Advanced Biofuels and Biorefinery Platforms

Modeling and control of biomass parameters in a multi-feedstock biorefinery
Tolutola Oyetunde, Masdar Institute, Abu Dhabi

Background

Without a doubt, energy will be a key driver of the future global economy. Biomass is the fourth largest energy source after coal, oil and natural gas. It is the most important renewable energy option and could potentially all global energy demands. Transforming this potential to reality would involve building biorefineries that are both economically viable and environmentally sustainable based on local availability of biomasses and suitable conversion technologies.

Key challenges to achieving this, among others, are the low volumes of biomass available (which limit the economy of scale), seasonality of some biomasses and the cost of transportation and storage.

One potential solution is multi-feedstock biorefineries where different biomasses are treated together.

Investigating the effect of the mix of biomasses, the amount of each biomass purchased and/or stored and biomass costs on the economics of a multi-feedstock biorefinery as well as effective control strategies for minimizing the impact of these parameters on biorefinery profitability is the subject of this work.

Methodology

A multi-feedstock biorefinery was simulated for Abu Dhabi municipality. Five different biomasses which were actually ‘waste’ streams from local industries: date seeds, date cake, food waste, camel dung and waste cow milk. Realistic volumes for each biomass based on current data were used in the base case. The volumes of biomass were randomly varied between plus or minus 50%. A control strategy based on a combination of classical process control and adaptive neuro fuzzy logic is proposed to limit the effect of both biomass composition, availability and prices on the biorefinery output quality and productivity. The amount of each biomass purchased and stored are used as dynamic decision variables.

Results, conclusions and perspectives

Preliminary results that variation in biomass volumes and prices have a significant impact on the overall biorefinery profitability (variation in gross margin of more than 20%). This underscores the need for a suitable control strategy. The ‘ease of
control’ could serve as an important factor in choice of biomasses in a multi-feedstock biorefinery subject to local constraints (availability, ease of storage, cost of transportation/storage, and seasonality etc.) A comparison of different control strategies is planned for future work.

Kinetic study and optimization of asphaltene biodegradation using microorganisms by response surface methodology
Hossein Salehizadeh¹, University of Isfahan, Tina Tavasoli¹, Abbas Shojaosadati²

Asphaltenes are complex fraction of heavy oil with high molecular weight petroleum hydrocarbons. Microorganisms can degrade asphaltene to small parts using enzymatic reactions. In this study, asphaltene biodegradation by mixed culture and Bacillus lentus was optimized using response surface methodology with designing three factors at five levels. To estimate the response, the quadratic polynomial models were found to be significant. The confidence coefficients (R²) were calculated as 0.863 and 0.887 for B. lentus and mixed culture, respectively. The optimum values of pH, salinity and asphaltene concentration for asphaltene biodegradation were obtained as 6.4, 76 g L⁻¹ and 12 g L⁻¹ for mixed culture and 6.7, 76 g L⁻¹ and 22 g L⁻¹, for B. lentus at 40 °C, respectively. Under optimum conditions, the highest asphaltene biodegradation was achieved up to 48% by mixed culture of bacteria in bubble column bioreactor during 60 days. The kinetic parameters were determined using experimental data and the Model Maker 3.0.3 software package (Cherwell Scientific Publishing Ltd.). The R² values for kinetic models including Monod, Moser, Tessier, Contois and Logistic were obtained as 0.8711, .8534, 0.9092, 0.46 and 0.5266, respectively. Tessier model with highest R² value was selected to calculate the kinetic coefficients of B. lentus. The asphaltene biodegradation by bacteria was subjected to Tessier model and the kinetics parameters μmax, Ks and Yx/s were determined as 0.31 day⁻¹, 39.19 g L⁻¹ and 0.21, respectively. Conclusively, asphaltene biodegradation promises high potential biotechnological applications for use in oil industries and environmental remediation.

Keywords: Asphaltene, Biodegradation, Optimization, Response Surface Methodology, Kinetic parameters, modeling

Optimization of C/N ratio for maximum oil Production from oleaginous yeast Rhodosporidium toruloides and its exploitation to Produce FAME (Biodiesel)
Saurabh Saran, Technology Based Incubator, Biotech Centre, Delhi

Fossil fuel reservoirs are continuously depleting, generating various environmental concerns like accelerated global warming, elevated levels of greenhouse gas (GHG) emissions, depletion of crude oil reservoirs and high energy prices. This situation has given rise to a growing worldwide interest in renewable energy resources such as biomass-based bio-fuels. Realizing the importance of lipid produced by
oleaginous yeasts and the necessity of developing bio-based fuels using fermentation, the present investigation was carried out with an objective of increasing the amount of lipid accumulated within the selected oleaginous yeast Rhodosporidium toruloides. Optimization of lipid production by R. toruloides revealed that the lipid production by this strain was highest in shake flask at 250 rpm and at 25 °C in medium containing 55 g/L glucose, 2.0 g/L yeast extract, 1.75g/L NaNO3, 6 g/L KH2PO4, 0.3 g MgSO4•7H2O and pH 6.5. Under these optimal conditions, when the C/N ratio of the medium was 65, R. toruloides produced lipid of 6.5 g/l, which was 43.7 % of dry biomass (14.91 g/l) after 48 h of cultivation. These optimized conditions were put to test in a scale up in a 30L fermenter with an agitation speed of 250 rpm, an aeration rate of 0.6 vvm and 25 °C throughout the cultivation. This yielded in a final lipid of 12.5 g/l, which was 53.51% of dry biomass (23.36 g/l) after 60 h of cultivation. The major fatty acids of the cellular lipid were oleic acid (45.02), palmitic acid (22.91), linoleic acid (18.1%) and steric acid (11%). The fatty acid profiles of R. toruloides revealed that its lipid was similar to vegetable oil with four major fatty acids including oleic, palmitic, linolenic and stearic acid. These fatty acids can further be transesterified for biodiesel (FAME) production.

