Advanced Biofuels and Biorefinery Platforms

Is Technology Integration the "Right" Value Proposition for Rural Sustainability?

Mojgan Kavoosi, BECii Corp.

Collaboration is a powerful new sustainable business model. Biowaste to Energy for Canada Integration Initiative (BECii) Corp. is an industry led, industry-focused initiative building a commercial scale Integrated Bio-Refinery™ demonstrating waste-to-profit strategies. Located in rural Hairy Hill, Alberta, this integrated technology cluster consists of a 2.5 MW biogas power plant and a 40 M L/year ethanol production facility co-located with a 36,000 head of cattle commercial feedlot operation. This Integrated Bio-Refinery™ is both an effective waste management strategy, processing over 260 tons/day of manure and municipal source separated organics (SSO) waste, and an example of an innovative model for rural sustainability. This collaborative framework produces cleaner air, lower cost energy, increased local employment opportunities, and provides the feedlot with benefits that allow it to be one of the lowest carbon footprint commercial feedlots in Canada. In this presentation, we will discuss the synergies involved in the Integrated Bio-Refinery™, some of the technical challenges encountered during integration, and the economics surrounding this collaborative model. In addition, we will briefly touch upon other innovative synergistic partnerships under consideration for addition to this Integrated Bio-Refinery™.

Potential of thin stillage as a low-cost medium for direct bioconversion of cellulose into ethanol and hydrogen

Rumana Islam, University of Manitoba

An ideal fermentation medium for commercial-scale production should be inexpensive, able to supply essential growth nutrients, and be readily available. Thin stillage (TS) generated in dry-grind ethanol facilities is rich in many essential macro- and micro-nutrients. Drying of TS to produce dried distillers grains with solubles (DDGS) consumes over half of the thermal energy required by an ethanol plant. Therefore utilization of TS was investigated as an alternative nutrient medium for Clostridium thermocellum DSM 1237 during direct fermentation of cellulosic substrates into ethanol and hydrogen. Various concentrations (5 - 400 g/L) of TS were used to support growth C. thermocellum on cellulose (10 g/L), replacing all ingredients of the regular growth medium except buffering agents. Cultures with 50 g/L TS showed the best performance representing 100% and 81% of H2 and ethanol respectively produced by cultures on the regular growth medium. Cell growth monitored using a quantitative polymerase chain reaction (qPCR) technique showed a slower growth of C. thermocellum with increasing concentration of TS in the culture media. Magnesium supplementation of 50 g/L TS medium resulted up to 59% more ethanol compared with the unsupplemented TS medium.
Microbial demetallization of metallic compounds in crude oil

Hossein Salehizadeh, University of Isfahan

Out of 19 microorganisms isolated from polluted soils of the Isfahan Refinery Company in the center of Iran, a strain identified as Aspergillus sp. (designated MS-100) was selected based on the capability of utilizing vanadium oxide octaethyl porphyrin (VOOEP) as sole carbon source. The degradation percentage of VOOEP before optimizing was 37% at 20 °C after 7 days. The optimum values for pH, temperature and VOOEP concentration obtained were 5.5, 30 °C and 20 mg/l, respectively. The UV-visible spectrophotometric experiments and HPLC analyses confirmed the degradability of VOOEP in crude oil up to 50-55% under optimum conditions during 7 days. The release of vanadium (0.96/2 mgl-1) into the aqueous phase was proved using atomic absorption spectroscopy. Briefly, Aspergillus sp. was exhibited a high potential to utilize VOOEP as a model for protoporphyrins metallic compounds in crude oil and released considerable amount of vanadium in aqueous phase.

Production of ethanol from corn stalks using an engineered strain of Saccharomyces cerevisiae

Qiuwei Zhao*, Institute of Microbiology, Chinese Academy of Sciences

Cellulosic ethanol has been widely regarded as an attractive alternative fuel, due to the sufficient supply of feedstocks, the rapid advance on pretreatment, and the decreased cost of biomass hydrolysis. Efficient co-utilization of xylose and glucose is critical for cellulosic ethanol production. Recently, many efforts had been made to introduce the xylose metabolic pathways into S. cerevisiae. However, most of the engineered S. cerevisiae strains cannot metabolize xylose well under anaerobic condition. In this study, we engineered S. cerevisiae by introducing xyl1 and xyl2 genes from Pichia stipitis and overexpressing its own xk gene. The engineered strain was subjected to continuous evolution with dissolved oxygen concentration gradually decreased. The resulted adaptive strain, S. cerevisiae W32N55, could efficiently metabolize xylose under static condition, and tolerate the inhibitors in cellulosic hydrolysate. Furthermore, a fermentation-membrane pervaporation coupling process was developed and applied in the fermentation of the hydrolysate of steam exploded corn stalks. Using such a process, the final ethanol concentration reached 70 g/L, with a yield of 220 kg ethanol per ton steam exploded stalks.

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

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

Biodiesel from high free fatty acid rice bran oil using heterogeneous catalyst
Rajiv Arora, Shaheed Bhagat Singh State Technical Campus

Most of the biodiesel production is by process of alkaline catalyzed transesterification on edible oils. The cost of these edible oils is quite high. Cost of waste and high free fatty acid (FFA) acid oils is almost one half to one third that of refined oils, but the production cost increases due to additional steps for converting free fatty acids to esters. The FFA of the oils should be less than 2% for further transesterification to biodiesel. In the present work, the esterification process for the reduction in FFA of rice bran oil with 70% FFA using strong homogeneous aluminum doped sulfated zirconia has been investigated. The various process variables like temperature, catalyst concentration, amount of methanol and reaction time were optimized with the objective of producing low FFA oil. In the transesterification step, MgO impregnated with KOH was prepared by wet impregnation method and this alkali-doped metal oxide catalyst was evaluated for activity in the transesterification of rice bran oil to biodiesel. Also these catalysts appeared to be promising candidates to replace conventional homogeneous catalysts for biodiesel production as the reaction times are low enough to be practical in continuous processes and the preparations are neither prohibitively difficult nor costly. Also the heterogeneous catalysts
can be separated from the final product by filtration which prevents the consumption of large volumes of water.

