretreatment based on dilute sulfuric acid hydrolysis of APP was optimized for yield of glucose in relation to three independent variables: acid concentration (0.5-2% w/v), operating time (5-30 minutes) and temperature (80-100oC) using response surface method. A 23 central composite design (CCD) was used to code the three variables at five levels. The optimal acid hydrolysis conditions obtained through canonical analysis of response surface method were: acid concentration - 1.86%; time - 14.35 minutes; and operating temperature - 97.44oC. Additionally, the effect of laccase, a polyphenol oxidase, at three levels (10, 20 and 30 mg/25g fresh weight biomass) on polyphenol oxidation of acid hydrolyzed APP will be presented. The final yield of 18% fermentable sugars (glucose, fructose, galacturonic acid) was obtained after multi-step hydrolysis using commercial cellulase, pectinase and β-glucosidase at dosage of 9, 38 and 8 units/g FW, respectively. The other optimum conditions were temperature of 40 oC, pH at 4.0 and 24 h of reaction time. The fermentable sugars such as glucose, fructose and galactose, obtained from enzymatic hydrolysis, can be further converted to bio-ethanol using specific strains of yeast such as Saccharomyces cerevisiae and bacteria such as Escherichia coli. These fermentable sugars can further be converted into bio-ethanol and organic acids using specific yeast and bacteria.

Application of Photo-Fenton Process to Treatment Waste Generated in Step Hydrolysis of Bagasse of Sugar Cane for Bioethanol Production

Ivy Oliveira, Escola de Engenharia de Lorena EEL-USP

The study of viable technologies for ethanol produced from biomass hydrolyzates in recent decades has been the object of worldwide research. The environment concerning and the development of technologies that can mitigate the ecological problems have been widely investigated. This project is a proposed to evaluate the environmental impact of waste generated during the production process through the hydrolysis of sugarcane bagasse for ethanol production. In this step is generated phenolic compounds that are harmful to the environment . In preliminary studies, there was a mild sulfuric acid hydrolysis of the hemicellulose fraction of bagasse and another product generated in this reaction (cellulignin) was submitted to basic hydrolysis. The waste generated at this stage (residual lignin) was quantified and characterized using environmental techniques proposed in Article 18 of CESTEB, aiming to evaluate their environmental impact. For this purpose, we used advanced oxidation processes (AOP) based on the application of photo-Fenton (Fenton's reagent / UV). The experiments were performed in batches according to a factorial design L9 (Taguchi Method) operating on three levels to optimize the treatment according to the following parameters: temperature (° C), pH, Fenton's reagent (Fe2+/H2O2) and ultraviolet irradiation. The environmental parameters analyzed were total phenols, total organic carbon (TOC) and chemical oxygen demand (BOD). Analysis of total phenols showed the maximum removal of about 98 % when it was used the experimental conditions of temperature 35 ° C, pH 2.5, in Fenton reagent volumes of H2O2 and Fe2+ respectively, 144 mL and 153 mL, and UV irradiation at 16 W of power. This combination showed the best result in the degradation of phenolic compounds present in the residual lignin. The COT had 95.75% removal of organic matter and COD to 96.90%. It was found that the degree of recalcitrance of organic matter in waste rightly DBO5/DQO

Salt-Free Production of Gamma-Aminobutyric Acid from Glutamate Using Glutamate Decarboxylase Isolated from Escherichia coli

Keehoon Won, Dongguk University-Seoul

Recently there has been a tremendous demand for chemicals and plastics derived from renewable resources, biomass. Nylon 4, which is a linear polymer of butyrolactam or 2-pyrrolidone, is a novel biobased and biodegradable polymer with excellent properties. The monomer for polyamide 4 can be readily produced from cyclization of gamma-aminobutyric acid (GABA), which is a chief inhibitory neurotransmitter and has a lot of physiological functions in humans. From an industrial point of view, the production of GABA from the cheap renewable source such as L-monosodium glutamate (L-MSG) is a completely reasonable and valuable process. GABA is produced through CO2 release from the a-carboxyl group of glutamate, which is well catalyzed by pyridoxal phosphate-dependant glutamate decarboxylase (GAD) widely distributed in living cells of various creatures from bacteria to mammalians. We have conducted the production of GABA from L-MSG using GAD partially purified from Escherichia coli. Almost complete transformation of the substrate into the target product was achieved when a low concentration of L-MSG was used in a buffer solution with an acidic optimum pH for enzyme activity. However, at higher substrate concentrations, the reaction conversion was found to considerably decrease, which turned out to be because the pH of reaction mixture increased beyond the optimum as the enzymatic reaction progressed. Under these conditions, buffer solutions failed to maintain the pH constant due to their limited buffering capacity. For this reason, addition of conventional acids such as hydrochloric acid to the reaction medium during the reaction has been attempted. However, this method brings about formation of a significant amount of salts, which can cause a serious problem in

separation and purification of GABA after the reaction. In this respect, a simple and effective method for maintaining the pH acidic during the GABA production catalyzed by GAD without the salt formation has been devised in the present work for the first time.