Development of CBP Biocatalysts for Industrial Ethanol Production
Justin van Rooyen, Mascoma Corporation

Development of CBP Biocatalysts for Industrial Ethanol Production Mascoma Corporation, a Lebanon, NH, based biotechnology company, has developed a suite of technologies implemented in Saccharomyces cerevisiae to improve the economics of industrial ethanol production. We have focused on bringing together the secretion of enzymes important for the breakdown of polymeric carbohydrates found in biomass, with the necessary metabolic pathways to ferment monomeric sugars derived from that biomass to ethanol in high yield in robust strain backgrounds. Several strains of yeast have been developed for the U.S. based corn ethanol industry that secrete amylase enzyme to reduce exogenously added enzyme requirements, and reduce the formation of the byproduct glycerol. In cooperation with Lallemand, Inc., we have deployed our strains to more than 10% of the U.S. corn ethanol industry and produced in excess of 600 million gallons (~2.2 billion liters) of ethanol with these strains to date. Mascoma has also developed strains of yeast targeted towards the emerging 2nd generation ethanol industry. Technologies for extremely robust and rapid xylose fermentation, high level cellulase and hemicellulase secretion, and glycerol reduction will be discussed. The combination of these technologies allows for increased yields and/or decreased enzyme requirements for the processing of lignocellulosic feedstocks, and can represent value creation in excess of $0.40/gallon of ethanol. Taken together, our developments have shown that consolidated bioprocessing (CBP) via S. cerevisiae is not only feasible, but the preferred biotechnology solution for biomass conversion.
Development of a Consolidated Bioprocess for Cellulosic Ethanol
Anand Ghosalkar, Praj Matrix-The Innovation Center (a division of Praj Industries Ltd.)

Development of a Consolidated Bioprocess (CBP) is considered as the “Holy Grail” for cellulosic ethanol. Production of cellulosic ethanol in a single step process with a mild pretreatment followed by fermentation with a microbe capable of producing cellulase enzymes as well fermentation of different monomeric and oligomeric sugars to ethanol is expected to eliminate costs associated with exogenous enzymes as well as detoxification costs of removing inhibitors generated by harsh pretreatment. However, there are several challenges with respect to the development of a commercial process like associated nutrients costs, microbe’s ability to handle high solids and sterility requirements are rarely addressed in the initial stages of the development of a CBP process. Efforts at Praj Matrix-The innovation center to develop a CBP process using clostridium phytofermentans and associated research challenges will be discussed.

Effect of acid-catalyzed organosolv pretreatment conditions on the characteristics of ethanol organosolv lignin
Ho-Yong Kim, Seoul National University, Seoul, South Korea

In this study, the characteristics of ethanol organosolv lignin (EOL) as a function of acid-catalyzed organosolv pretreatment conditions (pretreatment time and concentration of acid catalyst) were investigated. Stems of twenty years old Liriodendron tulipifera (chemical composition: glucan: 37.13±0.07, xylan: 16.94±0.03, Klason lignin: 22.86±1.40, acid soluble lignin: 3.71±0.12) were dried and milled to a particle size of < 0.5 mm. Ethanol organosolv pretreatment was conducted in a 500 ml reactor at various conditions (reaction temperature: 100-200°C at 10°C intervals, acid concentration: 0.5, 1, 2% sulfuric acid, reaction time: 10 min, S/L ratio: 1/10). Pretreated materials were filtered and divided into pretreated solid residue and liquid hydrolysate fractions. Liquid hydrolysates and the water used to wash pretreated solid residue were mixed and centrifuged. The resulting precipitate, referred to EOL, was collected and weighed after freeze-drying. The EOL yield was calculated based on the initial input of wood meal of Liriodendron tulipifera and weight of EOL collected after precipitation.

The step of EOL formation during acid-catalyzed organosolv pretreatment can be divided into three stages: 1) isolation of S unit lignin, 2) degradation with condensation of S unit lignin, and 3) maintenance of lignin structure. Importantly, the order of these stages can vary depending on reaction temperature and acid concentration. The isolation stage of S unit lignin ranges from between 120°C and 140°C with 0.5% and 1% acid, and between 120°C and 130°C with 2% acid. During the isolation stage of S unit lignin, EOL yield, Mw, polydispersity, methoxyl group (OMe) contents, phenolic-OH contents (0.5% acid concentration only), and NBO products of S unit lignin are critically enhanced. EOL is formed primarily by
cleavage of lignin-hemicellulose structures in the secondary wall below 140°C, and thus exhibits a high S/V ratio. The stage of degradation with condensation of S unit lignin ranged from 140°C to 170°C with 0.5% acid, from 140°C to 160°C with 1% acid, and from 130°C to 150°C with 2% acid. During this stage, the Mw, polydispersity, and NBO products of S unit lignin decrease drastically while EOL yield, OMe content, and phenolic-OH content are largely unchanged. Specifically, EOL underwent both cleavage of lignin structural linkages and condensation. In all of the conditions described above, EOL appeared to be stable, and was maintained without significant changes.

The characteristics of EOL were significantly altered by the pretreatment conditions, and thus controlling the pretreatment process is necessary for efficient EOL production. Furthermore, several special characteristics of EOL make it potentially useful for a number of applications as well as lignin depolymerization. Further analyses of EOL products are necessary to establish their usefulness and potential economic impact.

Metabolic Engineering of Escherichia coli for n-propanol production
Kajan Srirangan, Department of Chemical Engineering, University of Waterloo

Background:

While most resources in biofuels were directed towards implementing bioethanol programs, 1-propanol has recently received attention as a promising alternative biofuel. Nevertheless, no microorganism has been identified as a natural 1-propanol producer. In this study, we manipulated a novel metabolic pathway for the synthesis of 1-propanol in the genetically tractable bacterium Escherichia coli.

Results:

E. coli strains capable of producing heterologous 1-propanol were engineered by extending the dissimilation of succinate via propionyl-CoA. This was accomplished by expressing a selection of key genes, i.e. (1) three native genes in the sleeping beauty mutase (Sbm) operon, i.e. sbm-ygfD-ygfG from E. coli, (2) the genes encoding bifunctional aldehyde/alcohol dehydrogenases (ADHs) from several microbial sources, and (3) the sucCD gene encoding succinyl-CoA synthetase from E. coli. Using the developed whole-cell biocatalyst under anaerobic conditions, production titers up to 150 mg/L of 1-propanol were obtained. In addition, several genetic and chemical effects on the production of 1-propanol were investigated, indicating that certain host-gene deletions could abolish 1-propanol production as well as that the expression of a putative protein kinase (encoded by ygfD/argK) was crucial for 1-propanol biosynthesis.

Conclusions:
The study has provided a novel route for 1-propanol production in E. coli, which is subjected to further improvement by identifying limiting conversion steps, shifting major carbon flux to the productive pathway, and optimizing gene expression and culture conditions.

**Study on Production of Biodiesel by Lipase-catalyzed Transesterification of Inedible Oil**

*Zunxi Huang, School of Life Science, Yunnan Normal University*

Energy and environment was a prerequisite for the socioeconomic development. Recently, with the continued development of the socioeconomic, energy deficit and the environmental pollution had become a severe problem which troubled worldwide. To reduce the impact of energy deficit and environmental pollution on the economic development, it is very important to study on development and application of renewable energy. Biodiesel was an alternative liquid fuel, which made from biological sources such as vegetable oils, animal fats or waste cooking oils by transesterification. There are two common approaches which can make biodiesel including chemical method and enzymatic catalyzed method. Compared with chemical method, enzymatic catalyzed method has its own advantages, such as mild reaction conditions, low alcohol used level, easy glycerol recovery, no waste material production and so on. Based on these advantages, enzymatic catalyzed approach has been paid more attention. However, both the high price of lipase and lacking of oil feedstock obstruct the industrialization of enzymatically catalyzing technique on a large scale. In this study, we chose the *Jatropha oil* and restaurant waste oil as the raw materials to make biodiesel using homemade lipase. We have obtained the optimal process conditions and a new lipase catalyst which will provide the experimental basis and theoretical data for the biodiesel production.