**International Regulation of Industrial Biotechnology**


Uses of genetically modified organisms in production of fuels and chemicals are rapidly moving towards commercialization in the United States and elsewhere in the world. Such projects may face differing regulatory requirements in different regions of the world, with considerable differences likely to be found between different countries. Although regulatory regimes, including oversight over biotechnology, are well established in many developed nations, this is likely to be less true in nations in the developing world, where many biofuel projects may be located. Many countries have based biotechnology regulations on the principles of the Cartagena Protocol on Biosafety, but within the developing world, approaches to implementing the Protocol differ widely, and many signatories of the Protocol have yet to establish national regulations. This presentation will give an overview of the regulations that industrial biotechnology projects using modified microorganisms, plants or algae might face in different countries or regions of the world, but may also discuss applicability of other regulatory programs such as the U.S. Renewable Fuel Standard and the EU Renewable Energy Directive (RED). To the extent possible, the presentation will include one or more case studies of successful interactions with government agencies in the U.S. or elsewhere in the world, including examples of Microbial Commercial Activity Notices (MCANs) reviewed by the U.S. Environmental Protection Agency for fuel or chemical projects. Recommendations for winning strategies for dealing with regulatory agencies will also be presented. David J. Glass, Ph.D., with over twenty five years experience with the industrial uses of biotechnology and microorganisms, is an independent consultant specializing in renewable fuels and industrial biotechnology regulatory affairs. Dr. Glass has longstanding experience with the biotechnology regulations of the U.S. EPA and U.S. Department of Agriculture, extensive familiarity with international biotechnology regulation as well as renewable fuel standards and other fuel-related regulation in the U.S. and elsewhere in the world.

**Feasibility and Environmental Impacts of the Production of Biodiesel from Grease Trap Waste**

*Megan E. Hums, Drexel University*

Biodiesel is a renewable fuel that can be produced from a variety of vegetable oils, animal fats, and waste greases. In 2013, the United States produced 1.2 billion gallons of biodiesel primarily from refined soybean oil; although biodiesel producers struggle to compete economically with petroleum diesel because the cost of soybean oil dominates the production costs. The use of grease trap waste as a feedstock has lower feedstock cost and potentially lower environmental
impacts than agricultural oils, but requires additional or alternative steps for pre-treatment, conversion to biodiesel, and biodiesel purification. Research at Drexel has demonstrated the technical feasibility of production of biodiesel from grease trap waste; however, commercial feasibility of producing biodiesel from grease trap waste is limited by the variability of its lipid content, which ranges between 2-20%, by volume. This poster presents a process for conversion of grease trap waste to biodiesel including the separation of lipids from grease trap waste via heating and settling, esterification of lipids with methanol and sulfuric acid catalyst in a bubble column reactor to produce crude biodiesel, and the purification of crude biodiesel through washing and wiped film distillation. This research includes both techno-economic analysis and life cycle assessment to compare the energy requirements and select environmental impacts of biodiesel produced from grease trap waste to the impacts of low-sulfur petroleum diesel and biodiesel from common feedstocks used in U.S. markets. Material and energy balances in conjunction with experimental data and ASPEN simulations are used to estimate raw material requirements, utilities, and waste generation of the biodiesel production process. Results show that biodiesel produced from grease trap waste with lipid content above 10% by volume is competitive with other biodiesel processes both energetically and environmentally. At lower lipid content, the separation of lipids from grease trap waste can be difficult and costly which could eliminate the environmental benefits of producing biodiesel from waste feedstocks.

Algae, Specialty Crops, and Biomass Supply

Global Evaluation of Biofuel Potential from Microalgae

Jeffrey Moody, Utah State University

The evaluation of microalgae based biofuel production systems through lifecycle, technoeconomic, and resource assessments have based growth models on the extrapolation of laboratory-scale data due to the immaturity of the technology. This type of scaling leads to large uncertainty in the results due to the inaccurate modeling of the current near-term productivity potential which typically serves as the functional unit. This study integrates a large-scale validated outdoor microalgae growth model that utilizes 21 species and reactor specific inputs to accurately account for biological effects such as nutrient uptake, respiration, and temperature with hourly historical meteorological data from around the world to determine the current global productivity potential. A global map of the microalgae lipid and biomass productivity has been generated based on the results of annual simulations at 4,388 global locations spread over the 7 continents. Maximum annual average yields between 24-27 m3•ha-1•yr-1 are found in Australia, Brazil, Colombia, Egypt, Ethiopia, India, Kenya, and Saudi Arabia.
with the monthly variability (minimum and maximum) yields of these locations ranging between 14 and 33 m\(^3\) ha\(^{-1}\) yr\(^{-1}\). A scalability assessment which leverages geographic information systems data to evaluate geographically realized microalgae productivity, energy consumption, and land availability has been performed highlighting the promising potential of microalgae based biofuels compared to traditional terrestrial feedstocks. Results show many regions can meet their energy requirements through microalgae production without land resource restriction. Discussion focuses on sensitivity of monthly variability in lipid production compared to annual average yields, biomass productivity potential, effects of temperature on lipid production, and a comparison of results to previous published modeling assumptions.