Effect of Nutrient Source on Ethanol Production During D-xylose Fermentation by Candida Shehatae
Kelly Dussan, University of São Paulo - EEL-USP

Effect of nutrient source on ethanol production during D-xylose fermentation by Candida shehatae Kelly J. Dussán1, Eduardo P. Machado1, Samira P. Silva1, Carlos A. Rosa2, Silvio S. Silva1. 1 Departamento de Biotecnologia, Escola de Engenharia de Lorena, Universidade de São Paulo, Lorena, SP, 12602-810, Brazil, Tel.:+55-12-3159 5146; E-mail address: silvio@debiq.eel.usp.br 2 Departamento de Microbiologia, ICB, C.P. 486, Universidade Federal de Minas Gerais, Belo Horizonte, MG, 31270-901, Brazil The effect on D-xylose utilization and the corresponding ethanol production by Candida shehatae (UFMG HM 52.2) were examined with different nutrient sources. These included organic (yeast extract and urea) and inorganic (ammonium sulphate) sources. The ethanol production performance by this yeast strain was assessed using detoxified sugarcane bagasse hemicellulosic hydrolysate (g L-1): xylose (47,0), glucose (6,0), arabinose (5,0). Inoculum was prepared in 125 mL Erlenmeyer flasks containing 50 mL of YPX medium: xylose (30.0 g L-1), yeast extract (10 g L-1) and peptone (10 g L-1). The flasks were incubated in rotary shaker at 30°C, 200 rpm, for 24 h. The cells were recovered by centrifugation at 2600 xg for 15 min, washed and resuspended in sterile water. The fermentation experiments were carried out in 250-mL Erlenmeyer flasks containing 100-mL of the detoxified hydrolysate, with the fixed concentration of inoculum level (1,0 g L-1) and yeast extract and ammonium sulphate (3 g L-1) and yeast extract and urea (3 g L-1) at 30°C, 200 rpm for 72h. Improved ethanol production (Yp/s), yield coefficient (grams product/grams substrate), 0.378) was observed when organic nutrient sources were used. With an initial sugar concentration of 47 g L-1, the maximum ethanol concentration and cell number of the fermentation medium with urea and yeast extract as nutrient sources (10.39 g L-1 and 2.84 g L-1, respectively) were higher compared with ammonium sulphate and yeast extract (7.03 g L-1 and 2.25 g L-1, respectively). It was concluded that production of high levels of ethanol could be achieved by supplementing urea and yeast extract as nutrient sources during ethanol fermentation from sugarcane bagasse hemicellulosic hydrolysate. These results may be useful in the optimization of alcohol production by C. shehatae during fermentation of D-xylose. The authors express their acknowledgments to the CNPq, CAPES and FAPESP, for the financial support to this work.

Expression of an Evolved Recombinant Xylanase in Pichia Pastoris and its Application in Biobleaching of Bagasse Pulp
Kugen Permaul, Durban Univeristy of Technology

A xylanase gene from Thermomyces lanuginosus was modified by directed evolution and expressed in Pichia pastoris under the control of a constitutive promoter. Xylanase yield of 160 IU/ml in BMGY medium without zeocin after 56 h was only slightly lower than cultures supplemented with the antibiotic. Xylanase production by recombinant P. pastoris was scaled-up in a 5 L fermenter containing 1% glycerol, and highest xylanase production of 139 IU/ml was observed after 72 h. Further studies carried out in a fermenter under controlled pH (5.5) yielded a maximum xylanase production of 177 IU/ml after 72 h. The biobleaching efficacy of the crude xylanase was also evaluated on bagasse pulp and a brightness of 47.4% was observed with 50 IU of crude xylanase per gram of pulp, which was 2.1 points higher in brightness than the untreated samples. Reducing sugars (24.8 mg/g) and UV absorbing lignin-derived compounds values were considerably higher with xylanase-treated samples. Thus, P. pastoris is a suitable alternative candidate for recombinant xylanase production and the biobleaching

experiments on bagasse pulp revealed that the crude xylanase has potential application in enzyme-aided bleaching of non-woody plant fibres.

A Facility to Engineer Microbes Producing Fine and Specialty Chemicals
Meredith Fischer, Ginkgo Bioworks

Ginkgo BioWorks is a synthetic biology company dedicated to making the engineering of organisms predictable. Here we describe our newly-opened, 11,000 sq ft organism engineering facility in Boston, MA. This is a first of its kind, highly-automated facility that is uniquely positioned to operate at the scale and with the resources necessary to design, develop and deliver commercial-ready engineered organisms. The facility includes:

- Custom computer-aided manufacturing (CAM) software that coordinates the activities of liquid-handling robotics, handles the output of data from our assay platform, tracks samples, and allows for monitoring of process throughput. We have generated over 30,000 biological samples to date.
- Custom computer-aided design (CAD) tools that optimize organism, pathway and part design schemes.
- A clean interface to our suppliers of synthetic genes. Ginkgo has ordered over 200,000bp of synthetic DNA in the last year and maintains statistics on provider turn-times and costs to manage our supply chain.
- Proprietary one-pot DNA assembly technology that we have shown to assembly up to 10 fragments in a single *in vitro* reaction, using sub-parts ranging from 51 to 3648 bp. We have automated the construction of such fragments using Tecan and Biomek robotics platforms.
- Assay platforms including mass spec-based metabolomics are integrated into our system to measure the performance of candidate organisms.

