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™.

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(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

David Glass, D. Glass Associates, Inc.

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.

The Electrobiome^{TM}: a Microbial Platform for the Electrosynthesis of Chemicals and Fuels from CO2.

Harold May, Biomedicine & Environmental Science Center, Medical Univeristy of South Carolina (MUSC)*

The Electrobiome^{TM} is for the chemical, fuel, polymer, and carbon capture/sequestration industries. It is a microbial community platform that lives and operates within an electrochemical bioreactor where it uses electricity to capture and convert CO2 into chemicals and fuels. Unlike present synthesis methods, it avoids the use of fossil carbon, food crops, arable land; and it consists of inexpensive, self-replicating microbes (not precious, rare-earth catalysts). The system has been continually operating for more than two years, which demonstrates sustainability that far surpasses other electrosynthetic microbiomes. As an added benefit, the electrical input into the ElectrobiomeTM can be intermittent, allowing it to run only when low-cost electricity is available. Production rates have reached >1 kg of H2 or acetic acid per m3 reactor vol per day at a Coulombic efficiency (electrons cpatured in product) of >90%. Total electrons passed through the ElectrobiomeTM indicate that acetate production could reach a kg/m3/h, which would capture 1.5 kg of CO2 per kg of acetic acid generated. Other products include formic, propionic, butyric, and isobutyric acids, and the entire collection of products may be used as feedstocks for the biological production of liquid hydrocarbons and bioplastics. Markets for all of the ElectrobiomeTM products are sizeable and growing (annual global: H2 to $118B by 2016, fatty acids to $13B by 2017, and bioplastics in US to $7.7B by 2016). Further growth for most of these markets will depend on the availability of oil, gas and coal, which are scarce in many countries and are an environmental concern, particularly as a source of fossil carbon. The cost of the electricity (@$0.05/kWh) delivered to the ElectrobiomeTM to produce 1 kg of product has been <$3 for H2 and $0.35 for acetic acid, which places the system within range of becoming cost competitive. The H2 may be used directly as a stationary or transportation fuel (1kg of H2 ≈ 1 gal gasoline equivalent) or for further chemical or biochemical processing. Formic and acetic acids are used as food additives and preservatives or to produce adhesives, plastics, paints, and dyes. All of these products may be used as feedstocks for the production of bioplastics, and in another proprietary process the inventors are modifying the electrobiome to produce liquid hydrocarbons. The intellectual property associated with the ElectrobiomeTM is aggressively being protected through patent filings, including application PCT/US2013/060131. For more information please visit www.electrobiome.org.

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Electrofermentation to Produce Fuels from Carbon Dioxide

Dan Derr, Logos Technologies*

The ability to convert renewable electricity and captured carbon dioxide into liquid transportation fuels is a novel approach to biofuels production. This approach has a number of high risk technical challenges that need to be overcome for the economical production of fuel; however the possible efficiency gains are large enough to necessitate exploration. If the electricity is renewably generated from non-bio (e.g., solar, wind, or tides) sources the energy inefficient photosynthetic step is taken out of the equation. Higher efficiency means land, water, and nutrients necessary are also lower. Logos Technologies has designed and demonstrated a technology which takes advantage of widely available electricity and carbon dioxide for the production of biofuels. The technology is based on an electrofermenter – a fermenter that generates hydrogen and oxygen in situ. Hydrogen and carbon dioxide gases are consumed by a microbe in the production of biofuels. The microbes, Ralstonia organisms provided by Steve Singer at the Joint BioEnergy Institute, grow and produce methyl ketones in the presence of an applied electric field. This engineered Ralstonia eutropha has had a modification to the fatty acid synthesis pathway that enables production of methyl ketones from hydrogen, oxygen and carbon dioxide. These methyl ketones can be used as drop-in replacements for petroleum derived fuels. We will discuss the design and operation of the electrofermenter. Specifically, we will demonstrate growth of the Ralstonia organism in the electrofermenter, including the optimum seed train approach and strategies for reducing the effect of growth due to the oxygen produced at the counter electrode. We will also discuss our progress in demonstrating methyl ketone production. Techno-economics will be presented showing the commercial potential of the electrofermenter in alternative biofuel production.

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Novel Bioreactor Designs for Rapid Methane Fermentation

Dr. Joshua Silverman, Calysta Energy, Inc.

Methane-based fermentation is fundamentally different from 'traditional' glucose-based fermentation for several reasons, including: the low solubility of methane in aqueous solution, the need to co-feed multiple (explosive) gasses at high mass transfer rates while simultaneously allowing efficient extraction of CO2, and significant heat loads generated from the metabolism of the high-energy methane substrate. These issues are more similar to those faced in

'traditional' chemical reactor design where many approaches to mitigate the above issues have been developed.

Conventional stirred tank bioreactors are energy-intensive, typically operated at atmospheric pressure with poor mass-transfer, and do nothing to mitigate the explosive mixture issue. Air-lift reactors show poor mixing rates, poor heat transfer, and are unlikely to support the high growth rates needed for a commercial process. Currently, the U-loop fermenter is the only example of a purpose-built, commercial scale, operating methanotroph fermenter. However, capital costs are very high, suggesting that the plant has been overdesigned.