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**Cloning and nucleotide sequence of the maltohexaose-producing amylase gene from Bacillus cereus strain HLSSD-5**

*Xiaoyan Wang*

The *AmyHLSSD-5-2* gene encoding a maltotetraose-producing amylase (exo-maltotetrahydrolase) from *Bacillus cereus* strain HLSSD-5 was cloned by the shotgun cloning method. The positive transformant carrying *pUC19-HLSSD-5-2* with amylase activity was selected from the complete genome library of *B. cereus* strain HLSSD-5. It contained a 3226-bp Sau3AI-BamHI fragment that had a 1542-bp open reading frame encoding a precursor (513 amino acid residues) of the secreted amylase. The precursor had a signal peptide of 39 amino acid residues at its amino terminus. A region in the primary structure of this amylase exhibited homology to corresponding regions of other amylases. The deduced mature protein had 474 amino acids, a molecular weight of 53.9 kD, and an isoelectric point (IP) of 5.435. The amylase gene was expressed in *Escherichia coli* BL21 using the prokaryotic
expression vector pET28a+. The optimal conditions for the starch-degrading activity were a temperature of 50°C and pH 6.8. The enzyme activity rapidly decreased when the temperature increased above 60°C. The enzyme produced maltohexaose and maltopentaose by hydrolyzing the soluble starch. The enzyme was found to be a glucan 1,4-α-maltohexaosidase (EC 3.2.1.98) and was named AmyHLSSD-5-2. The GenBank accession number of this enzyme is ACQ73554.

Authors: Xiaoyan Wang, YunJuan Yang, Runchi Gao, Changlong Liao, Bo Xu, Yueling Mu, Shi-long Li, Zunxi Huang

Production of diesel and jet fuel intermediates using hybrid gasification-syngas fermentation

Raymond L. Huhnke

Butanol and hexanol are considered intermediates for the production of diesel and jet fuels. These alcohols can be produced using hybrid gasification-syngas fermentation technology. The hybrid conversion process begins with gasification of biomass such as dedicated crops, plant residues and municipal solid waste to syngas (primarily CO, CO2 and H2) followed by the conversion of syngas using acetogens to produce alcohols and organic acids. A mixed culture of acetogens consisting initially of 56% Alkalibaculum bacchi CP15 and 34% Clostridium propionicum was investigated. Using a syngas consisting of 40% CO, 30% CO2 and 30% H2, results showed that the mixed culture produced ethanol, propanol and butanol from syngas. Previous research showed that the monoculture of CP15 only produced ethanol. To examine the ability of the mixed culture to convert carboxylic acids to alcohols, propionic, butyric and hexanoic acids were initially added into the fermentation medium. The mixed culture was 50% more efficient in converting these acids to their respective alcohols compared to the CP15 monoculture. In addition, the synergy of the mixed culture resulted in over 60% more alcohol production from syngas and acids added than the monoculture of CP15. The conversion efficiencies of propionic, butyric and hexanoic acids to their respective alcohols with the mixed culture were 83%, 75% and 91%, respectively, which shows its potential to make diesel and jet fuel intermediates.

Mapping The Emerging Technolgical Innovation System of the US Military Aviatiob Biofuel After the 2012 Congressional Provisions: Defining Actors, Networks and Institutions

Mohamed Leila, University of McGill

Second generation biofuels are produced from non-edible agricultural feedstock and has been proven via Life Cycle Assessment LCA analysis to possess less negative environmental impacts than fossil fuels. Military and Civil Aviation sectors are one of the highest consumers of liquid fossil fuels and thus these sectors play an important role investing in biofuel alternatives. In 2012, the US congress passed provisions exempting the Department of Defence DoD from section 526 of Energy
Independence and Security Act EISA relaxing biofuels procurement constrains related to economic and environmental consideration. The policy reform thus initiated Military Biofuel Initiative setting percentage biofuels acquisition goals for each service. The USAF is historically the single largest renewable energy consumer in the US federal government and thus is expected to provide a powerful market signal by demanding large amounts of Military Aviation Biofuels to meet its 2016 goal of 50% domestic fuel requirements from biofuels. Technological Innovation Systems and functions of innovation theoretical framework developed by Jacobsson, Bergek and Hekkert was used to analyse the elements of this emerging technological system. Actors, Networks and Institutions were mapped to provide researchers interested in military aviation biofuels technological assessment with the incumbent technologies in this emerging technological system. Results showed that there are three technological pathways that the DoD as a prime mover is interested in acquiring in spite of the variation of their production costs and environmental impacts. These pathways are the Fischer-Tropsch Synthesized Paraffinic Kerosene (FT SPK) developed by Sasol, Hydrotreated Renewable Jet (HRJ8) of Honeywell UOP Ecofining® Process and Alchol to Jet ATJ8 developed by Gevo. Future research projects will involve performing cradle to grave Life Cycle Assessment of these products to evaluate their energy balance, environmental and economic impacts.

Feedstock Production and Utilization

Development of a Novel Revolving Algal Biofilm Cultivation System for Easy Biomass Harvest

Martin Gross, Iowa State University

Current algal cultivation has been mainly performed in open ponds or closed photobioreactors in which algal cells are suspended in liquid and harvested through sedimentation, filtration, flocculation, and/or centrifugation devices. The objective of this research was to develop a novel attached algal cultivation system to reduce the biomass harvest cost. In the attached growth system, algal cells were attached to a material that was rotating between nutrient-rich liquid phase and carbon dioxide rich gaseous phase for alternative absorption of nutrients and carbon dioxide. The algal cells were harvested by scrapping from the attached surface, and thus, the expensive harvest procedures commonly used in a suspension cultivation system can be avoided.

Compared to the suspended culture systems, the proposed system has several advantages: (i) the biomass can be in-situ harvested DURING the culture process, rather than using an additional sedimentation or centrifugation unit for harvesting (harvested cells have water content similar to that of cells after centrifugation); (ii) the culture enhances the mass transfer by directly contacting algal cells with CO2 molecules in gaseous phase, while traditional suspended culture systems have to
rly on the diffusion of CO2 molecules from gaseous phase to the liquid phase which is often limited by low mass transfer rate; (iii) the culture system only needs a small amount of water and land area through utilizing the unique design that only requires the bottom of the reactor system to be bathed in the liquid, this allows for algae cultivation to maximize area by actually growing vertically vs only horizontally.