Ginkgo has engineered organisms for government agencies (DOE, DOD, and NIST) and is now specifying organism engineering projects for specialty chemicals and high value small molecules. To date, we have successfully engineered industrial strains to make use of H2S as well as C1 metabolites as part of the DOE Electrofuels program. We also added a heterologous pathway to an industrial host and demonstrated initial production of a valuable active pharmaceutical ingredient (API) in just one month.

Genotype Effects on Physical Properties of Soybean Stem Fibres in Composite Materials
Muhammad Arif, Plant Agriculture, University of Guelph

The current study was initiated to investigate the influence(s) of plant genotype on the performance characteristics of fibres extracted from soybean stems after incorporation into a polypropylene (PP) matrix. Soybean stem fibres from a recombinant inbred (RI) population (from a RG10 x OX948 cross) of 50 lines were evaluated across four environments in Ontario to investigate the genetic influence on plant fibre performance in a PP-based composite. The stem fibres were incorporated into PP at 20% (wt/wt) by extrusion at 190°C and 40 rpm with a conical twin-screw extruder. Test samples were injection moulded at a barrel temperature of 190°C and a tool temperature of 50°C with an injection period of 15 sec at 100 psi. The samples were annealed at 150°C for 10 minutes in an air circulating oven and cooled to room temperature. The composites were tested for their flexural, tensile and impact properties. Dry soy stem fibres from different genotypes were significantly different for their cellulose, hemicellulse, lignin and free phenolic contents. The composites prepared with these fibres were also significantly different for a number of physical properties. Soy stem fibres improved flexural strength up to 28%, flexural modulus by 49%, tensile strength by 7%, tensile modulus by 69% and impact strength by

7% compared to pure PP. On a genetic linkage map with a total length of 1663 cM based on 153 simple sequence repeat (SSR) genetic markers distributed over 20 linkage groups we identified 84 Quantitaive Trait Loci (QTL) that affected flexural, tensile and impact properties of the composites. Some of the QTL explained as much as 39% variation in the composite traits. The flexural, tensile and impact strengths of the composites were positively correlated with the cellulose content of the fibres. The impact strengths values of the composites were negatively affected by the hemicellulose and free phenolic contents of the fibres. Fibre free phenolic contents were also negatively correlated with flexural modulus values of the composites. This study will provide information required by plant biologists to identify fibre-related genes and breed soybean varieties with fibres that produce superior composites.

Next-Generation Sequencing in Industrial Biotechnology: Genedata Selector™ for Optimization of Microbial Production Strains

Nadim Jessani, Genedata AG, Switzerland

The new era in industrial biotech research is increasingly driven by cost-efficient next generation sequencing (NGS) technologies. Workflow-based, scalable software systems are required for systematically managing large data volumes produced by next-generation sequencers and for interpreting genotype data across complex production strain ancestries. The design of improved production strains based on NGS data is of major interest to Evonik Degussa, a leading producer of L-amino acids by microbial production strains derived from C. glutamicum and E. coli. To support this innovative research at Evonik Degussa, Genedata Selector™ has been developed which provides a fully automated data analysis pipeline that compares unlimited number of strain genomes resulting from random mutagenesis campaigns and directed strain engineering strategies. Genedata Selector™ is successfully used to systematically analyse and annotate microbial strains including automatically identifying and categorizing point mutations in their genetic and biological context. This process is assisted by tailored viewers that drill down to raw sequences. The process also predicts the mutations' influence on gene products (e.g. modifying an enzyme's active site) or on gene regulation (e.g., altering a transcription factor's DNA binding site). This study demonstrates how next-generation sequencing and Genedata Selector™ can support rational genomic design strategies for developing strains to optimize yield of L-amino acids, with applicability to other biotech products such as vitamins and enzymes.
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The Genome and Transcriptome of a Thermophilic Fungus, Reveals its Potential for Biomass Conversion

Nokuthula McHunu, Durban Univeristy of Technology

The drive towards green technology has created a need to find microbes that can fulfil this purpose. Thermomyces lanuginosus is a thermophilic fungus that can degrade plant biomass. Because of this ability, it has been identified as one of the organisms that can have various industrial applications. Although a few proteins from this fungus have been cloned and used commercially, the vast majority is still unknown. In order to identify new protein candidates and understand the biochemical interactions, the T. lanuginosus genome was sequenced and assembled forming a genome size of 23.3 Mb containing 30 scaffolds. Protein prediction identified 5,105 candidates as protein-coding genes and these genes models were supported by expressed sequence tag and transcriptomic data. The total number of biomass degrading and related proteins that fall into the CAZY family was 224. Most of these proteins were similar to other filamentous fungi proteins. As this fungus is a thermophilic fungus, proteins that

are related to temperature control would be vital for survival. Interestingly out of the 46 proteins identified with this biological function were linked to DNA and nucleic acids. This could point to the importance of maintaining genetic material integrity at high temperature. The genome sequencing of this fungus has provided valuable information obtained that can be used for various biotechnological application.