The objective of this project is to develop key bioreactor technology to enable efficient methane-to-biofuel fermentation processes. Although sugar-based fermentation is well-established with a variety of off-the-shelf reactor technologies available, relatively little effort has been expended to address gas-fed fermentations. This is significant because gas-fed fermentations present a number of unique challenges which must be addressed, such as low rates of heat and mass transfer and accumulation of explosive gas mixtures. By utilizing the project team's unique expertise in reactor design in the chemical industry and in methanotroph fermentation, we plan to develop specialized reactors that can serve as the basis for the production of a variety of fuels and fuel precursors via bioconversion of methane. Redesign of the bioreactor will be performed using computational fluid dynamics (CFD) software FLUENT. A bench-scale version of the new design will be built and tested using Calysta's target strain to demonstrate operating parameters and performance metrics.

The bioreactor technologies developed in this project will position methane as a new biological feedstock for the domestic, cost-competitive production of biofuels (referred to as a biological gas-to-liquids (BioGTL™) process). Importantly, the project team will make the developed reactor technology available to the research community at large via partnering and toll manufacturing. The availability of gas-fed reactors on a 'pay-as-you-need' basis is expected to significantly reduce the hurdles for research groups to move from lab-scale to process validation, resulting in a faster path to market for new methane-based bioprocesses.

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Bio-GTL: Novel Biocatalyst For Conversion Of Natural Gas Into Diesel Fuel

Richard Bolin, NREL

A team led by University of Washington and including the National Renewable Energy Laboratory, LanzaTech and Johnson Matthey was awarded ARPA-E funding to develop a process for the biological conversion of natural gas to liquid fuels (Bio-GTL) as part of the REMOTE program. This project is designed to capture and valorize methane from natural gas that is

currently wasted through venting and flaring and thus add to our national energy portfolio and mitigate greenhouse gas emissions. Our approach is to engineer methanotrophic bacteria for the production of lipids, develop a scalable, low-cost low-energy cultivation system and to develop a catalytic process to convert the lipids to liquid fuel.

Commercial lignocellulosic biofuels in Brazil boosting future development opportunities

Viviane Serpa Muller, Novozymes Latin America LTDA

Biomass to biofuels is a reality in Brazil. The commercial bio-route process to transform biomass into ethanol incorporates the use of Novozymes enzymes. The first step has been taken to produce biofuels and biochemical from lignocellulosic feedstock materials at commercial scale. It is time to take a look into alternatives and details to advance even further into the sustainable bio-economy. The way sugar cane is harvest in association with an integration of 1G with 2G can also play an essential roll on future developments. Biomass is readily available in Brazil, manly sugar cane bagasse and sugar cane straw. The comparison of the hydrolysis of these two pretreated feedstock materials can make all the difference to the cost of the final products from the 2G process. This is just one example of a topic to be taken into seriously consideration and comprehended from different angles. The success of these 2nd generation processes is sparking more and more opportunities for ways lignocellulosic bio-products can be made.

Techno-economic Analysis of Jet Fuel From Camelina

Robert H. Natelson, North Carolina State University

A team of NCSU scientists are genetically modifying camelina sativa (a short growing interval, cool season oilseed crop) to improve its stress tolerance, increase its seed yield, improve oil composition, and increase limonene content: in summary, to improve camelina as a source of jet fuel precursors. We are building the techno-economic tools for investors and adopters to evaluate commercialization of the camelina to jet fuel production system. The tools will include user-friendly modules for analyzing costs of crop production, oilseed transportation, seed-to-oil processing, and oil-to-fuel refining. Two different oil-to-fuel refining technologies are assessed: the commercialized hydrodeoxygenation process and the NCSU-patented hydrolysis and catalytic decarboxylation process. A module for analyzing returns of jet fuel and co-products (diesel, gasoline, LPG, camelina meal, and dry ice) is also included. Many risks such as crop yield, and decision variables such as refinery location and size, and oil profile can be analyzed and managed through the model. As anticipated, yield has a large effect on reducing crop production and transportation costs. Depending on the transgenic outcomes and other uncertainties such as camelina meal value, many parameters in the model can be optimized for

reduced risk and increased probability of profitability. Preliminary results indicate that with transgenic improvement, camelina has an increased potential to be a profitable biofuel crop.

Mild Biomass Liquefaction Process for Economic Production of Stabilized Refinery-Ready Bio-Oils

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Previous attempts to scale up and commercialize biomass liquefaction processes, such as PERC, LBL, and Shell HTU have not been successful due to severe process conditions and a lack of focus on both the process and product. The goal of this work is to commercialize a cost-effective low severity methanol-based solvent liquefaction process to convert woody biomass to stabilized bio-oils that can be directly blended with petroleum refinery hydrotreater/cracker streams for production of gasoline and diesel range hydrocarbons. The low severity and cost is being achieved by using mild conditions, maximizing the solids processing, minimizing catalyst use, minimizing solvent consumption, and minimizing wasted organics. The process is being optimized at laboratory scale. Several laboratory scale tests of the process have been conducted over a range of statistically designed test conditions. The results of these tests show that there is significant effect of reaction conditions on biomass conversion and bio-oil yield. At optimum reaction and separation conditions, 98 % of the biomass was converted and over 55 % of the biomass was converted to stabilized bio-oil. A commercial embodiment has been developed and a technical and economic analysis and life-cycle assessment of the process is being carried out. Stabilized bio-oil samples in sufficient quantities have been produced simulating the commercial embodiment for hydro-treating studies.

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Algae, Specialty Crops, and Biomass Supply

Global Evaluation of Biofuel Potential from Microalgae

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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 m3•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.