**Microalgae to Biofuels: Lifecycle Assessment of Alternative Conversion Technologies**

*Eddy Bennion, Utah State University*

The high solar energy conversion efficiency of microalgae has led to the evaluation of it as a feedstock for the commercial production of renewable transportation fuels and bio-products. Technological challenges currently exist in the conversion and stabilization of microalgae based bio-oils. This research uses a systems engineering approach, validated through experimental data, to evaluate multiple conversion processes of microalgae biomass to biofuel. Pyrolysis and hydrothermal liquefaction are the two conversion processes that this research focuses on followed by supercritical stabilization. A system boundary of “cradle to pump” was defined and includes biomass cultivation, dewatering, conversion, stabilization, conversion, and transportation and delivery. Contrary to traditional solvent extraction systems, pyrolysis and hydrothermal liquefaction both have the capability of improving yields through the conversion of non-lipid constituents to bio-oil. The resulting bio-crude is further processed to a drop in bio-oil through supercritical fluid processing techniques which stabilize the bio-oil followed by processing to fuel through transesterification. Results are presented on the metrics of net energy ratio and greenhouse gases. The net energy ratios from “cradle to pump” are 1.58 and 1.36 using pyrolysis and hydrothermal liquefaction as the conversion methods, respectively. The net energy ratio is defined as the energy going into the system divided by the energy coming out. A sensitivity to process parameters for identification of future research and development focus areas is presented with results directly compared to tradition fuels and currently published literature data.

**Plantain and Banana Fruit as Raw Material for Glucose Production**

*Sergio H. Duque Q.*

The plantain and banana fruit are characterized as a rich source of lignocellulosic material and starches. These fruits are among of the most important agroindustrial products in the world, which reached 37.2 million tons of plantain and 102 million tons of bananas in 2012. Likewise,
12% of plantain production is lost, 26.46% of the bananas produced to export were rejected and 6.67% of the total banana produced corresponds to waste. Moreover, the glucose is one of the most important multi-transformable raw materials, which allow the production of other add-value compounds. Hence, more than 20 derivates compound can be obtained either by fermentative or chemical way. In this work plantain and banana fruit were considered for glucose and xylose production. All these in order to generate: food application components directly, biodegradable materials and bioenergy. Experiments of the transformation stage were developed. The glucose production was achieved using enzymatic hydrolysis by alpha-amylases for pulp transformation and cellulases. Additionally, Industrial scale processes were proposed and simulated using Aspen Plus software for sugars production and add value-add components production. As results 316 kg glucose/Ton of plantain pulp and 57 kg glucose/Ton peel were obtained, 238 Kg of ethanol/Ton plantain pulp, 2 kg H2/Ton banana peel, 25 kg Acetic acid/Ton banana peel, 5 kg CH4/Ton banana peel and 153Wh/kg of plantain peel by gasification among others were obtained. The complete utilization of agro-industrial crops, produce value-added raw materials of high industrial application, which produce additional profits for farmers and reduces the underutilization of crops.

Renewable Chemical Platforms and Biobased Material

Method of preparative scale production of immobilized lipases destined for bioconversion, biotransformations and biorefining processes

Lukasz Stanczyk, Institute of Technical Biochemistry, Lodz University of Technology

Lipases are one of the most important and promising groups of catalysts used in industrial biotechnological processes. They are particularly attractive since they catalyze various reactions in aqueous and non-aqueous conditions, and exhibit the high catalytic activity and exquisite substrate selectivity, stereoselectivity and enantioselectivity. Furthermore, immobilised lipases can be reused, e.g., in continuous manufacturing processes often lasting several months, thereby saving energy and reducing wastes. The potential of lipases has been only partially exploited, mainly because of high costs of their purified preparations. Robust and inexpensive lipolytic preparations, well suited to technological conditions have been still prospected for. Certain whole-cell biocatalysts, like mycelium-bound lipases, which are presented in this work, meet these requirements. The main objective of presented project is the development of a method of large laboratory scale production of microbial lipase preparations, e.g. from lipolytic Mucor circinelloides and Mucor racemosus strains, originating from pure culture collection at ITB LUT. At IBT LUT it was developed the method of production of two forms of immobilized lipases, potentially useful in various branches of industry: cosmetic, pharmaceutical, production
of biofuels, food processing etc. Their properties were also characterized. These immobilized whole-cell preparations are as follows: 1. Mycelium of Mucor filamentous fungi, immobilized in a porous carrier in the form of uniform thin foams with open porosity and the large internal surface – for industrial applications this lipase may be adapted to the needs of a user. 2. Dehydrated and ground Mucor mycelium (particles of around 3 µm in diameter), additionally stabilized - for industrial applications in the form of water and/or organic solvents-insoluble powder. Key elements of the technology include: - activation of fungal strains for efficient production of the lipase (for transesterification of lipids with aliphatic alcohols and hydrolysis of lipids) – biosynthesis of the mycelium-bound enzyme is induced using selected esters, - selection of porous carriers (pore size, shape and dimensions of the porous carrier) useful for the immobilization of Mucor strains and checking the usefulness of the immobilized lipase preparations in selected manufacturing processes; - optimization of culture medium composition, and agitation and aeration modes, - development of methods of preparation and standardization of lipase preparations (conditions of mycelium de-fatting and de-hydration).