Long-Term Yield of Giant Reed, Switchgrass and Mimosa for Biomass Production in Alabama
Ping Huang, Auburn University

Numerous technologies that can convert cellulosic biomass into various liquid biofuels are currently under development, making production of cellulosic biomass more attractive than ever. Giant reed (*Arundo donax*) and switchgrass (*Panicum virgatum*) have been extensively evaluated for biomass production in southern Europe and the United States, respectively, both with very favorable results. Mimosa (*Albizia julibrissin*) is another potential perennial, woody cellulosic energy crop with high yield potential. However, long-term (> 10 years) yield data for the three crops are lacking. Therefore, experiments were conducted to provide comparative data for these crops at the same location over an 11-year period. Results indicated that giant reed and mimosa provided much higher biomass yield than switchgrass: average annual dry biomass yields for giant reed, mimosa and switchgrass were 36.0, 32.3 and 24.7 Mg/ha (16.0, 14.4 and 11.0 tons/acre), respectively. In contrast to traditional summer row crops such as corn, cotton and soybeans, rainfall and age of stand did not have significant effects on biomass yields of the three perennial energy crops evaluated.

Solid State Cultivation of Aspergillus Niger for Citric Acid and AMG Production on Sugarcane Bagasse with Vinasse
Reinaldo Bastos, CCA/UFSCar

Solid state cultivation (SSC) has been defined as the process involving growth of microorganisms on support solids in absence of free water. However, the substrate must possess enough moisture to maintain metabolism of microorganisms. SSC has built up credibility in recent years in biotech industries due to potential applications, offering higher yields compared to submerged microbial process. Nowadays, several agro industrial residues have been used as solid support for SSC, generally impregnated by nutrient solution. Sugarcane bagasse and vinasse are mainly by-products from sugarcane processing industry. Citric acid is a commercially valuable biotechnological product produced mainly by Aspergillus niger from sucrose or molasses. Amyloglucosidases (AMG) is amylolytic enzyme that hydrolyses single glucose units from the non-reducing ends of amylose and amylopectin in a stepwise manner, and produced glucose as the sole end-product from starch and related polymers. AMG (EC 3.2.1.3 or glucan 1,4-a-glucosidase) has been produced by SSC and submerged fungal cultivations. In this study, SSC are employed for citric acid and AMG production by Aspergillus niger from packed bed column filled sugarcane bagasse moistened with vinasse. Experiments were set up for 7 days at 25oC, particle diameter between 0.59 to 1.17m, in columns of 30mm diameter and 200mm bed height. Vinasse and inoculum suspension was impregnated in the bagasse particle until the initial moisture of 80g/100g (wet basis). Results indicated maximum of 3.2 mg citric acid per gram of dry bagasse in 3 days, with yield about 0.3 g.g-1 of glucose uptake, indicating non-limiting oxygen conditions. Maximum AMG production was obtained in 5 days (78 UI per gram of dry bagasse), with maximum enzyme productivity about 8 UI.g-1 dry bagasse. Enzyme synthesis probably was induced by organic matter of vinasse that moistening solid support. The research suggests that the main by-products of sugarcane processing could be used in SSC to obtain biotechnological products with high added value.

Non-conventional Biomass Production of Microalgae Aphanothece Microscopica Nägeli and Chlorella Vulgaris from Glycerol as Organic Substrate

Reinaldo Bastos, CCA/UFSCar

Heterotrophic cultivation of microalgae could be defined as the use of organic compounds for growth, in absente of light. This non-conventional cultivation overcomes deficiencies of illuminated autotrophic process, allowing reduction in costs. Under some heterotrophic cultures, the microalgal yields are consistent, reaching cell concentrations higher than photosynthetic conditions. However, these systems are limited to some species that can grow heterotrophically, and are susceptible to contamination and inhibition by excess of organic molecules. Glucose, glycerol and acetate have been mainly used as organic source. Studies about metabolism of glycerol assimilation are limited, but with a potential for biodiesel production by microalgae, it is important improve researches aiming the use of glycerol as substrate for microbial process. The aim of this work was to study heterotrophic cultivation of cyanobacterium Aphanothece microscopica Nägeli and chlorophyceae algae Chlorella vulgaris. Optimal glycerol concentration was previously evaluated in mixotrophic cultivation, resulting 0.005 and 0.01M for Aphanothece and Chlorella, respectively. Biomass profiles suggest the high inhibition by glycerol concentration higher than optimal value. Heterotrophic experiments showed maximum specific growth rate of 0.02h-1 for Aphanothece and 0.09h-1 for Chlorella, with maximum cell concentration about 30 hours to both of microalgae. Moreover, results indicated maximum glycerol removal about 48 and 31% for Aphanothece and Chlorella, respectively. Results suggest that glycerol could be used as organic source for non-conventional heterotrophic growth of Aphanothece microscopica Nägeli and Chlorella vulgaris, minimizing impact of biodiesel by-product and improving microalgal biomass production.

Using Degenerate Primers for the Isolation of Cold-adapted Pullulanase Gene from Indigenous Exiguobacterium sp. SH3

Reza Heidary, National institute for Genetic Engineering and Bio

Psychrotrophes are organisms that thrive in cold environments. One of the strategies for their cold adaptation is the ability to synthesize cold-adapted enzymes. These enzymes usually display higher catalytic efficiency and thermolability at lower temperatures compared to their mesophilic and thermophilic counterparts. In this work, a psychrotrophic bacterial isolate, designated as SH3, was selected for the cloning of the gene encoding Pullulanase. Based on 16S rRNA gene sequence analysis, this isolate was identified as a species of the genus Exiguobacterium. Using degenerate primers designed based on sequence similarity, the partial sequence of the Pullulanase gene was PCR amplified. The sequence of the amplified fragment was determined and used for homology search using Blast program suite at NCBI. The results showed that the amplified fragment has maximum homology with a putative Pullulanase gene of Exiguobacterium sibericum. This promising result is going to be extended by future experiments using splinkerette PCR and Vectorret PCR technique to isolate the whole ORF coding for the expected Pullulanase gene.