Renewable Chemical Platforms and Biobased Material

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

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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)

Thermostability properties of a xylanase XynA from Caldicellulosiruptor sp. F32: important influence of carbohydrate binding module and non-regular region amino acids

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Xylanase is one of the key enzymes in the degradation of lignocellulosic biomass. We described here the characteristics of xylanase XynA from extremely thermophilic anaerobic bacterium Caldicellulosiruptor sp. F32 and its thermostability properties. XynA wild type protein WT has a specific activity at 2601 U/mg when using beechwood xylan as enzymatic substrate, with the optimal temperature at 75 °C and pH 6.0. Truncation mutant protein TM1 without carbohydrate binding module (CBM) exhibited a great improvement in the specific activity (215.8 U/nmol vs. 94.7 U/nmol) and thermal stability (t1/2 of 48 h vs. 5.5 h at 75 °C) compared with WT. Site-directed mutagenesis at N-terminal amino acids located in non-regular region were carried out. The residual activity of mutated protein TM1AA2,3TS is 76 % after heat treatment at 75 °C for 48 h, while protein TM1 only have 50 %. The thermal stability of XynA and its mutanted proteins were consistent with its melting temperature (Tm) investigated using differential scanning calorimetry (DSC). Hydrolysis products from beechwood xylan catalysed by WT were mainly composed by cellobiose to cellopentose. These results demonstrated that XynA is a thermostable xylanase with high catalytic stability, meanwhile, CBM and non-regular region amino acids are critical for the thermostability of the protein which make XynA an interesting enzyme for biotechnological application.

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Specialty Chemicals, Pharma Intermediates, Food Ingredients

Antiparasite Activity of Chitosan Prepared from Shrimp Shell Waste

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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 deacetyled 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.

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

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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.

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 impoved 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

Patenting Innovation in Industrial Biotechology

Michael Harlin, Neal, Gerber & Eisenberg

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 prefered 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 to 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 biopolimero 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% 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.
Acknowledgements: The aurhors are grateful to the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and to Centro de Pesquisas da Petobras (CENPES) for financial supports.

Malathion Biodegredation: 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-Emmet-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 (50-250oC). 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 (50-250oC). 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

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

Gabriela Dziworska, Lodz University of Technology, Lodz, Poland

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 agrotextiles. 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 patatoes, cereal grains or beet pulp are employed as starting biomass. The tactic polylactide will be further utilized for fiber formation and thermoforming. Co-polyesters 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 co-polyesters. 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

Dr. Shalini Singh, Lovely Professional Universit, Punjab, India

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 prefered 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.

A presentation on biobased economy applications in the eu and in taiwan on waste microalgae culture with biofuels production study.

Renee Yuan, Tamkang University

Facing limited resources on Earth against sustainable development of our economy, the perception in the EU of high technologies role for our future generations has been polarized due the very fast developments of creative and innovative systems to find viable transition within two decades. Biotechnologies progress shows promising potential to reduce C, Waste excess, Global warming risks, and grow this new Sector of economy. Biotechnological conversion from Biomass into agro-foods, nutraceuticals, industrial chemicals, bio energies forms a primary resource in terms of entropy could reverse situations for present threats: environmental, climatic, social and economic. It will change the way we live and work if potential for sustainable production and conversion of biological material is fully exploited and evaluated to address to long term visions – arable and livestock farming, forestry, food, aquaculture, chemical industry, materials manufacturing and energy supply. Further will assess a Microalgae production techniques from waste water of biofuels .“The study of Bioenergies production from Microalgae culture on wastewater”. TW sewage treatment (nitrogen, phosphor, organic carbon, GHG, conversion into feedstock biodiesel, coproducts,high lipid content & growth microalgae) associated to power plant emissions & production controls. The

race for 3rd generation biofuels industrialization engage closer assessment of changes & choices to happen during process and their side effects.

Acetylation of Corn Cob Hemicellullose in the Presence of Potassium Acetate Salt to Reduce Their Water Sensitivity

Merve Akkus, Middle East Technical University

Sustainable sources of materials are much needed at present as the fossil fuels are being depleted and lignocellulosic biomass has become the main focus of the developing bio-refining industry. Many lignocellulosic biomass studies have been focused on ethanol production from cellulose; however co-utilization of lignin, hemicellulose and cellulose is necessary from the point of a realistic application. In particular, the use of lignocellulosic biomass for the production of food packaging has recently received interest due to the ecological problems of the petroleum based packaging materials. One of the main obstacles in the application of hemicelluloses for packing is their inherent hydrophilic nature. This hydrophilicity makes biopolymer films sensitive to water which affects their functionality negatively limiting their usage. Acetylation, which is a widely used technique for the modification of cellulose and starch, can be used analogously for the hydrophobic modification of hemicellulose. A classic procedure involves the acetylation with acidic or basic catalysts like sulfuric acid and pyridine. In this study, the effect of potassium acetate salt on the acetylation of hemicellulose was investigated without the use of any catalyst. Hemicellulose was alkaline extracted from an agricultural waste, corn cob. Potassium acetate salt was formed by the neutralization of potassium hydroxide with acetic acid during alkaline extraction. It is a common step to remove these salts from the structure by a desalting step like washing or dialysis but for this study, desalting step was excluded and salted hemicellulose polymers were obtained. Hemicellulose polymers were reacted with acetic anhydride at different temperatures for various reaction periods. It was found that, water solubility of salted hemicellulose was reduced by 50% after acetylation at 120oC for 30 minutes whereas the reduction was only about 2% for the reference sample (acetylated desalted hemicellulose). When conditioned in 90% relative humidity for 24 hours, salted hemicellulose polymers absorbed nearly %60 less moisture than their reference counterparts. ATR-FTIR analysis also verified the acetylation reaction and hydrophobic modification of corn cob hemicellulose was achieved by a very simple acetylation reaction without the use of any solvent or catalyst. By this way, it was also shown that better products can be obtained without time consuming and costly desalting steps.