Specialty Chemicals, Pharma Intermediates, Food Ingredients

**Antiparasite Activity of Chitosan Prepared from Shrimp Shell Waste**

*Rym Salah-Tazdait, Mouloud Mammeri University of Tizi-Ouzou, Algeria*

Chitin is found especially in the structure of the shell of crustacean, cuticles of insects and cell walls of fungi. The waste of this natural polymer is a major source of surface pollution in coastal areas. Chitosan is obtained by the thermochemical deacetylation of chitin. It has been proved to be biologically renewable, biodegradable, biocompatible, non-antigenic, non-toxic and biofunctional. In the present study, chitin was chemically extracted from shrimp shells. The obtained chitin was deacetylated by NaOH to prepare chitosan. Then, chitin and chitosan were characterized. Further, antiparasite activity of chitosan was evaluated using Leishmania infantum LIPA 137 and Leishmania infantum LIPA 155/10, two reference strains isolated from patients in Pasteur institute from Algeria. The results showed effective antileishmanial activity of against Leishmania infantum LIPA 137, but no antileishmanial activity of chitosan against Leishmania infantum LIPA 155/10. It was also demonstrate that Leishmania infantum LIPA 155/10 is resistant to leishmaniasis drug glucantime® and Leishmania infantum LIPA 137 is sensitive to glucantime®. Further studies are necessary to determine the in vivo activities and applications of chitin and derivatives, in particular, in the design of new lines of drugs for use in the treatment of leishmaniasis and hopefully eradication. Keywords: antiparasite, chitin, chitosan, Leishmania infantum, leishmaniasis, waste.
Clarity of fruit juices is desirable to maintain an aesthetically pleasing quality and international standards. The most commonly used enzymes in juice industries are pectinases. A partially-purified pectinmethylesterase from tomato was entrapped in calcium alginate beads and used for juice clarification. The activity yield was maximum at 1 % (w/v) CaCl2 and 2.5 % (w/v) alginate. The immobilized enzyme retained ~ 55 % of its initial activity (5.68 x10-2 units) after more than ten successive batch reactions. The K??m, pH and temperature optima were increased after immobilization. The most effective clarification of fruit juice (% T620 ~ 60 %) by immobilized enzyme was at 4 °C with a holding time of 20 min. A viscosity drop of 56 % and an increase of 264 % in filterability were observed. The juice remains clear after two months of storage at 4 °C.

**Construction of reductive pathway in Saccharomyces cerevisiae for effective succinic acid fermentation at low pH value**

*Jianmin Xing, Institute of Process Engineering, Chinese Academy of Sciences*

Succinic acid is an important precursor for the synthesis of high-value-added products. Saccharomyces cerevisiae is a suitable platform for succinic acid production because of its high tolerance towards acidity. In this study, a modified pathway for succinate production was established and investigated in S. cerevisiae. The engineered strain could produce up to 6.17 ± 0.34 g/L of succinate through the constructed pathway. The succinate titer was further improved to 8.09 ± 0.28 g/L by the deletion of GPD1 and even higher to 9.98 ± 0.23 g/L with a yield of 0.32 mol/mol glucose through regulation of biotin and urea levels. Under optimal supplemental CO2 conditions in a bioreactor, the engineered strain produced 12.97 ± 0.42 g/L succinate with a yield of 0.21 mol/mol glucose at pH 3.8. These results demonstrated that the proposed engineering strategy was efficient for succinic acid production at low pH value.

**Matrix Entrapment of Cellulose Degrading Endo (1?4) β-D-Glucanase from B. licheniformis KIBGE-IB2 for Continuous Industrial Use**

*Asad Karim*, KIBGE, University of Karachi

Endo (1?4) β-D-glucanase [EC 3.2.1.4] is a type of cellulase that randomly cleaves the β-(1?4) glycosidic linkages in cellulose polymer chain. Endo (1?4) β-D-glucanase is widely used in several industries including biofuel, food, textile, paper and pulp industries. There are several limitations in using soluble enzymes because they show low stability under harsh operational conditions and the product recovery becomes difficult and unfeasible for continuous usage.
Immobilization technology is one of the solutions that not only overcame the aforementioned problems but also made the process more cost effective. Therefore, the current study was designed to study the effect of immobilization using entrapment technique for endo (1\4) \( \beta \)-D-glucanase produced by B. licheniformis KIBGE-IB2. For this purpose non toxic, non protein reactive biopolymer known as agar-agar was used. A maximum immobilization yield of 66.0% was achieved using agar-agar. The enzyme entrapped in this matrix exhibited broader pH and temperature activity profile as compared to the soluble enzyme. The temperature optimum for the soluble enzyme was 60°C and it shifted to 70°C after entrapment. This immobilization also showed greater thermal stability up to 80°C as compared to the soluble enzyme. It was observed that the entrapped enzyme could also be used repeatedly for the degradation of Carboxymethyl cellulose (CMC). The enzyme in agar-agar gel retained its activity even after 8 cycles of usage. The results indicate a possibility of employing matrix entrapped endo (1\4) \( \beta \)-D-glucanase from B. licheniformis KIBGE-IB2 for various industrial purposes.

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**Entrapment of Enzyme within Calcium alginate beads: An Efficient Method for increasing the catalytic performance of industrially important Maltase**

Muhammad Asif Nawaz*, KIBGE, University of Karachi

Maltase catalyzes the degradation of maltose into glucose and plays a central role in food industries. Partially purified maltase from Bacillus licheniformis KIBGE-IB4 was immobilized within calcium alginate beads using entrapment technique. The immobilization parameters such as sodium alginate and calcium chloride concentration were optimized and 4.0 % sodium alginate and 0.2 M calcium chloride was found to be optimum for maximum immobilization of maltase. The calcium alginate beads having 2.0 mm bead size showed higher activity. The optimum reaction time and temperature for maximum catalytic activity of maltase was increased after immobilization, whereas the optimum pH was remained same for both free as well as immobilized enzyme. The stability of maltase against different temperatures was increased after immobilization and immobilized maltase showed higher resistance against different temperatures as compared to free maltase. The immobilized maltase showed admirable recycling efficiency and retained more than 60 % of its initial activity after third cycle. The strategy of immobilization of maltase within calcium alginate beads was seem to be cost effective technique to develop maltase bioreactor for the commercial utilization in various food industries.