Optimization of Medium for Pullulan Production Using a Novel Strain of Exiguobacterium sp. SH3

Reza Heidary, National institute for Genetic Engineering and Bio

The aim of this work was to study the effects of cultural parameters on Pullulanase production of Exiguobacterium sp. SH3. This bacterium produces an interesting cold-adapted Pullulanase with high catalytic activity at temperatures approaching freezing cond ition. To this aim, the combinatorial effects of seven cultural parameters including: time (h), temperature(c), pH, shaking speed (rpm), yeast extract (g/l), tryptone (g/l), starch (%) were analyzed at two levels using Plackett–Burman and Central

composed design. The results indicated that the effects of 3 factors including temperature (25 and 40 °C), yeast extract (1 and 3 g/l), and shaking speed (100 and 180 rpm) were statistically significant resulting in improved amylase productions. Temperature at lower level while yeast extract and shaking speed at higher levels were more suitable for Pullulanase production and secretion by this bacterium. The results revealed that cold condition is more suitable for Pullulanase production by this psychrotrophic bacterium. Yeast as a general growth-improving factor and shaking as an important factor for aerobic organisms at higher levels were effective. the newly psychrophilic Pullulanase SH3 seems to be suitable biocatalyst for practical use in liquefaction of starch at low temperature, detergent and textile industries.

Food Vs. Renewable Chemicals: BIO's New Industry Position
Rina Singh, Biotechnology Industry Organization & Paul Winters, Biotechnology Industry Organization

BIO's Industrial and Environmental business unit has analyzed the supply and demand for conventional feedstocks for the production of renewable chemicals. The adoption of biotechnology enables more sustainable techniques – such as no-till cultivation, and several other cost advantages – that further reduce the need for petrochemical inputs. The presentation will show that sustainable production of renewable chemicals must include profitability for growers and add value for agricultural products. Industrial biotechnology offers state of the art innovative solutions for sustainability.

Performance of Stirred Airlift Bioreactor Using Experimental Dates and CFD Simulation
Sergio Santos de Jesus, University of Campinas

Bioreactors are used in all kinds of bioprocess, including those for making vaccines, antibiotics, amino acids, and many other high-value products such as the carotenoids and also in the biodiesel production. Stirred tank and airlifit bioreactors are commonly used for the industry. However, few studies have been carried out on hydrodynamic and mass transfer for the hybrid bioreactors, i.e. stirred airlifit. The objective of this work was to verify the performance in a mechanically stirred airlift bioreactor in term of hydrodinamic (gas hold-up and mixing time) and mass transfer coefficient (kLa) using experimental dates and computational fluid dynamics simulation (CFD). The experiments were carried out in a airlift bioreactor (3,5 L) with internal recirculation (a concentric draft-tube airlift vessel device); the agitation is carried out through a turbine Rushton impeller located along with the gas sparger in the region comprised in the riser. The bioreactor was sparged with air under different velocities (0.003 to 0.021 ms-1). Water was used in this work a fluid model. The simulation was carried out using ANSYS 5.0 software. Gas hold-up was determined by the manometric method (Chist, 1989), the mixing time was determined with the acid tracer method and the mass transfer coefficient (kLa) was determined by the dynamic method, which the experimental data were adjusted based on the equation proposed by Bang et al. (1998). The kLa is obtained from the basis of Higbies's penetration theory on the simulation study. The experimental results were similar to CFD simulation results, the kLa values varied from 0.004 to 0.021 s-1, which the stirrer speed is directly related to the increase of the kLa value.

Bio (Industrial) Applications and Economic Perspectives of Sugarcane Feedstock in Brazil
Silvio Silva, University of São Paulo - EEL-USP

Brazil is the largest producer of sugarcane in the world followed by India and China. Sugarcane bagasse and leaves are the main residues along with molasses which have profound applications in bio-industrial sector. Due to the significant advances made in biomass processing and fermentation, several value-added products can be obtained from these residues adopting biotechnological routes. These products