Lignin Valorization by Catalytic Depolymerization in Supercritical Ethanol

Tamas I. Koranyi, Department of Mechanical Engineering, Eindhoven University of Technology, Eindhoven, The Netherlands

We developed novel chemistry for the second most abundant but least valorized biomass component lignin and showed how it can be valorized into a number of useful chemicals and fuel additives. Our strategy is to depolymerize lignin in ethanol, which can also be derived from biomass, using a Cu-modified hydrotalcite as the catalyst. Significantly, we show that one-step valorization of soda lignin in supercritical ethanol using CuMgAlOx catalyst results in high monomer yield (23 wt%) of lignin products without coke formation. Aromatics are the main components, almost half of this monomer fraction being free of oxygen. These BTX (Benzene-Toluene-Xylene)-type streams have value as chemicals or may alternatively serve as fuel additives to gasoline. The oxygenated fractions are highly alkylated and may be used as base chemicals or as additives to increase the octane number of gasoline. Besides these promising results in terms of lignin valorization, we show how the use of ethanol as a solvent results in high monomer yield, deoxygenation and, importantly, protection of the monomers and intermediates from repolymerization.

Colchicine Pathway N-Acetyltransferase: Identification, Cloning and Expression

Ganapathy Sivakumar, Arkansas Biosciences Institute, Arkansas State University

Gloriosa superba is a perennial climbing tropical plant for the production of colchicine, which has potential antimitotic activity. Advanced colchicine-based enzyme and nanotechnology could lead to the design of cancer therapeutics. In order to improve natural isomer raw colchicine for drug production, understanding of the biosynthetic pathway genes is necessary. We have identified the colchicine pathway final step's likely candidate gene, N-acetyltransferase (NAT) which catalyzes the acetyl transfer from acetyl-CoA deacetylcolchicine to colchicine. We report the cDNA establishment from G. superba and identification of NAT from the G. superba RNA transcriptome database. In addition, putative NAT open reading frame cloning into three different Gateway destination vectors, pDEST-17, pBAD-DEST49 and pDEST-HisMBP and the expression of the recombinant NAT in E. coli. This study not only characterizes the colchicine pathway genes but also could provide new insights into elucidating the colchicine metabolism in G. superba.

Intertidal Marine Environments as a Biotechnological Source of Microbial Biosurfactants

B. Otto Ortega-Morales

Microbial communities are ubiquitous in marine intertidal environments. These communities, which grow preferentially as biofilms on natural and artificial surfaces, carry out key processes contributing to the functioning of coastal environments and providing valuable services to human society, including carbon cycling, primary productivity, trophic linkage, and transfer and removal of pollutants. The fluctuating conditions of the intertidal zone make it an extreme environment to which intertidal biofilm organisms must adapt at varying levels. This study was

performed to determine the potential of tropical intertidal biofilm bacteria as a source of novel exopolymers (EPS). Here we report the results of chemical characterization and evaluation of biotechnological potential as biosurfactants of two EPS produced by intertidal biofilm bacteria. Isolates MC3B-10 and MC6B-22, identified respectively as a Microbacterium sp. and Bacillus sp. by 16S rDNA and cellular fatty acids analyses, produced different EPS, as evidenced by colorimetric and gas chromatographic analyses. The EPS produced by MC6B-22 appears to be a polysaccharide dominated by neutral sugars but with significant concentrations of uronic acids and hexosamines, while EPS produced by isolate MC3B-10, here termed microbactan, was further analyzed to characterize its molecular weight, ionic character and toxicity, along with its bioemulsifying potential for hydrophobic substrates at a range of temperatures, salinities and pH values. Analyses showed that microbactan is a high molecular weight (700 kDa), non-ionic molecule. Gas chromatography of the lipid fraction revealed the presence of palmitic, stearic, and oleic acids; thus microbactan may be considered a glycolipoprotein. Microbactan emulsified aromatic hydrocarbons and oils to various extents; the highest emulsification index was recorded against motor oil (96%). The stability of the microbactan-motor oil emulsion model reached its highest level (94%) at 50 °C, pH 10 and 3.5% NaCl content. It was not toxic to Artemia salina nauplii. The chemical nature, not toxicity and stability of microbactan suggest its potential application in bioremediation of marine environments and in cosmetics.