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Scale up of chito-oligomer production via bacterial fermentation
Hendrik Waegeman, Bio Base Europe Pilot Plant

Chito-oligomers constitute an interesting class of specialty carbohydrates, among other applications used in plant protection and wound healing products. Today’s commercially available chitosans are produced chemically from chitin isolated from shrimp shell wastes. They can be well defined concerning their degree of polymerisation and degree of acetylation, but they are invariably characterised by a random pattern of acetylation (PA), despite this influences the activity greatly.

Synthetic Biology and Genomics Research

Accelerating Microbial Metabolism by Controlling Redox Potential during Fermentation Process
Yanping Zhang*, Institute of Microbiology, Chinese Academy of Sciences

Many important biochemical reactions are catalyzed by reductases or dehydrogenases, whose activities are dependent on the availability of NADH and the ratio of NADH to NAD+. In a previous study, we found that the intracellular NADH/NAD+ ratio was sensitive to the level of extracellular redox potential (also named oxidoreductive potential, ORP). To investigate the mechanism of ORP control, we applied comparative proteomic analysis and genomic-level metabolic flux analysis. Using Klebsiella oxytoca as a model, we found that the proteomic profiling was changed in response to the extracellular ORP level. Furthermore, we found that the metabolic flux via pyruvate dehydrogenase increased significantly under low-ORP condition. It might result in increased availability of NADH. The combined analyses revealed the relationship between ORP control and metabolism alteration. Controlling redox potential during the anaerobic and micro-aerobic fermentation processes was shown an efficient strategy to accelerate microbial metabolism. It has successfully improof the fermentative production of 1,3-propanediol by Klebsiella oxytoca, butanol by Clostridium acetobutylicum, and increased the biomass yields of Lactobacillus and Bifidobacterium.

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Growing Global Markets
Industrial biotechnology is fueling one of the greatest booms in global innovation in all of history. Innovation lies at the heart of every new company and new initiative in the field. Those innovations, and the investment and creativity that goes into them, can be protected by patents in the US and throughout the world -- at least in most cases. However, there are some discoveries that are not eligible for patenting, and premature disclosures of inventions can damage or destroy the opportunity to patent those inventions. Different countries have different standards when it comes to evaluating the inventiveness of an innovation and whether it merits a patent. Business people, scientists, attorneys, and all involved in the innovative process need to have some understanding of the patent laws of the US, along with similarities to and differences from the patent systems in Europe, China and other major markets. This paper and presentation will educate attendees about patenting innovations in industrial biotech throughout the world.

**Academic Research Presentations**

*A novel perfusion system for enhanced therapeutic protein production from mammalian cells*

*Pradip Roychoudhury, Indian Institute of Technology, Delhi*

The demand for therapeutic proteins has been increasing at a pace faster than that at which the new production technologies are being developed. Among the various production technologies presently being used, perfusion culture technology is preferred over others because it enables selective cell retention for achieving high cell density and consistent product quality. In addition, it improves productivity and the economic outlook of the process. However, there is scope for improving perfusion technology to achieve higher protein production as the existing cell retention modules are prone to clogging during long term operations. Considering this, we developed a novel spinfilter module using a silk membrane possessing superior cell retention properties. With this module, we achieved higher viable cell retention and lesser fouling compared to stainless steel modules that are normally used for such processes. It was possible to operate the perfusion culture of hybridoma cells (HB8696) for monoclonal antibody production uninterrupted for more than 200 h with the silk spinfilter. A performance comparison of silk spinfilter with the stainless steel spinfilter showed a 57.4% increase in monoclonal antibody production. The spinfilter module is suitable for perfusion culture of both adherent and non-adherent mammalian cells.
Biopolymers Production by Xanthomonas campestris PV campestris From Glicerine, by-products of biodiesel production

Francisca Pessoa de França, Universidade Federal do Rio de Janeiro

The growing interest for biofuels makes biodiesel a renewable alternative, biodiesel came to supply part of the expectations of the energy segment, however, its production has increased in recent years, generating as a consequence, large amounts of glycerol, the main by-product of the biodiesel production process; Glycerol, can replace carbohydrates usually employed as a source of carbon, in the cultivation and growth of some microorganisms for the production of bio-products. The xanthan gum is an extracellular biopolymer consisting of glucose, mannose, and glucuronic acid, high molecular weight anionic, capable of forming high-viscosity aqueous solutions, produced by Xanthomonas campestris pv campestris, The biosurfactant are compounds of microbial origin, produced extracellularly or as part of the cell membrane by different microorganisms. This work aimed to evaluate the effect of substituting, supplementing and potentiality of glycerol as carbon source on production of xanthan gum and biosurfactante. The medium of production were composed of different concentrations of glucose and glycerol (g / L) in 100 ml of mineral medium, as a carbon source, with stirring speed of 180 rpm at 28° C for 96 hours, using the bacteria Xanthomonas campestris pv campestris. The study was done using experimental planning 22 with 4 experimental conditions and 3 central points, in order to evaluate various components such as the gum production (g/L), and the biosurfactant, that was evaluated through the index of emulsification and 24%. The study demonstrated that the maximum production of gum (6.48 g/L) was at a concentration of 1.5% glucose and glycerol. When the mineral medium had a low content of glucose, the gum production was reduced, however, the formation of biosurfactant was relatively high with 55% for emulsification index with an aviation kerosene. The observed results indicated a possible application of the bacteria using waste by-products from biodiesel industry.