The attached growth system was optimized for improved biomass productivity. The major operational parameters optimized include biofilm attachment material, harvest duration, rotational speed, and CO2 concentration. It was found that the optimal attachment material was duct cotton, harvest duration of 7 days, rotational speed of 4 rpm and atmospheric CO2 concentration. These parameters resulted in a biomass productivity of 10.5 g·m-2·day-1. A compositional analysis (lipid, protein, carbohydrate, ash) was conducted to compare the biofilm based system and standard suspended cultivation system. The compositional analysis showed that algae grown using the attached system has 5% more protein, and 9% more carbohydrate and 14% less lipid, than suspended culture of the same species of algae. The algae grown in the attached growth system produced a higher content of 5 of the 9 essential amino acids, threonine, valine, isoleucine, leucine, phenylalanine, methionine and histidine. Two had no significant difference, while lysine and tryptophan had a lower concentration than the suspended systems. A fatty acid analysis was also conducted but no significant difference was observed. Overall, the results indicated that the attached growth is a promising algal culture system for an improved biomass productivity with high nutritional value.

**Estimating National Yield Potentials for Priority Biomass Feedstocks:**
**Results from the DOE-Sun Grant Regional Feedstock Partnership**
*Terry Nipp, Sun Grant Association*

The Department of Energy (DOE) and the land grant universities of the Sun Grant Initiative (SGI) have worked together over the past seven years in a Regional Feedstock Partnership that supports research on the development of biomass feedstocks. Teams of leading scientists from across the country have worked together to examine the production opportunities and challenges of biomass feedstocks that include switchgrass, miscanthus, sorghum, energycane, CRP lands, corn stover and cereal residues, willow and polar. The SGI has supported over 130 field research sites with locations with primary and affiliated location in 90% of the states. Concurrent with the field research, regional GIS teams have developed models to estimate the current yield and production potentials of these major feedstocks across the landscape.

The Western Sun Grant Region, led by Oregon State University collaborated with Oak Ridge National Laboratory (ORNL) to incorporate the results of the biomass fieldwork into the PRISM-EM climate and crop model to provide the best current estimates of national yield potentials for these bioenergy feedstocks. PRISM-EM is a
semi-monthly FAO-style water balance simulation, which tracks precipitation input, evapotranspiration, and soil moisture depletion. An estimate of monthly relative yield (0-100 percent) is the product of the water stress coefficient and a temperature growth curve. In what is known as a "limiting factor" approach, the final relative yield is the lowest of the modeled yields resulting from the water balance simulation, plant injury curves for summer heat and winter cold, and growth constraints due to soil pH, drainage, and salinity. PRISM-EM is driven by PRISM temperature and precipitation data, prepared at a twice-monthly time step on a regular grid across the US. PRISM is a state-of-the-science climate mapping technology that produces several major spatial climate datasets for the US, including official maps for the US Department of Agriculture.

Terry Nipp will provide an overview of the national mapping process, with an in-depth presentation provided about the yield potential and the results of the fieldwork for a representative sample of the feedstocks.

**Certified Biomass Procurement Specialist on-line course**

*Mark Hall, Auburn University*

This free online course is funded by a grant from the United States Department of Agriculture’s National Institute of Food and Agriculture. The course is a part of the Southeastern Partnership for integrated Biomass Supply Systems.

This course is designed to train you to work with farmers and landowners to produce switchgrass that will be used as the feedstock for a biorefinery.

The idea for this program came from observing the poultry industry. In the poultry industry, poultry companies contract with growers to grow the chickens they need. Farmers cannot show up at the processing plant any time they want with whatever kind and size of chicken they happen to have. The poultry companies hire specialists that work with the growers. These specialists make sure that they have the exact type and the exact size of chicken the processor needs at the time they need it.

As a certified biomass procurement specialist, graduates will work for a biorefinery that’s making fuel or other bio-products. Like the poultry field specialist, you’ll insure that your employer has the inputs it needs to keep the plant running. Graduates will work with the farmers and landowners to produce switchgrass in an environmental and socially accountable way that meets the specifications of the biorefinery. These specifications are sure to include size, amount and delivery time. The course consists of four separate units with the objective of giving students a working understanding of the fundamentals of sustainable feedstock production practices.
Estimating National Switchgrass Yield Potential: Results from the DOE-Sun Grant Regional Feedstock Partnership
Vance Owens, North Central Sun Grant Center, South Dakota State University

The Regional Feedstock Partnership is a collaborative research effort between the US Department of Energy and the Sun Grant Initiative. Various herbaceous energy crops are being investigated as part of this partnership at diverse locations throughout the USA. Specifically, the research reported in this poster demonstrates the potential for switchgrass production across various regions of the USA. Switchgrass is being evaluated in AL, IA, NY, OK, SD, and VA on field-scale plots (10 ha minimum). Data collected from national, replicated switchgrass field trials will help us better understand benefits and limitations of this species grown in diverse geographic and climatic conditions, and develop potential supply curves for advancing the bioenergy industry. Switchgrass researchers from each location represented in the trial, in conjunction with members of Oregon State University’s PRISM Climate Group and Oak Ridge National Laboratory scientists, have developed a best-guess map of the potential relative yield distribution of switchgrass across the lower 48 states under long-term, average climate conditions, using the PRISM-EM environmental suitability model. This map was developed at an in-person meeting in order to answer questions about the PRISM-EM suitability model, run the model on the fly to produce draft maps, get immediate feedback, revise model parameters accordingly, and iterate to a final relative yield map. Following this meeting, the PRISM Climate Group members incorporated estimates of long-term farm yield at field trial points supplied by the group to transform the best-guess relative yield map into an actual yield map that best reflects the current knowledge of experts across the US. This poster will present results from the yield mapping exercises.

Nitrogen Application and Harvest Timing Affect Biomass Yield and Composition on CRP Grassland
Chengci Chen, Montana State University

Conservative Reservation Program (CRP) grassland has potential to be used for biomass feedstock production. However, management strategies for soil fertility and harvest timing are needed for sustainable production. A replicated study was conducted from 2009-2011 to investigate the biomass yields and compositions of CRP grassland with mixed alfalfa and grass vegetation in central Montana, as affected by nitrogen (N) application rates and harvest timing. Nitrogen was applied in the spring of each year at 0, 56, and 112 kg N/ha and biomass was harvested at peak production and after frost kill. Biomass yields varied from year to year. Nitrogen and harvest timing had significant effects on biomass yields. Averaged over three years, biomass yields were 3479, 3762, and 3998 kg/ha at 0, 56, and 112 kg/ha N rates, respectively. The biomass yield was 4105 kg/ha at the peak production stage, compared with 3387 kg/ha at the frost kill. Nitrogen application and harvest timing also significantly affected the species compositions. The
proportion of alfalfa significantly decreased from 49% to 35% and the grass significantly increased from 51% to 65% when the N rate increased from 0 to 56 kg/ha, and the species compositions did not change when N rate further increased from 56 to 112 kg/ha. At the peak production stage, the proportion of alfalfa and grass was 52% and 48%, respectively, compared to 29% and 71% at frost kill. Less alfalfa proportion in the biomass at frost kill was due to the senescence and drop of alfalfa leaves.