Biodegradable polyesters from agro-industrial by-products

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Polyhydroxyalkanoates (PHAs) are biodegradable bioproduced polyesters that have different mechanical, chemical and thermal properties depending on the monomeric composition and polymeric structure. Poly-3-hydroxybutyrate (P(3HB)), the most common type of PHA, is synthesized in bacterial cells under unbalanced growth conditions and accumulates as intracellular carbon and energy storage. Being biocompatible when adequately processed, certain co-polymers (e.g. poly(3-hydroxybutyrate-co-4-hydroxybutyrate) (P(3HB-co-4HB)) and the homopolymer P(4HB) have interesting properties for specific uses in the medical and pharmaceutical areas. In fact, in 2007, the FDA approved the clinical application of P(4HB) ("TephaFLEX® Absorbable Suture" and "BioTREKTM Bioabsorbable Septal Repair"). Although P(3HB) and other PHAs are currently being bioproduced by several companies worldwide (e.g. Biomer, Metabolix, Procter&Gamble, Tianan, PHB Industrial…), the high production costs limit further market penetration to replace conventional, petrobased plastics. For high scale production, 48% of total production costs is ascribed to the raw materials, in which the carbon source for growth and polymer accumulation accounts for 70 % to 80 %. Therefore, the carbon source choice is a key factor for PHAs industrial production. At IST, two by-products were tested

as low-cost feedstocks for PHA production: a)crude glycerol rich phases (GRP) from a biodiesel plant b)wheat straw lignocellulosic hydrolysates (LCH). (a) High cell density cultures of Cupriavidus necator DSM 545 fed with GRP were used to produce P(3HB) and the copolymer P(3HB-co-4HB), co-feeding gamma-butyrolactone (GBL) as the precursor of 4HB units. Studies on the effect of several cultivation parameters (e.g. dissolved oxygen) led to process optimization. The highest Prodvol attained on GRP were 1.1 gPHA•L-1•h-1 (P(3HB)) and 0.80 gPHA•L-1•h-1 (P(3HB-co-4HB) with a 4HB molar % of 12.3). Selected co-polymers were further tested for scaffold fabrication by electrospinning for mesenchymal stem cells proliferation studies. (b) LCH were prepared from wheat straw by biorefinery.de.GmbH. Biomass was pretreated using the AFEX process followed by enzymatic hydrolysis and a concentration step. After studies for strain selection, the hydrolysates were fed as carbon source to the best PHA producing strain (Burkholderia sacchari DSM 17165, able to produce PHA from glucose and xylose) cultivated in bench scale stirred tank reactors, under controlled conditions. Feedback from bench scale assays allowed for LCH improvement (sugars:organic acids:inhibitors ratio) by biorefinery.de.GmbH. Cell density and P(3HB) Prodvol obtained were similar to those reached in control cultivations with mixtures of commercial sugars. Additionally, fed-batch strategies for the production of P(3HB-co-4HB) on glucose and GBL were developed, indicating that the strain is able to accumulate the copolymer at various 4HB %s with high Prodvol (up to 0.8 gPHA•L-1•h-1) using wheat straw hydrolysates as major carbon source. Acknowledgements: Studies on GRP were financed by the EU Integrated Project BIOPRODUCTION (contract nº 026515-2) and those on cellulosic hydrolysates are funded by the EU Collaborative Project BUGWORKERS (contract nº 246449); C. Almeida, J. Cavalheiro, T. Cesário and F. Ferreira were supported by Fundação para a Ciência e Tecnologia, Portugal (SFRH/BPD/26678/2006, SFRH/BD/45266/2008 and SFRH/BPD/68587/2010,IF/00442/2012)

Potentials of Arrinrashow Clay in Wet Litter Management in Poultry Production.

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Poultry production activities within our communities are characterized by the consistent release of foul odor. The feedback from questionnaires administered indicates that most Residence within 100meters radius, were displeased with the offensive atmosphere of these environment. Our approach at solving this problem is an attempt from the wet Litter management from the feed perspective. Beneficiated Arrinrasho Clay (BAC) was incorporated separately into four different commercially available poultry Feed within Jos, central Nigeria. The BAC and feed combinations gave BACF1 BACF2 BACF3 BACF4 and the Feed Control (FC). They were administered within a controlled regime and at liberty. Observation was for twelve weeks and the birds were investigated for growth rate, weight gain, mortality rate, physical features, vital organ morphology, activity and toxics. The Broilers presented normal growth

rates, the weight gain were steady and rose from averages of (2.45, 2.87, 2.37, 2.46 and 2.11)kg to (3.80, 4.45, 3.67, 3.81 and 2.87)kg for each group respectively as at the seventh and twelfth week. The examinations of the vital organs at the National Veterinary Research Institute, (NVRI) at Vom, showed normal features and no toxins have been identified in all the five groups studied. The patterns of accumulation of fatty tissues were peculiar to the components of the parent feed before incorporation of BAC. The mortality observed was under 5%. The causes of the death were not tied to the incorporated BAC. The mortality was traced to crowding of the birds and other mechanical accidents. The litters from the birds fed with the incorporated BAC were excreted in distinct solid form. These droppings formed air spaces which assisted in accelerating their drying up process. The wet litter observed in the control experiment lacked air spaces, drying was slow, this condition aided the release of foul odor. The atmosphere within our farm house was devoid of the characteristic odor of a poultry environment. Acknowledgement: The University of Jos Senate Research Grant Committee for partially financing this project.