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Malathion Biodegradation: Evaluation of its use as phosphorous and sulphur sources

Djaber Tazdait, Mouloud Mammeri University of Tizi-Ouzou

Malathion S-[1, 2- di (ethoxycarbonyl) ethyl] dimethyl phosphorothiolothionate; CAS No 121-75-5; C10 H19 O6 PS2] is one of the most widely used organophosphate insecticides throughout the world. It is commonly used to control mosquitos and a variety of insects that attack fruits, vegetables, landscaping plants and shrubs. Removal of this pesticide can be attained by physical-chemical and biological processes. Several studies have examined the degradation of malathion by microbes and most of these studies were carried out using pure cultures. Little
information is available concerning degradation of malathion by activated sludge culture. In most studies of xenobiotic degradation in general, and malathion degradation in particular, the compounds under consideration have been supplied to microorganisms exclusively as sources of carbon. But their utilization as source of phosphorus and sulphur has been less well studied until now. Therefore, in this study, the biodegradation of malathion using acclimated activated sludge culture was achieved. The ability of mixed microbial community to use malathion as a source of phosphorus and sulphur nutrition was also evaluated. The result showed the potential for using local activated sludge for malathion biodegradation. On the other hand, the acclimated activated sludge could use malathion as its sole phosphorus source but could not use it as its sulphur source.

**Microwave-assisted synthesis of n-butanol directly from bioethanol using bulk MgO**

Dr. Idan Chiyanzu, North West University

Bio-butanol is an attractive alternative drop-in fuel as replacement for fossil-based petroleum in internal combustion engines. The application of bulk magnesium oxide (MgO) catalyst for n-butanol production was investigated in this study. The phase purity of the catalyst was confirmed by X-ray diffraction (XRD). Transmission Electron Microscope (TEM) was used to observe the morphology and size of the catalyst. Nitrogen adsorption and CO2 desorption using Brunauer- Emmett-Teller (BET) analysis were done to confirm the surface area of nanoparticles of the MgO. Thermogravimetric analysis (TGA) provided insight into the decomposition process of the catalyst at different temperatures. The effect of the catalyst on n-butanol yield was studied by varying the mass loading (0.1-0.5 g), and the microwave temperature (50-250°C). The average particle diameter for MgO ranged between 50-200 nm. The MgO surface area obtained from BET studies was found to 44 m²/g. Ultimately, the microwave method was compared to n-butanol production using a hot plate thermostat at different temperatures (50-250°C). The study will highlight the effect of different amounts of MgO as a catalyst on microwave-assisted butanol productivity from bioethanol. Keywords: Bulk MgO, Microwave, n-butanol

**Aerobic methane-oxidizing communities in saline alkaline and arable soils**

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The former lake of Texcoco is located in the Valley of Mexico City (Mexico). This is a unique and extreme soil, highly saline-sodic, with electrolytic conductivity (EC) up to 150 dS m⁻¹ and pH up to 10.5. The composition of aerobic methane-oxidizing bacteria (MOB) communities was studied in three soils from the former lake Texcoco (Mexico) and compared to that in two arable soils. The MOB were identified on the basis of comparative sequence analysis of the
pmoA gene, that encodes for a subunit of the particulate methane monooxygenase, a key enzyme in the aerobic methane oxidation process. The sequences from the arable soils belonged to type I and II methanotrophs. The clones were closely related to JR-2, JR-3, USC-? and USC-a clusters, that have previously been detected in upland soils. In the saline alkaline soils, a novel group of sequences related to the Nitrosococcus-like clade was detected (> 92% of clones sequenced). These data indicated that the methanotrophic communities in the soil from the former lake Texcoco differed from those in arable soils and further experiments are needed to confirm that the methanotrophic community found in Texcoco soils oxidizes methane under adverse conditions.