Renewable Chemical Platforms

Synthetic cell Factories for a Sustainable Future
Eric Althoff, Arzeda Corp.

We enable sustainable chemistry -- Almost all of today’s chemicals that make our entire modern world (fibers, polymers and plastics, solvents, paints) are made from seven key building blocks obtained from petroleum. This is not sustainable, has a negative impact on our environment and our energy independence. The emergence of industrial and synthetic biotechnology, of which Arzeda is a leading player, has the potential to radically transform the chemical industry and make it sustainable.

We replace entire factories with designer cellular factories -- Arzeda designs novel cell factories that incorporate synthetic enzymatic pathways to make any molecule of interest to the chemical industry, as opposed to simply optimizing existing cell to have them produce what they already know how to produce.

We don’t improve, we create! -- Whereas other industrial biotechnology companies rely on natural diversity exclusively, we compute new enzymes and new pathways using proprietary algorithms and cloud computing. Each of our potential solutions are tested experimentally to create novel cell factories that are further developed in partnership with (petro)-chemical companies. Arzeda has successfully employed this partnership model with products in development with INVISTA and DuPont Pioneer.

Effect of glucose on glycerol bioconversion in unaerated continuous cultures by Lactobacillus reuteri
Jyostsna Jolly, Department of Biotechnology, Indian Institute of Technology-Madras, India

1,3-propanediol (1,3-PD) is a valuable molecule, which in the past was produced by chemical methods. Nowadays, it can be produced from renewable resources using microorganisms. 1,3-PD has very promising properties that could be used in polymers, cosmetics, health care and food applications. For the past few years, 1-3-PD is used as a monomer in the production of aromatic polyester called Polytrimethylene terephthalate (PTT), commercially known as Sorona polymer.
Sorona, is a biodegradable polymer which has great potential in textiles, carpets and upholstery manufacturing. In nature, 1,3-PD is produced from anaerobic glycerol fermentation. Most of the microorganisms which synthesize 1,3-PD in high amounts belong to 2nd hazard group, which limits their use in industrial production. In this work, 1,3-PD production in a GRAS (Generally Recognized As Safe) organism, Lactobacillus reuteri, utilizing glucose and glycerol as a co-substrates, was investigated. The influence of molar ratios of glucose and glycerol on growth and production of 1,3-PD was studied in continuous unaerated culture. At all dilution rates (D) studied, cell biomass and 1, 3-PD increased steadily with increasing molar ratios along with glycerol uptake rate. Product concentration as high as 26.6 g/l was obtained at a dilution rate 0.05 h⁻¹, while the volumetric productivity was the highest at 0.15 h⁻¹ (2.2 g/l/h) dilution rate, when the molar ratio was 0.3. Enhanced glycerol metabolism correlated positively with the concentration of glucose. NADH produced during glucose metabolism was preferentially reoxidized to NAD by the reduction of glycerol to 1,3-PD. At low dilution rates, 1, 3-propanediol production was favored, whereas at higher dilution rates lactate production increased. At lower dilution rates higher glycerol consumption, lactate utilization and increased production of acetate was observed. It is likely that at low dilution rate, the conversion of lactate to pyruvate generated NADH and pyruvate was further metabolized to acetate in order to gain one mole of ATP. The generated NADH was reoxidized to NAD via 1,3-PD generation pathway resulting in higher conversion of glycerol to 1,3-PD. While at higher dilution rate, glycerol consumption was less and the lactate produced from glucose metabolism was not utilized for NADH formation, resulting in lower 1,3-PD being formed. Higher molar ratios of glucose/glycerol did not hinder the conversion of glycerol to form 1,3-PD and metabolic activity of cells was also found to increase with increase in molar ratios.

**In silico evaluation of 3-Hydroxypropionic production pathways in industrial microorganisms**

*Balaji Balagurunathan, Institute of chemical and Engineering Sciences, A*STAR, Singapore*

Sustainable production of 3-Hydroxypropionic acid (3-HP), a valuable platform chemical, is gaining much attention recently as a precursor for the synthesis of acrylic acid and other specialty chemicals. Several pathways have been proposed for the production of 3-HP and several microorganisms are currently being explored for the production of 3-HP [1, 2]. Theoretical yields and thermodynamic feasibility of some of these pathways have been already explored [3]. However, the actual biomass coupled yield and yield under different cultivation conditions has to be established. Malonyl-coA reduction pathway and glycerol dehydration pathway are the most prominent pathways for 3-HP production. However, few alternative pathways are also under active investigation. In this work, the advantages of the various pathways for 3-HP production and the organism specific efficiency of the proposed pathways were analyzed. Three industrial microorganisms have been

**Synthetic Biology, Algae and Marine Biotech**

**Production of Natural Vitamin A From The Microalgae Living in The Dead Sea**

*Sadeq Emeish, Al-Balqa' Applied University, Faculty of Engineering Technology , Department of Chemical Engineering*

This study investigates the feasibility of β-carotene production from Dunaliella salina isolated from the Dead Sea employing a number of interdependent steps and focusing on the laboratory scale cultures. Then the produced β-Carotene was subjected to enzymatic oxidation using the enzyme 15,15' β-β carotene dioxygenase to produce tretinoin (vitamin A). The produced vitamin A was verified using proper analysis. Dunaliella salina was isolated from the Dead Sea and cultivated using a certain media until the cell count was 6 million cell/ml, then it was centrifuged and extracted using organic solvents and oils like jojoba oil and ethanol. The extracted β-carotene was subjected to enzymatic oxidation in a bioreactor under inert atmosphere of nitrogen. Freeze drying step was performed to obtain vitamin A as powder. The produced vitamin A powder was of a high purity, and it has a very strong economic potentiality.