From wheat straw hydrolysates to homo- and co-polyhydroxyalkanoates

M. Teresa Cesário, IBB- Instituto Superior Tecnico

Wheat straw is an abundant renewable agricultural residue with a low economic value and is normally used as cattle feed. It is however a potential source of sugars and it can be upgraded namely in the bioproduction of polyhydroxyalkanoates (PHAs). PHAs are biodegradable, environmentally friendly and biocompatible thermoplastics that can be synthesized by various microbial strains as intracellular storage materials of carbon and energy. Depending on the monomeric composition and chain molecular weight these polyesters present different mechanical and thermal properties and have multiple fields of application. PHAs have been used as packaging materials, drug delivery carriers and as biomedical implant materials A major drawback for its commercialization is the high production cost. To decrease raw materials costs, processed lignocellulosic raw materials can be used as the C-source. Wheat straw lignocellulosic hydrolysates (LCH) were prepared (biorefinery.de GmbH) by pre-treating this residual biomass using the AFEX process followed by enzymatic hydrolysis and a subsequent concentration step. A high sugar concentration solution with low titers of inhibitory compounds is produced that can be used as carbon source for polyhydroxyalkanoate production. The strain Burkholderia sacchari DSM 17165 was selected due to its ability to convert both glucose and xylose into poly-3-hydroxybutyrate (P(3HB)). Biopolymer production was carried out in fed-batch using 2L stirred-tank reactors (STR). A feeding strategy was implemented to overcome carbon catabolite repression leading to a polymer concentration of 84 g/L, corresponding to a P(3HB) accumulation in the cells of 68%. Polymer yield and productivity were 0.22 g P(3HB)/g sugar and 1.6 g L-1h-1, respectively and are the highest so far attained when using LCH as carbon

source. Poly(3-hydroxybutyrate-co-4-hydroxybutyrate) copolymers exhibit attractive thermal and mechanical properties as the presence of the 4HB monomer reduces the melting temperature, the polymer crystallinity and provides higher flexibility, thus facilitating plastic processing. These copolymers find applications in the medical and pharmaceutical fields. Shake flask trials using gamma-butyrolactone (GBL) as precursor of 4HB synthesis have shown the ability of B. sacchari to produce P(3HB-co-4HB). Fed-batch cultures using glucose as carbon source (control) and GBL were developed to achieve high copolymer productivities and 4HB incorporations. Overall productivity and copolymer composition depend on the addition strategy of both C- source and precursor. Using a DO-stat feeding strategy for glucose and a continuous addition of GBL after a manual pulse, the attained P(3HB-co-4HB) productivity and 4HB molar % were 0.7 g/(L.h) and 4.7 molar %, respectively. When LCH were used as C-source under the same feeding conditions, these values were 0.5 g/(L.h) and 5.0 molar %, respectively. Lignocellulosic agricultural residues were shown to be ugraded with high yields and productivities to value-added biocommodities using a biorefinery approach.
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An industrial protocol for in vitro production of psoralen: a highly medicinally potent bioactive compound from an endangered leguminous taxon- Psoralea corylifolia Linn.

Dr Shahnaz Subhan, Amity Institute of Biotechnology, Noida, India

Psoralea corylifolia (Linn.) is enlisted as rare and endangered leguminous taxon. Four species are found in India of which P. corylifolia is medicinally valuable plant. It is used in indigenous medicine as laxative, aphrodisiac, anthelmintic, diuretic and diaphoretic and specially recommended for the treatment of vitiligo, leprosy, psoriasis and inflammatory diseases of the skin in ayurvedic as well as allopathic system of medicines. The medicinal value is due to the presence of furanocoumarin especially a psoralen in seeds that cost around US $ 4634/- per gram (Sigma-Aldrich Chemical Company, USA). In recent time, it is compulsory to have some alternative source of these seeds that produce the highly important bioactive compound known as Psoralen. First time, a novel and simple protocol has been successfully developed for in vitro production of psoralen through rapid and recurrent callus cultures from one week old cotyledon of Psoralea corylifolia Linn. Amongst the various auxins and cytokinins tried alone or in combinations, 10 µM 2,4-dichlorophenoxyacetic acid (2, 4-D) supplemented to Gamborg et al. (1968) basal medium (B5) was optimum for inducing profuse amount of callus in cent percent cultures of cotyledon. The cotyledon calluses were subcultured continuously at an

interval of 35-40 days on 5µM 2, 4-D medium up to two year and good amount of callus proliferation was achieved in cent percent cultures. However, some cultures were maintained under optimum cultural condition on B5+5µM 2, 4-D+5µM BA with 0.5% polyethylene glycol (w/v) up to 3 months. These callus cultures showed the maximum psoralen content of 16.15 mg/g FW. Psoralen has been detected time to time from calluses of different time durations grown on different type of culture media under optimum cultural conditions through HPLC by using calibration curve of standard psoralen. Psoralen estimation was also done for mature seeds samples collected from various localities of central India.. Till date, there is no report of in vitro production of Psoralen , therefore, the present study becomes globally novel, simple and reproducible in which cotyledonary callus cultures were used for the continuous production of psoralen at commercial/ industrial scale.

Homologous and heterologous expression of dehydrogenases and oxidoreductases of Ralstonia eutropha H16

Petra Koefinger

Ralstonia eutropha is a Gram-negative, strictly respiratory facultative chemolithoautotrophic bacterium which can use H2 and CO2 as sole sources of energy and carbon in the absence of organic substrates. It has attracted great interest for its ability to degrade a large list of chloroaromatic compounds and chemically related pollutants. Furthermore it was already applied for the production of biodegradable polymer polyhydroxyalkanoates on an industrial scale. R. eutropha serves as a model organism for the mechanisms involved in the control of autotrophic carbon dioxide fixation, hydrogen oxidation and denitrification. In our project we are interested in establishing specialized R. eutropha based cell factories by genetic engineering. The particular interest is constructing cells efficiently performing oxidoreductase reactions by overexpression of homologous and/or heterologous enzymes. One of the main types of oxidoreductase reactions is performed by dehydrogenases, particularly alcohol dehydrogenases, which have a wide range of possible biotechnological applications. Biotransformations involving the interconversion of alcohols, aldehydes and ketones have great potential for the commercial production of pure optically active compounds and also for other processes such as the treatment of industrial effluents. The genome of R. eutropha H16 contains a remarkable diversity of oxidoreducteases. A selection of alcohol dehydrogenases as well as short chain dehydrogenases of R. eutropha H16 was cloned and expressed in native versions in Escherichia coli. Their activity was analyzed by NAD/NADH dependent enzyme activity assays with different substrates. Currently we are working on the homologous expression of these enzymes in R. eutropha H16 and their functional analysis.