**Biomass as a source of feedstocks for the preparation of environmentally friendly polymer materials**

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This poster presentation contains the main ideas of the POIG project BIOMASA partially financed by the European Union within the European Regional Development Fund. The aim of the project is utilization of various kinds of plant biomass and textile waste materials by their transformation with biotechnological methods, involving either enzymatic or microbial processes, into fibrous polymer materials. Cellulose nanofibres For the preparation of cellulose nanofibres, a cellulose-rich plant biomass is being utilized, including grass and straw of various cereals as well as waste fibres from textile industry (cotton, linen). The biomass is first pretreated with physical and/or chemical methods including boiling, steam-explosion or treatment with certain chemicals. Multienzyme complex obtained from Aspergillus niger mould is utilized as the main enzymatic tool. The fibrous materials and composites prepared within this project on the basis of abovementioned intermediates will be further utilized for obtaining new functional textiles and nonwovens with potential sanitary or technical applications, such as sweat-absorbing textile inserts, sanitary textiles, filtration materials, geotextiles and agrotecxtiles. Within this project, the processes of ageing and controlled biodegradation of prepared materials will be studied, as well as the conditions of their recycling and possible use of degradation products in agriculture. Tactic polylactide The synthesis of tactic polylactide is being performed by chemical polymerization of L,L-lactide, prepared from L-lactic acid. The latter is obtained by stereoselective fermentation of plant biomass, after its saccharization by appropriate enzymes (Aspergillus niger preparations). The microorganisms (bacteria), used for the fermentation, were selected by classical microbiology methods from the environment. In this case potatoes, cereal grains or beet pulp are employed as starting biomass. The tactic polylactide will be further utilized for fiber formation and thermoforming. Co-polysteres The third path involves utilization of various oil-plant biomass, which on sequential treatment with lipase preparations obtained from Mucor circinelloides and Mucor racemosus moulds
(transesterification with 2-methylbutanol) and dimerization of obtained esters (cycloaddition) are transformed into dimeric esters containing fatty acid residues. These will be co-polymerized with appropriate reagents in order to produce new biodegradable aliphatic-aromatic copolyesters. The polyesters will be utilized as fillers for the preparation of various fibrous polymers and composites. Concluding remarks The project is being realized by Polish Consortium with the Lodz University of Technology being the leader. The methods of preparation of polymer fibrous materials and composites elaborated within this project will positively influence development of knowledge-based economy and will increase the innovativeness of connected areas of research and production. The main recipients of elaborated methods will be producers of fibers and nonwovens from thermoplastic materials, sanitary textiles, filtration materials, geotextiles, agrotextiles and packing materials. Acknowledgment The Project (POIG 01.01.02-10-123/09) is partially financed by the European Union within the European Regional Development Fund.

**Effect of Metabolic Regulators in the Production of Alpha-Amylase by Aspergillus sp. Using Lignocellulosic Substrate for Potential Industrial Applications**

*Shalini Singh, Lovely Professional University*

The concerned paper talks about the influence of antibiotics on production of alpha-amylase by Aspergillus fumigatus NTCC1222, Enzymes—the biocatalysts, have changed the definition of industrial growth and development by envisaging the features of ‘environment friendliness’ and ‘cost-effectiveness’ to conventional chemical based products and processes. Government regulations and public awareness have shifted the focus of researchers worldwide to develop products and processes that offer environment friendly alternatives to already existing chemical processes. Thus, large scale production of enzymes an area of major interest for researchers. Since biotechnological applications require large amounts of low cost enzymes, one of the appropriate approaches is the search for powerful enzyme producers and the utilization of the potential of lignocellulosic wastes/by-products, which may contain significant concentrations of soluble carbohydrates and inducers of enzyme synthesis, ensuring efficient production of various enzymes. Alpha-amylase is one of the most important industrial enzymes with numerous industrial applications and its production is influenced by a number of factors including, metabolic regulators. In the current study, the influence of antibiotics on alpha-amylase production by indigenously isolated fungal strain Aspergillus fumigatus NTCC1222, under solid state fermentation conditions using cheap, readily available lignocellulosic substrate (wheat bran), was studied. The fermentation medium was supplemented with variable concentrations of antibiotics (streptomycin, tetracycline and chloramphenicol) and the amylase activity of same was compared with unsupplemented fermentation medium. Simultaneously, the effect of antibiotics on fungal growth was also monitored. Amylase activity was found to
improve for streptomycin-supplemented fermentation medium at a concentration of 80µg/mL while it decreased at other concentrations used. On the contrary, tetracycline and chloramphenicol decreased the amylase activity as compared to unsupplemented fermentation at all concentrations used. Interestingly, the growth of the test fungus improved in the presence of streptomycin at all concentrations, improved at 80 (+28.43%) and 100 (+17.73%) µg/mL of tetracycline but decreased in the presence of chloramphenicol at all concentrations used, as compared to unsupplemented fermentation medium. The study signifies the influence of antibiotics on fungal amylase production and fungal growth in the presence of cheap substrate as the carbon source.

**A novel perfusion system for enhanced therapeutic protein production from mammalian cells**

Prof. P.K. Roychoudhury, Indian Institute of Technology

The demand for therapeutic proteins has been increasing at a pace faster than that at which the new production technologies are being developed. Among the various production technologies presently being used, perfusion culture technology is preferred over others because it enables selective cell retention for achieving high cell density and consistent product quality. In addition, it improves productivity and the economic outlook of the process. However, there is scope for improving perfusion technology to achieve higher protein production as the existing cell retention modules are prone to clogging during long term operations. Considering this, we developed a novel spinfilter module using a silk membrane possessing superior cell retention properties. With this module, we achieved higher viable cell retention and lesser fouling compared to stainless steel modules that are normally used for such processes. It was possible to operate the perfusion culture of hybridoma cells (HB8696) for monoclonal antibody production uninterrupted for more than 200 h with the silk spinfilter. A performance comparison of silk spinfilter with the stainless steel spinfilter showed a 57.4% increase in monoclonal antibody production. The spinfilter module is suitable for perfusion culture of both adherent and non-adherent mammalian cells.

**Production of lactic acid production in a novel fermentation and separation integration system**

Xuerong Xing, Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences

Lactic acid is an important platform chemical. A novel lactic acid fermentation and separation integration system which combines microbe fermentation, cross flow microfiltration and product adsorption processes was set up in this study. The novel approach can overcome product inhibitory and enhance cell growth period from 41 h to 120 h. The final improved lactic acid production increased 1.26-fold, up to the final titers of 183.4 g/l with an overall yield of
0.97 g/g glucose and 1.53 g/l/h productivity. The experimental results indicated that the system could be economically viable for continuous production of lactic acid at high level.