**Studies on the bioactivity of different solvents extracts of selected marine macroalgae against fish pathogens**

*Mary Ghobrial, National Institute of Oceanography and Fisheries, Kayet-bay*

Selected species of marine benthic algae belonging to the Phaeophyceae and Rhodophyceae, collected from different coastal areas of Alexandria (Egypt), were investigated for their antibacterial and antifungal, activities against fish pathogens.
In vitro screening of organic solvents extracts from the marine macroalgae, Laurencia pinnatifida (Hudson) Stackhouse, Pterocladia capillaceae (Gmelin), Halopteris scoparia (Linnaeus) Kützing, Stepopodium zonale (J.V. Lamouroux), and Sargassum hystrix var. fluitans Børgesen, showed specific activity in inhibiting the growth of five virulent strains of bacteria pathogenic to fish Pseudomonas fluorescens, Aeromonas hydrophila, Vibrio anguillarum, V. tandara, Escherichia coli and of two fungi Aspergillus flavus and A. niger.

Acetone and ethanol extracts of all test macroalgae exhibited antibacterial activity, while acetone extract of S. hystrix displayed the highest antifungal activity. Macroalgal extracts inhibited bacteria more readily than fungi, besides, the extracts of the Rhodophyceae species showed the greatest activity against current test bacteria rather than fungi. Cluster analysis revealed the general response of the tested pathogens to the action of the different algal extracts. Composition of the most potent algal extracts included acetone extracts of L. pinnatifida, P. capillaceae and S. hystrix and ethanol extract of P.capillaceae was determined using GC-MS. The present study provides the potential of red and brown macroalgae extracts for the development of anti-pathogenic agents for use in fish aquaculture.

A novel strategy for synthesis of magnetic Fe3O4 nanoparticles using chitosan
Hossein Salehizadeh, University of Isfahan

This research describes a novel strategy for synthesis of magnetic Fe₃O₄ nanoparticles under mild conditions using chitosan. Chitosan polysaccharide cross linked by formaldehyde was used as stabilizer for synthesis of magnetic Fe₃O₄ nanoparticles. Magnetic nanoparticles with average size of 9.8 nm were synthesized by co-precipitation method using iron salts in optimum Fe³⁺:Fe²⁺ molar ratio of 2:1. The properties of magnetic nanostructures were determined by modern methods such as FTIR, X-ray diffraction, TEM and AFM. Briefly, the results showed that chitosan can be used as green stabilizer for agglomerate-free synthesis of Fe₃O₄ nanoparticles. Chitosan stabilized magnetic nanoparticles have great potential for biotechnological and biomedical applications such as selective bioseparation of biomolecules, drug delivery and cancer therapy, immunoassay, and for MRI bioimaging as enhanced contrast agent.

Keywords: Biopolymer, Chitosan, Magnetite, Nanoparticles, Nanotechnology, Synthesis

Assessment of Water Use for Microalgae in Open Pond in Southwest USA
Jalal Rastegary, New Mexico State University, Institute for Energy and the Environment
Renewable energy resources will prove to be vital in the coming decades as global population increases and as developing countries expand their energy use. Currently, biofuel is made from a variety of feedstocks such as corn and soybean which are concentrated into pure vegetable oils and processed into biodiesel. Use of food crops for biofuel production has raised a major concern in terms of food prices and competition with land and fresh water needed for agriculture. Microalgae are becoming one promising alternative feedstock for biofuel production and have the potential to replace fossil fuel. The southwestern US is becoming one of the areas that has attracted a lot of attention for microalgae production due to its favorable climatic conditions. One of the major limiting factors for expansion of microalgae in the southwest can be lack of water and the amount of water required for microalgae production in open ponds are not known.

An experiment was conducted growing microalgae Chlorella sorokiniana UTEX 1230 in both outdoor and indoor open ponds at Aggie Mountain on the campus of New Mexico State University. The experiment ran for a period of two weeks during June using geothermal and fresh water. Data was collected on parameters such as water temperature, evaporation, pH, biomass production and turbidity. The results of the findings will be presented at the conference.

Production of biofuels in E.coli using an engineered Cyanobacterial biosynthetic pathway enzyme
Navya Menon, The University of Manchester

The increased demand for an alternative form of fuel has brought about some urgency to explore various metabolic pathways and enzymes in several microbial species for hydrocarbon production. In recent years, cyanobacteria have emerged as an attractive microbial host and engineering cyanobacteria to produce “drop in” fuels such as propane and butane has a greater and wider scientific impact. Whilst appealing, practicalities for producing biofuels in cyanobacteria remain challenging, requiring the identification and engineering of natural biocatalysts and their integration into metabolic processes. Cyanobacterial hydrocarbon biosynthesis arise from the fatty acid metabolism involving a potential enzyme, aldehyde deformylating oxygenase (ADO), which catalyses the decarbonylation of long chain fatty aldehydes to alkanes, mainly in the conversion of octadecanal (C17H35CHO) to heptadecane(C17H36) and formate. Thus, the main objective is to identify the structure and mechanism of ADO as well as other important enzymes involved in hydrocarbon biosynthesis. Optimisation of these enzymes using protein engineering to alter substrate specificity and incorporating the resulted modified enzymes in E.coli using various synthetic biology tools is attempted.

The crystal structure of ADO revealed the presence of an adventitious fatty acid ligand in the active site. A more versatile approach was taken by designing site directed mutagenesis of the residues (Val41 and Ala134) positioned at the entrance
of ligand binding pocket. This was carried out in order to develop an active mutant without any adventitious ligand and optimise ADO for shorter chain aldehydes. A detailed substrate profiling and kinetic characterisation was done from C4-C18 chain length aldehydes. The GCMS characterisation and crystal structure of these mutants did not show any adventitious ligand, while the substrate profiling of these variants showed increased substrate specificity towards shorter chain aldehydes. The Ala134Phe variant also showed a higher propane production compared to wild type ADO, indicating that the removal of adventitious ligand from the active site was beneficial. The present work is now focussed on incorporating these variant ADOs to biofuel production pathway in E.coli for further optimisation.

Curation of lipid pathways in a genome-scale model of Saccharomyces cerevisiae metabolism for application in identifying metabolic engineering strategies to increase biodiesel production