Versatile and stable vectors for efficient gene expression in Ralstonia eutropha H16

Steffen Gruber

The gram-negative ß-proteobacterium Ralstonia eutropha H16 is primarily known for polyhydroxybutyrate (PHB) production and its ability to grow chemolithoautotrophically by using CO2 and H2 as sole carbon and energy sources. Up to now some basic systems for targeted genetic manipulation of this bacterium were already established. However, the majority of metabolic engineering and heterologous expression studies conducted so far rely on a small number of suitable expression systems. Particularly the plasmid based expression systems already developed for the use in R. eutropha H16 suffer from high segregational instability and plasmids loss after a short time of fermentation. In order to develop efficient and highly stable plasmid expression vectors for the use in R. eutropha H16 a new plasmid design was created including the RP4 partitioning system, as well as various promoters and origins of replication. The application of minireplicons derived from broad-host-range plasmids RSF1010, pBBR1, RP4 and pSa for the construction of expression vectors and the use of numerous, versatile promoters extend the range of feasible expression levels considerably. Moreover, the implementation of the RP4 partition sequence in plasmid design increased plasmid stability significantly and enables fermentations with marginal plasmid loss of recombinant R. eutropha H16 for at least 96 hours. The utility of the new vector family is demonstrated by providing expression data with different model proteins.

Technical Presentations

Enhanced Biofuels Production from Lignocellulosic Biomass by Microwave-assisted Pretreatment

Wei Huang, BeijingResearch 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.

A Dynamic Imaging Cell Monitoring System for Real-Time Analysis of Algae-to Biofuel/Bioproduct Production

Victoria Kurtz, Fluid Imaging Technologies, Inc

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.

Innovative lab automation solution for culture dish handling and imaging using the PetriJet Technology platform

Felix Lenk, Technische Universitaet Dresden, Department of Bioprocess Engineering

Petri dishes are a user-friendly and easy method for in vitro cultivation of not only plant cell and tissue cultures [1]. Specific growth parameters, productivity and the influence of agar are only a small set of parameters which can be investigated by using petri dishes for cultivation. In larger screening experiments the manual and mostly only qualitative analysis is limited by the number of laboratory staff. Based on the constant development in laboratory automation an innovative solution to these problems is now for the first time readily available even for small and middle scaled laboratories with special requests due to their individual research profiles. With the presented fully automated, bench-top solution towards the handling of culture dishes, the experimental efforts can be reduced significantly and the sample throughput can be increased. For a continuously monitored, systematic, non-destructive and quantitative analysis of the culture dishes an automatic image recognition and analysis has been implemented [2]. Therefore, in co-operation with Wimasis GmbH, Munich, a customized image recognition solution was created. Any customer related requirement for the analysis can be implemented into the algorithm. For long term experiments every culture dish gets labeled with a QR-code. With this system the culture dish can be tracked immediately by the algorithm and the new results can be displayed with respect to previous images. The precise analysis delivers reproducible results. These might be used for the simulation of tissue or cell growth of the investigated cultures [3]. The laboratory bench-top device PetriJet can analyze up to 100 culture dishes within one hour. The user interaction for PetriJet handling is limited to starting, loading and unloading the device with a set of 20 petri dishes each. Currently, with dimensions of 800 x 600 x 400 mm the PetriJet is designed especially for small and medium scaled laboratories and reveals an ideal possibility for systematic and quantitatively analysis of samples on culture dishes. Literature: [1] Steingroewer, J., Bley, T., Georgiev, V., et al., Bioprocessing of differentiated plant in vitro systems. Eng. Life Sci. 2013, 13(1), 26–38. [2] Lenk, F., Vogel, M., Bley, T., et al., Automatic image recognition to determine morphological development and secondary metabolite accumulation in hairy root networks. Eng. Life Sci. 2012, 12(6), 588–594. [3] Lenk, F., Sürmann, A., Oberthür, P., et al., Modeling hairy root tissue growth in in vitro environments using an agent-based, structured growth model. Bioproc. Biosyst. Eng. 2013, DOI: 10.1007/s00449-013-1088-y

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Genedata Selector™ Application Suite: From Single Genome Integration to Full Microbiome Characterization

Lisa Kenney, Genedata

The rapid evolution of Next Generation Sequencing (NGS) technologies has led to an explosion in the volume of data and the types of applications used in various business industries (e.g. Agribusiness, Biofuels, Industrial Biotechnology, Dairy, Cosmetics and Pharma). In order to get the full value of NGS, bioinformatics tools for data management, storage, and analysis must address different workflows and provide the flexibility to integrate proprietary and public information in an open and scalable environment. Furthermore, an investigation into molecular mechanisms of intra- or intercellular communication requires a functional reconstruction and annotation of the genomes.