Technical Presentations

*Enhanced Biofuels Production from Lignocellulosic Biomass by Microwave-assisted Pretreatment*

*Wei Huang, Beijing Research Institute for Nutritional Resource*

Lignocellulosic biomass are not easily utilized by microorganisms due to their physical shielding of cellulose imparted by the non-digestible lignin. Therefore, there is a great interest to develop an efficient pretreatment technique to disrupt recalcitrant structures of lignocellulosic biomass and improve renewable energy production. Microwave energy can efficiently penetrate plant materials and produce a volumetrically distributed heat source, and the highly localized temperature and pressure can cause serious destructive effect of cellulose structure, and therefore enhance resolvability of lignocellulosic materials. Microwave irradiation has been successfully integrated with alkali/acid pretreatment process to enhance enzymatic hydrolysis of lignocellulosic biomass for ethanol fermentation and biogas production. The aim of this poster is to identify the problems related to the microwave assisted pretreatment process with emphasis placed on developing novel strategies for biogas production from lignocellulosic biomass.

*Conversion of various oils using lipolytic fungal biomass in non-water systems*

*Miroslawa Szczesna-Antczak, Institute of Biotechnology and Food Sciences, Lodz University of Technology, Lodz, Poland*

Bioeconomy has been one of most dynamically developing sectors of EU economy. Its basic goal is the replacement of fossil fuels with biomass as a renewable source of valuable products and/or feedstocks for industry. Foundation of the industry on natural resources and bioprocesses is the prerequisite of sustainable development. Presented results were achieved within the frames of a research project with an acronym: Biomass, entitled “Application of biomass in production of environmentally friendly polymer materials”, which has been realized by a consortium of several research institutions in Poland: Lodz University of Technology, Institute of Biopolymers and Chemical Fibers in Lodz, Centre of Molecular and Macromolecular Studies of Polish Academy of Science, University of Agriculture in Krakow and Central Mining Institute in Katowice. One of objectives of tasks 2.2 & 3.2 of this project is the development of a chemo-enzymatic method of oleaginous biomass conversion into biodegradable components of
aliphatic-aromatic polymers for fabrication of agro-textiles. The team from the Institute of Technical Biochemistry (ITB) LUT devised a biocatalyst, which is inexpensive and highly active in non-aqueous systems (task 2.2), and optimized conditions of its effective usage in processes of oil bioconversion (mainly rapeseed, sunflower, soybean and waste oils) into esters of aliphatic primary alcohols (also branched) or structured SUS-type triacylglycerols (saturated-unsaturated-saturated acid bound to glycerol) (task 3.2). The latter may be further converted into dimers and macrodiols, which will be used in polymerization processes. Immobilized in porous carriers, whole-cell (mycelial) preparations of intracellular lipases produced by oleaginous and lipolytic fungal strains from the culture collection at ITB, which are robust and highly active in non-aqueous systems, have been used to develop semi-continuous transesterification processes, e.g. plant oil alcoholysis by 2-methylbutan-1-ol (or other medium-chain alcohols) and acidolysis by saturated fatty acids (especially palmitic and stearic). Operational stability of these biocatalysts in column PBR reactors (working volume of 0.2-0.5L) either with petroleum ether used as a solvent (or without it), under suitable process (acidolysis and alcoholysis) parameters reaches about half a year (or more) without any decrease in bioconversion yield. Identification of crucial parameters deciding of transesterification processes efficiency and high stability of the biocatalyst guarantees the successful up-scaling of these processes. Keywords: bioconversion, alcoholysis, acidolysis, whole-cell lipase preparation, high operational stability Acknowledgement The project BIOMASA (POIG 01.01.02-10-123/09) is co-financed by the European Union within the European Regional Development Fund (in the frames of Operation Program Innovative Economy 2007-2014).

Investigation of yeast performances in the fermentation of first generation feedstocks

Rishi Jain, Praj Matrix – The Innovation Center

Usage of first generation feedstocks such as cereals and molasses is a source of potable ethanol and currently is also being sourced as fuel ethanol. Starch from grains such as cassava, corn, sorghum, sweet potato and wheat is the source of sugars that is fermented to ethanol. Molasses is the non-crystallizable residue left over after sucrose purification from sugarcane or sugar beet juice. Unlike starch from grain feedstocks, the quality of molasses varies a lot depending on the harvesting stage of the crop, amount of sugar extracted and the techniques used to extract the sugar. The focus of this presentation will be on the challenges associated with yeast performances in the fermentation of first generation feedstocks. Specific hurdles will be laid out not only with respect to the composition of these feedstocks but also with respect to the fermentation process parameters. A detailed review of research activities catering to these specific problems will be discussed. Possible solutions from a strain development perspective will be discussed that will combine classical methods as well as metabolic engineering techniques.
This presentation will detail a novel new system (patent applied) for real-time analysis and monitoring of algae production. The system uses in-flow digital imaging to capture images of all representative cells or other microorganisms in photo bioreactors or raceway ponds. Sophisticated image processing algorithms are used in real time to segment each microorganism from the background, and record over 30 size, shape and gray-scale measurements for each microorganism. Cell size and concentrations are produced in real-time, and are used for trend analysis. This system can be hooked into any part of the production flow loop for analysis at any point in the process. The system is Class I, Div. I compliant, and automatically cooled to maintain proper working temperature on-site. Using a unique auto-dilution system, the concentration is adjusted for optimum presentation of the microorganisms to the imaging system. Since every particle image and its measurements are saved by the system, it creates an ironclad audit trail for how data was recorded. Test data collected in the field will be shown illustrating typical results from the system. A short video will show how the system works in real-time, including how the particle images are acquired and measurements made. Finally the results of the analysis will be shown, detailing how the system can be used to monitor microorganism size and concentration, and in particular, identification of predators.