include ethanol, xylitol, organic acids, industrial enzymes, single cell protein and others. However, pretreatment and other coordinated process steps are crucial and decide the cost of overall process. Among all the products, ethanol as fuel derived from sugarcane residues got maximum attention in recent years due to the fast depletion of fossil fuel, price hikes and environmental concerns. In addition to this, many geo-political factors and employment issues have added significant momentum towards the development of biorefineries based on the successful exploitation of sugarcane residues. Our laboratory is dedicatedly working on the development of second generation fuel ethanol production and xylitol production from SB feedstock. We have been developing a dual acid-base pretreatment and hydrated ammonia based pretreatment technologies followed by enzymatic hydrolysis using commercial enzymes supplied by Dyadic Xylanase 2 XP CONC and Celluclast 1.5 L + Novozym 188. Dual acid –base pretreatment followed by enzymatic hydrolysis showed high hydrolytic efficiency (85%) than ammonia (72%). The obtained sugars are used for ethanol fermentation adopting modified fermentation strategies such as the implication of immobilized cells in different kind of bioreactors. For the fermentation, specialized xylose and hexoses fermenting yeasts are being used. For economic ethanol production, we believe that on site enzyme production using sugarcane bagasse and further cheap pretreatment and fermentation strategies could bring the price of ethanol competitive with gasoline. The commercial applications of by-products generated during biomass processing such as lignin, furfurals, and yeast cell mass will surely provide the economic and strategic benefits. Another striking solution is to use the land and utilities (boilers, chillers, air compressors) of conventional sugar and potable ethanol distilleries simultaneously processing the sugarcane residues for ethanol production. In addition to ethanol, harnessing of potential for the sustainable production of xylitol, industrial enzymes, organic acids, single cell protein are the pivotal in order to develop “Green economy”. High value based microbial metabolites such as antibiotics, peptides and a vaccine is also possible from the cellulosic fraction of sugarcane bagasse. In this line, there is R&D is required to develop the bioprocess for such kinds of high value-added products.

Mineral Composition of Four Edible Seaweeds and Antioxidant Activity and Phenolic Contents in their Methanolic Extracts

Solange Mussatto, University of Minho

The exploration and utilization of foods from non-conventional sources, of both terrestrial and marine origin, has been strongly encouraged for several reasons including the human population increase, the global climate change, and the diversification of terrestrial food resources for energy needs. Consumption of seaweeds in human diets is common in Asian countries, and some studies report these organisms as being excellent sources of bioactive compounds such as carotenoids, dietary fiber, protein, essential fatty acids, and vitamins. The present study aimed to determine the mineral composition of four edible seaweeds, as well as the antioxidant activity and total phenolic content in their methanolic extracts. The seaweeds used in the experiments included Sweet Kombu (Laminaria saccharina), Nori (Porphyra umbilicalis), Dulse (Palmaria palmata), and Sea Spaghetti (Himanthalia elongata). Mineral composition was determined by thermal gravimetric analysis (TGA), and the individual concentration of chemical elements was determined according to ISO/DIS 12914:2010 and ISO 22036:2008. Methanolic extracts were produced by mixing 1 g of ground seaweed (fine powder) with 20 ml of 90% (v/v) methanol, and the mixture was heated during 30 min in a water-bath at 70 ºC. After this time, the produced extracts were filtered through 0.22 µm membrane and stored at -20 ºC until further analysis. Total phenolic compounds in the extracts were determined by the Folin-Ciocalteu method, while the antioxidant potential was estimated by two different techniques: 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging and ferric reducing antioxidant power (FRAP). Twenty-five chemical elements including Ca, Na, K, Mg, Al, Fe, Ba, Sr, Mn, Cu, Zn, P, S, Cr, Sn, Pb, Co, B, I, Cd, Ni, Se, Ga, V, and Mo, were

identified in the mineral content of the seaweeds. Dulse seaweed presented the highest content of phenolic compounds (8.50 ± 1.69 mg GAE/g seaweed), while Sweet Kombu gave the lowest values (2.40 ± 0.45 mg GAE/g seaweed). The antioxidant activity by the FRAP and DPPH methods revealed Sea Spaghetti as being the seaweed with the highest antioxidant potential. These results are of interest since antioxidant compounds are beneficial for the human health, and antioxidant compounds obtained from natural sources are preferred for consumption than synthetic antioxidants. Acknowledgements: FCT (grant SFRH/BPD/38212/2007). Authors: Solange I. Mussatto, Silvia Martins, Mariana Guedes, Giuliano Dragone, José A. Teixeira Institute for Biotechnology and Bioengineering (IBB), Centre of Biological Engineering, University of Minho, Campus Gualtar, 4710-057, Braga, Portugal

Enhancement of Water Solubility for Poorly Water-soluble Materials in Supplements and Pharmaceuticals by Formation a Supramolecular Complex with Peptides
Tatsuya Oshima, University of Miyazaki

1. Introduction Generally, nutrients and pharmaceuticals are absorbed from gastrointestinal tract. If the materials are poorly soluble in digestive liquor, they are less absorbed in body resulting in the insufficient bioavailability. Therefore the enhancement of water-solubility for poorly water-soluble nutrients and pharmaceuticals is important to improve the bioavailability. In the present study, the supramolecular complexes between poorly water-soluble nutrients as well as pharmaceuticals and the mixture of peptides were prepared to enhance the water solubility and/or dispersibility. The water dispersibility of the supramolecular complex containing CoQ10 was enhanced compared to that of CoQ10 alone. Similarly, the aqueous solubilities of the complex containing curcumin and indomethacin were significantly enhanced compared with those of materials alone. 2. Experimental Bovine serum albumin and casein from milk were enzymatically hydrolyzed using a-chymotrypsin to obtain the peptides mixture. After lyophilization of the mixture, white powder containing peptides was obtained. An aqueous solution dissolving the peptides mixture was mixed with an organic solution containing CoQ10, curcumin, or indomethacin and lyophilized to obtain the conjugates. 3. Results and discussion The CoQ10/peptide complex A-Q10 reasonably disperses in aqueous media, suggesting that a hydrophilic conjugate containing CoQ10 and peptide was formed. As the filtrate of the mixture containing A-Q10 is turbid, the size of the CoQ10/peptide complex is partly less than 0.8 μm. Turbidity for the aqueous suspension of A-Q10 increases with increasing the amount of peptide. Additionally, the water solubility of curcumin is significantly enhanced by the formation of complex with peptides. The solubility of the curcumin/peptide complex under weakly acidic condition is much higher than that of curcumin alone. Similarly, the solubility of the indomethacin/peptide complex under weakly acidic condition is much higher than that of indomethacin alone. Thus the peptide mixture prepared as protein hyrdolysate was found to be available for enhancing the water solubility or dispersibility of poorly water-soluble nutrients and pharmaceuticals. This technique can be applied for various materials and would enhance their bioavailability.