Genedata Selector™ is an enterprise application suite which enables the management, integration and analysis of diverse data. Here we present four applications illustrating how Genedata Selector supports organizations with complex workflows involving sequence, omics- and phenotype data:

• Genome refinement of the recently published CHO genome and tools for the optimization of strains used in the production of therapeutic antibodies

• Genotype-phenotype based breeding strategies for plants and crops for a sustainable future.

• Identification of biomarkers for diagnostic tests, patient assessments and therapies of infectious diseases caused by pathogens such as Candida albicans

• Metagenomics analyses enabling taxonomic profiling of microbial communities in biogas processes, and discovery of new genes increasing the biomass degradation, methane yield or the production of side products.

Genotype-Phenotype Based Breeding Strategies for Plants and Crops Using an Enterprise Knowledge Management Suite

Thomas Hartsch, Genedata

Modern plant breeding and genetic engineering approaches for crop improvement drive an increasing need for scientists to gather and interpret genome sequence information. The large volumes of genome sequence data in the public domain, combined with increased throughput for sequencing proprietary lines, have provided plant researchers and breeders with more information than ever on which to base their hypotheses and decisions. This wealth of information also involves significant challenges, due to large sample sizes, complex genomes, and incomplete genome assemblies. Moreover, important information may not be organized or managed in a way that makes the data easily accessible or comparable. To make these data amenable to interrogations based on sequences, genes, and phenotypes, we developed a platform for genome management and analysis with agribusiness goals in mind. Genedata Selector for Agribusiness is an enterprise genome management system which is scalable,

includes interactive visualization tools, and can be used to guide the discovery of genotype-phenotype relationships.

In this study, we apply the platform to investigate barley, a highly repetitive 5.1Gb genome, and discover genes that could be targeted for beer production. As a first step, RNA-seq data was used to refine gene model predictions of the recently published barley genome. We then used the nucleotide sequence data to identify amylase homologs which could have a role in the malting process. Information for these genes is then annotated in the system, so that the genomic ranges can be quantified for downstream gene expression experiments. Next, we compared the amylase sequences to those in rice, bacteria, and fungi, to learn about conserved protein domains that could be targeted to modulate malting. At each step in the analysis, data are stored, tracked, and annotated through a central database, and all information is accessible and searchable from users' laptops via a friendly and feature-rich interface.

Metagenomics Analysis and Optimization of Biogas Production Processes

Tim Zeppenfeld, Genedata

Biologically derived methane (biogas) is a renewable energy source that plays an increasingly important role in satisfying worldwide energy demand. Optimizing the biotechnological production process of biogas depends largely on the selection of biomass and management of the biogas producing microbial community. For industrial biogas production, microbes can be used to break down plant material and animal waste into fermentable sugars. A production increase requires understanding the relationship between microbial populations and rate limiting molecular substrates.

New technologies, such as NGS (Next Generation Sequencing) combined with advanced bioinformatics solutions, can be used to taxonomically profile the microbial communities and identify novel genes and pathways in microbial strains implicated in biomass degradation and biogas formation.

Here we demonstrate Genedata Selector™, a comprehensive, scalable data management suite which stores, analyses and connects a broad range of data types, including genomes, metabolic pathways, phenotypes and patents. Big and complex data sets from NGS-derived metagenomes and transcriptomes are integrated in Genedata Selector and analyzed at different steps or time points of the biogas process and in response to different biomass types.

Genedata Selector provides:

• Full support of bioprocess engineering: from metagenomics, biomass to microbiome analysis
• Integrative, scalable, and secure strain management
• Complete set of software tools enabling analyses of raw sequencing data, omics data, and statistics

• Strain analysis tools to optimize feedstock and use of starter cultures in the fermentation process
• Decision support for production of biogas and bio-based materials

We demonstrate how this unique application suite is used for an RNA-centered meta-transcriptomic and genomic approach to simultaneously obtain information on both functional characteristics and (taxonomical) structure of microbial biogas communities, and how biomarkers are generated which are predictive for the state and composition of the biogas fermentation process.

The development of new biomarker-based tools and novel enzymes via metagenomic approaches may significantly contribute to the future of economical biogas production from renewable resources.

CAVER Analyst – Engineering of Enzymes by Modification of Access Tunnels

Ondrej Valina, Caversoft

The active site of enzyme is often located deeply inside a protein structure. Such positioning enables the formation of highly selective and specific reaction conditions for the reaction of the substrate with the enzyme. The deeply buried active sites of enzymes are connected with the external environment by the means of tunnels. The size, shape, physico- chemical properties and dynamics of the tunnels are for the catalytic characteristics of enzymes as important as the complementarity between the ligand and the active site.

This poster presents a software tool – CAVER Analyst - for protein tunnels analysis and visualization. The software enables to compute tunnels leading from the a deeply buried active site. Using the state-of-the-art CAVER 3.0 algorithms the tool is able to calculate tunnels in both static structures as well as molecular dynamic trajectory. The importance of tunnels is demonstrated in two case studies. Engineering enzyme activity by redesigning access tunnels of the haloalkane dehalogenase DhaA provides 32-fold increase in activity with toxic pollutant 1,2,3-trichloropropane and engineering enzyme stability by modification of residues lining the access tunnel of the haloalkane dehalogenase DhaA increased its melting temperature by 19°C and resistance to co-solvent DMSO 4000-fold.

The CAVER Analyst computes, analyses and visualizes the tunnels and also comprises many advanced features and techniques, which enable the users to explore and evaluate the tunnels of interests.