Hnin Aung, Cornell University

At the core of industrial biotechnology are the unique metabolic pathways that dictate the transformational power of industrial microorganisms. Over the past ten years there has been a rapid expansion of our knowledge of these metabolic pathways in part due to the generation of vast amounts of -omics data. The substantial information contained within these datasets have been extracted and structured into a mathematical format as genome-scale metabolic models. Genome-scale metabolic models are built using information on reactions catalyzed by the set of metabolic enzymes encoded by an organism’s genome. The reconstructed topology of the metabolic network in conjunction with additional constraints, such as maximum enzyme capacity and thermodynamics, can be used to define the feasible distribution of metabolic fluxes in a cell. This capability can be extended to the simulation of how genetic perturbations affect the flux space and can ultimately be used to guide strategies for metabolic engineering. In the interest of engineering Saccharomyces cerevisiae for increased productivity of triacylglycerol (TAG), we manually curated the representation of fatty acid, glycerophospholipid, and glycerolipid metabolism in a community-based consensus model of yeast metabolism. This process was undertaken to ensure an accurate representation of the aforementioned areas of metabolism in the model, which is vital for yielding simulation results reflective of actual biochemistry. The results of this effort include the addition of 11 genes which introduce the potential for catalytic activity previously unaccounted for in the model; the amendment of the representation of reactions involved in fatty acid synthesis, elongation, and desaturation and in breakdown and synthesis of TAGs; and a correction for cofactor balancing that enables simulation of β-oxidation of fatty acids. This resulting model considers 910 genes, 3498 reactions, and 2220 metabolites and has an overall accuracy of 90% for the prediction of whether a gene deletion is lethal or viable. In addition, we are using this model to assess pathway specific yield and efficiencies for producing more TAGs from lignocellulosic sugars. Our ultimate goal is to use this model to inform how we genetically engineer S. cerevisiae for biodiesel production.
Technical and Research Presentations

Biorefinery (lab scale) for low cost biodiesel production with zero waste production technology
Debarati Paul, Amity Institute of Biotechnology, Amity University

An oleaginous (fat accumulating) oxidative red yeast that can accumulate lipids and ß-carotene to more than 50% of its biomass when grown under different carbon and nitrogen ratios was isolated in the lab. It showed the capability to grow in various cheap agricultural raw materials such as sugar cane juice, molasses, extracts of vegetable and fruit peels for lipid production. There was concomitant production of beta-carotenoids (antioxidant) and finally biocompost was produced as an additional useful product.

The technology aims at recycling of all the waste/effluents generated during lipid or carotenoid production. Since the organism uses glycerol as a carbon source, this by-product of biofuel production will also be utilized for further cultivation of the organism. The other waste effluents (cell mass and liquid discharge) would be utilized as nutrient (nitrogen) source for re-generation of the yeast biomass in the bioreactor.

The proposed technology is novel bioprocess for the production of an important biofuel along with other invaluable components as by-products. It is also a low-cost technique in terms of availability of raw materials and processing. The zero-discharge aspect of this method makes it further lucrative because it is environment friendly. Preliminary results reflect upon the efficiency of the process as ‘good return on investment’ (ROI).

It has an advantage over algae, mycelial fungi and bacteria due to its unicellular and relatively high growth rate with utilizing low cost fermentation media and is also a good source of proteins, lipids, and vitamins if used as animal feed.

Immobilization of Yarrowia Lipolytica Lipase onto Magnetic Nanoparticles for Resolution of (R, S)-2-Octanol in Ionic Liquids
Liu Ying, Institute of Process Engineering

Ionic liquids (ILs) are known as molten salts, recognized as potential green solvents because of their unique properties such as nonflammability, no measurable vapor pressure, and a wide temperature range of liquid phase. ILs have been extensively employed as enzymatic reaction media and are still being taken an increasing interested in due to their widely tunable properties as regards to hydrophobicity, polarity, and solvent miscibility behavior through simple modification of the cation
and the anion. In the current study, Yarrowia lipolytica lipase (YLL) immobilized onto magnetic nanoparticles was used for resolution of (R, S)-2-octanol in ILs. A series of imidazolium ILs with various anions were tested, including dca-, PF6-, BF4- and TF2N-. In this case, the anions had a significant effect on the activity and enantioselectivity of YLL. Among the anions tested, dca- made YLL complete inactivation, while BF4- made YLL exhibit higher activity or enantioselectivity. [DMIM]BF4 was selected as the optimal reaction medium and provided an increased enantioselectivity by a factor of 2 without a decrease in activity. In addition, the magnetically immobilized YLL with high operational stability was easily recycled with an external magnetic field. These results indicated that the YLL-catalyzed resolution of (R, S)-2-octanol in ILs had a good potential in future application.

**Next-generation synthetic biology tools for rapid, efficient, cost-effective, HTP construction of Living Foundries**

*Sunil Chandran, Amyris*

Microbial production of any molecule requires re-writing the genetic code of the production host and repeated iterations of the design-build-test (D-B-T) engineering cycle. The D-B-T engineering cycle directly affects the time to market for any new product. Amyris has built a strain construction platform that rapidly accelerates cycle time at a high-throughput scale. This Automated Strain Engineering (ASE) platform, the first of its kind, has been immensely successful at lowering the threshold for building and testing strains with potential for further process improvements. Our goal is to reduce cost and cycle time, increase efficiency, and allow access to hitherto intractable hosts. Amyris, with support from DARPA has developed a new set of tools that will enable the synthetic biology community to make faster progress in the development of Living Foundries. In this presentation we will discuss the development of these new tools which include:

i) A genome annotation pipeline capable of processing raw next-generation sequencing data to standard parts within hours

ii) An alternate DNA assembly method capable of assembling large numbers of DNA parts with high efficiency

iii) A cost-effective next-generation sequencing pipeline to sequence thousands of DNA assembly in parallel

iv) Methods that allow us to achieve simultaneous multi-locus marker-free genomic integrations

Incorporation of these tools into our existing pipeline will usher in a totally new standard for a high-throughput strain construction platform capable of generating Living Foundries at a rate, scale, and cost unprecedented in the field.
Models of Company and Community College Partnerships to Provide Local Sources of Advanced Technology Biotechnicians to Support the Growth and Development of the Bioeconomy

Daniel Kainer, Lone Star Community College, The Woodlands, TX

The NBC2 originated in 2005 to support the education and training of advanced technology biopharmaceutical biomanufacturing technicians in locales across the northeast. Our methodology and curricular materials are now in use throughout the country. We help develop local partnerships amongst biomanufacturers, community colleges, four year colleges, universities and high schools and help to develop local career pathways in biomanufacturing. The NBC2 started by working with Lonza Biologics, a local company at the Portsmouth International Tradeport in Portsmouth, New Hampshire, to provide education and training and internships and apprenticeships utilizing the tools, processes and regulatory structure of the biopharmaceutical manufacturing industry. Skills developed in biopharmaceutical manufacturing work well in the crossover industries of the bioeconomy such as biofuels and the renewable chemicals industry. The bioeconomy currently represents 2% of the annual US GDP and is growing. The success of such companies and the growth of the bioeconomy is dependent of the hands-on skills of technicians that produce and analyze its bioproducts. This panel will follow technician education from the birth of the bioeconomy with the manufacture of human insulin in 1982. Dr. Wallman will talk about the early development of biopharmaceutical manufacturing education throughout the northeast and nation. Katrice Jalbert, Technical Writer, Lonza Biologics, Portsmouth, NH will provide the perspective of a student at a community college who received such training right after high school. Katrice will describe her career pathway from high school to apprentice and many positions at Lonza Biologics, a community college partner since 1994. Mike Fino, Director of Biotechnology at MiraCosta College, Oceanside, CA will trace the origin of his program and its support from Idec, now Genentech, in Oceanside and a few feet from his biomanufacturing education and training facility that is part of EDGE (Educating and Developing Workers for the Green Economy) to train technicians and professionals in biofuels and industrial biotechnology to support the strong and many pronged bioeconomy in San Diego, CA. Ikenna Nedosa, Operations Manager at Promethean Biofuels in Temecula, CA came into the community college biotechnology and EDGE biofuels certificate program with graduate degrees. He is now using the knowledge, technical and occupational skills set he learned at MiraCosta College as Operations Manager at Promethean Biofuels. Daniel Kainer, Director of the Lonestar Biotechnology Institute will describe innovative education and training methods adopted at Lonestar College-Montgomery, The Woodlands, TX that include internal internships devised through partnerships with local industry and though nurturing student interest.