Genedata Selector™: A Software System for Fungal Strain Optimization
Tim Zeppenfeld, Genedata AG, Switzerland

Next generation sequencing (NGS) has greatly increased the amount of data generated in genomic studies, in particular NGS applications such as genome and transcriptome sequencing. An integrative approach for management, analysis, and visualization of NGS data is required. In close collaboration with our customers we developed a flexible, scalable and integrative software solution called Genedata SelectorTM to provide a platform for genomic studies. Genedata Selector™ facilitates integration of public and proprietary data in one database and contains built in tools for data analysis and

visualization. With Genedata SelectorTM,the data analysis process of studies that generate complex and huge volumes of genomic data can now be streamlined and automated and allows scientists to focus on the interpretation of results. We illustrate how standard omics data together with NGS data of fungi can be used to elucidate the genetic variation, metabolic capacities, and stability of strains at a genome wide level. This is applied to select optimized fungi strains for antibiotic production. We also demonstrate a variety of data analysis tools in the context of applications in different industrial biotechnology segments such as bio-fuels, cosmetics, detergents, agricultural sciences, food, beverage and feed processing. Tim Zeppenfeld1, Thomas Hartsch1, Sebastien Ribrioux1, Asa Oudes2, Nadim Jessani2, Niko Bausch1, Julia Retey1, & Hans-Peter Fischer1 Genedata AG, Basel, Switzerland1, Genedata, Inc., San Francisco, USA2

Bioconversion of Crustacean Shell Waste Using a Novel Chitinase

Upasana Ramphal, Kugenthiren Permaul and Suren Singh
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Chitin is the most abundant biopolymer next to cellulose with an annual production of 1010 to 1011 tons per annum and IS now gaining attention due to the role of chitinase as a biocontrol agent and in many other biotechnological areas. Potential chitinase producers were isolated from soil (45 isolates) and screened on colloidal chitin agar. An actinomycete isolate was selected based on the largest zone of hydrolysis produced and was identified using 16S rRNA sequencing and shared a 99.41% 16S rRNA sequence similarity with Streptomyces kunmingensis NRBC 14463. A fermentation medium with 1% colloidal chitin induced maximum chitinase production. Mutations were induced by ethyl ethanesulfonate treatment and ultraviolet irradiation. Mutant U49 demonstrated 2.5-fold higher chitinase production than that of the wild type. The crude enzyme was characterized and the optimum pH and temperature was found to be pH 4.0 and 45°C, respectively, with a pH stability in the range of pH 3-7 and a molecular weight of approximately 56 kDa. The purified chitinase had a pH and temperature optima of pH 6.0 and 45°C and was stable over a pH range of 3-8 for 120 min. Bioconversion of shrimp and crab shells were carried out using the mutant U49 and the hydrolysates were analysed by HPLC. N-acetyl-β-D glucosamine (NAG) was detected, indicating the presence of an exochitinase when the crude chitinase was reacted with colloidal chitin. The crude chitinase enzyme also possessed anti-fungal activity against Fusarium moniliforme and Aspergillus niger.

Screening Australian Fungal Biodiversity for Novel Cellulolytic Capabilities

Victoria Haritos, CSIRO

Cellulase enzymes with improved activities toward complex lignocellulose such as eucalyptus wood chips are essential to reduce the costs of second generation biofuels. Towards that aim we have assessed the cellulolytic capabilities of an Australian wood-inhabiting fungal collection of ~2000 isolates and identified highly active strains based on ability to growth on untreated Eucalyptus globulus sawdust, filter paper, Avicel and newsprint. Forty-one of the revived strains (2.5%) showed a high potential for broad-based lignocellulosic saccharification. A major proportion of the 92 strains that displayed high growth scores on lignocellulose also released reducing sugars from pure crystalline cellulose, some exceeding the rate of Trichoderma reesei QM9414 under these conditions. The leading subset of fungi, selected on the basis of superior cellulase activity, cellulase gene sequence divergence and organism novelty and amenability to biotechnological production, have been grown in high density cultures at up to 10 L scale with induction of cellulase secretion. The performance of the novel fungal enzymes has been compared in laboratory-scale pilot trials against pretreated and untreated E. globulus woodchips

and the resultant liquor was fermented to ethanol. Selected fungi identified through the screening process show excellent promise for cellulase production and saccharification of complex biomass for biofuels production.

Commercial Scale Microbial Gas Creation Technology: Production Response of Coal Seams
William R. Mahaffey, Luca Technologies, Inc.

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Biogenic methane has enormous potential as a sustainable energy source and is found in a wide variety of subsurface, anaerobic, hydrocarbon bearing environments. Since 2003 we have been actively performing research, technology development and field scale proof-of-concept demonstrations that support the commercial viability of our technology. Numerous coal seams worldwide exhibit signatures of secondary biogenic methane production. The Powder River Basin (PRB) in Wyoming, has been one of the most important for demonstrating the efficacy of of this technology. The PRB had been previously developed for Coalbed Methane (CBM) production with over 30,000 wells drilled basin wide. It has been shown to be an active "geobioreactor" based on gas isotopic signatures of the CBM produced methane (δ^{13}C-methane -57‰, δD-Methane -320‰).

PRB coals are "alive" with active methanogenic communities and can be stimulated to create new methane from coal as demonstrated by coal conversion studies in laboratory experiments. Experimental data will be presented that shows new methane being created in real-time by activating the microbial communities with activation amendment packages and observing headspace methane accumulation in excess of the stoichiometric production from the nutrients. In addition, BESA inhibited cultures exhibit an accumulation of metabolic intermediates in coal slurries prepared with live formation waters but not in controls with active formation water only. Results from laboratory-field support research, and parallel field demonstration research will be presented, supporting commercial scale proof-of-concept. Quickly moving to projects of large scale, we have been able to demonstrate production of commercial quantities of new gas in multiple basins, using an enhanced in situ microbial based process we call "Methane Farming."

In 2006, eight (8) different nutrient formulations were emplaced in 102 CBM wells in the Powder River Basin in an attempt to create economic rates of methane gas by activating the natural microbial communities living within the coal seams. A follow on treatment was performed in 2007 by treating 32 wells within the treatment area with a single optimized activation formulation of nutrients. Push-Pull methods were used to deploy the treatments, CBM well operations and gas measurements were conducted according to industry standards, and the test area was compared to an offsetting control area also operated according to industry standards. Microbial and gas production data collected in the five years subsequent to initial treatment indicate that the treatments within the test area successfully influenced the microbial community. The averaged gas production and restoration response of all wells in the test area was significantly increased by 42.3 MMCF/well compared to the baseline decline trend and the control area. We will demonstrate, with results from a spatially discrete/temporally complete sampling program and 16S-rDNA Amplicon sequencing for microbial community analysis, that distinct shifts in the bacterial and archaeal population demographics occur. These shifts are associated with 2 of the 8 nutrient activation formulations are durable and associated with sustained enhanced gas production.

Production data from this program will be presented in the context standard reservoir engineering principles of analysis that support that the gas recovered is gas created by the restoration program. This analysis includes the evaluation of; gas/water ratio, timing of changes in gas response, perm testing and multiwall interference testing. In addition the analysis of reservoir volumetrics (historic production vs volume of the stored gas resource and mechanical aspects of Luca's treatment will also be addressed. These analyses will show that it is unlikely that significant volumes of un-drained or poorly drained sorbed methane existed prior to the restoration program activities.

Lipase-catalyzed Enantioselective Synthesis of D-lactide
Yong Hwan Kim, Kwangwoon University

R-lactide, a pivotal monomer for the production of PDLA or stereocomplex PLA was synthesized from alkyl R-lactate through lipase-catalyzed reaction without racemization. Among several kinds of lipase, only lipase B from Candida antarctica (Novozym 435; CAL-B) was active in reaction for R-lactide synthesis. Extremely enantiopure R-lactide was synthesized by the catalysis of CAL-B. Removal of by-product methanol was critical to obtain a high conversion of lactide. This novel synthetic method can supply the important monomer R-lactide that is required for the production of the widely recognized bio-plastic PDLA and stereocomplex PLA.

Disruption of Dolichyl-P-Man:Man(5)GlcNAc(2)-PP-dolichyl mannosyltransferase (Alg3) Alters the Growth and Development of Aspergillus Niger
Ziyu Dai, Pacific Northwest National Laboratory

In this study, the Dolichyl-P-Man:Man(5)GlcNAc(2)-PP-dolichyl mannosyltransferase (Alg3) gene was investigated for its roles in growth and development, protein secretion and metabolism in Aspergillus niger. Deletion of the Alg3 gene in A. niger resulted in an altered protein secretion pattern, a significant reduction of growth on complete medium and potato dextrose medium plates, and a substantial reduction of spore production on complete medium. In contrast, the Alg3 deletion led to significant accumulation of secondary metabolites with red brown color on complete medium plates and dark green color on malt extract agar plates. The Alg3 deletion also reduced growth on citric acid production (CAP) medium plates at different pH. In contrast, the Alg3 deletion triggered early spore germination and a substantially improved spore germination rate in CAP liquid culture medium. The results from this study show the involvement of Alg3 on the growth and development of A. niger. Authors: Ziyu Dai1, Uma K. Aryal2, Anil Shukla2, Wei-Jun Qian2 and Scott E. Baker1 (1 Fungal Biotechnology Team, Energy Process and Materials Division, 2Biological Science Division and Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, Richland, Washington 99352)