Cloning and nucleotide sequence of the maltohexaose-producing amylase gene from Bacillus cereus strain HLSSD-5

Xiaoyan Wang, Yunnan Normal University (Qactive)
The AmyHLSSD-5-2 gene encoding a maltotetraose-producing amylase (exo-maltotetraohydrolase) from Bacillus cereus strain HLSSD-5 was cloned by the shotgun cloning method. The positive transformant carrying pUC19-HLSSD-5-2 with amylase activity was selected from the complete genome library of B. cereus strain HLSSD-5. It contained a 3226-bp Sau3AI-BamHI fragment that had a 1542-bp open reading frame encoding a precursor (513 amino acid residues) of the secreted amylase. The precursor had a signal peptide of 39 amino acid residues at its amino terminus. A region in the primary structure of this amylase exhibited homology to corresponding regions of other amylases. The deduced mature protein had 474 amino acids, a molecular weight of 53.9 kD, and an isoelectric point (IP) of 5.435. The amylase gene was expressed in Escherichia coli BL21 using the prokaryotic expression vector pET28a+. The optimal conditions for the starch-degrading activity were a temperature of 50°C and pH 6.8. The enzyme activity rapidly decreased when the temperature increased above 60°C. The enzyme produced maltohexaose and maltopentaose by hydrolyzing the soluble starch. The enzyme was found to be a glucan 1,4-α-maltohexaosidase (EC 3.2.1.98) and was named AmyHLSSD-5-2. The GenBank accession number of this enzyme is ACQ73554.

Evaluation of second generation biofuels production from native halophytes by chemical-characterization of Salicornia sinus-persica
Ayah Alassali, Masdar Institute of Science and Technology

1. Introduction

Abu Dhabi exemplifies a coastal desert, where seawater could be used for salt-tolerant crops (halophytes) cultivation. The produced halophyte biomass could be utilized in feed, food and/or energy production, depending on its chemical composition. In this study the UAE native halophyte Salicornia sinus-persica was studied for its potential to be used as a feedstock for bioethanol production. Fresh Salicornia sinus-perica contains more than 65% of water. For such green biomass direct fractionation and fermentation can be advantageous. This allows for water preservation and the ability to run at lower dry matter in the fermentation step.

Chemical characterization and ethanol potential of the juice and fibers of the fractionated Salicornia sinus-persica was examined in this study.

2. Methodology

Two batches of Salicornia sinus-persica (washed and unwashed) were juiced, where two main fractions were obtained (juice and fibers). Washing of the fresh biomass
aims to reduce or remove the nonstructural ash (salt deposits). Both fractions were tested for their total dry matter and ash content. Sugar monomer composition was tested for both fractions applying acid hydrolysis as described in (Sluiter et al., 2008a).

The extent of glucan-to-glucose convertibility was tested for the juice by enzymatic hydrolysis (by simultaneous saccharification and fermentation (SSF)) and acid hydrolysis followed by fermentation by baker’s yeast. High Performance Liquid Chromatography was used for ethanol, sugars and other metabolites analysis as described in (Sluiter et al., 2008b).

3. Results

The juice fractions were found to represent 68.86 ± 1.78% of the unwashed batch and 74.09 ± 3.68% of the washed batch. The fiber fractions were found to contain 98.99% (unwashed biomass) and 99.52 % DM (washed biomass) of which 19.77% (unwashed biomass) and 19.73% (washed biomass) was ash. Dry matter content of the juices were found to be 13.06% (unwashed) and 11.58% (washed) of which 58.01% (unwashed) and 57.37 % (washed) was ash.

Sugar analysis revealed relatively low concentration of glucose, xylose, and arabinose in the juice fractions (8.40 g/L glu, 5.97 g/L xyl, and 3.42 g/L ara in juice of unwashed biomass and 7.39 g/L glu 4.87 g/L xyl, and 2.17 g/L ara in juice of washed biomass) and not much difference was observed between the washed and unwashed biomass. The fiber fractions contained 15.6 g/100 g DM glu, 11.55 g/100 g DM xyl, and 14.04 g/100 g DM ara for the unwashed biomass and 16.67 g/100 g DM glu 13.01 g/100 g DM xyl, and 13.82 g/100 g DM ara for the washed biomass. This is comparable to the lignocellulose content of the mature (dry) plant (Cybulska et al., 2013).

The highest ethanol yield in SSF with Baker’s yeast was achieved in juice extracted from washed biomass after enzymatic hydrolysis. In this experiment 129.61% of the theoretical yield (based on sugar analysis) was achieved. This shows that fresh juice of Salicornia sinus-persica is a good medium for yeast fermentation - but more work is needed to identify all fermentable sugars in the juice.

Authors: Alassali, A., Cybulska, I., Brudecki, G. P., Thomsen, M. H.

Life cycle assessment of an algae facade system
Kyoung-Hee Kim, University of North Carolina Charlotte

The construction and operation of buildings significantly contributes to resource depletion and greenhouse gas emissions. A challenge for the building design and construction industries and building owners is to provide healthy indoor
environments without depleting non-renewable energy resources or contributing to air pollution and global warming.

Our project focused on life cycle assessment of an algae facade system, situated in urban environment, as a way to promote building sustainability and improve indoor air quality. An algae façade system is an innovative façade system in that a bio-reactor system has never been integrated into a building envelope as a sustainable alternative.

Detailed analysis results on the life cycle assessment of an algae facade system will be presented.

**Models of Company and Community College Partnerships to Provide Local Sources of Advanced Technology Biotechnicians to Support the Growth and Development of the Bioeconomy**

*Sonia Wallman, NBC2*

The NBC2 originated in 2005 to support the education and training of advanced technology biopharmaceutical biomanufacturing technicians in locales across the northeast. Our methodology and curricular materials are now in use throughout the country. We help develop local partnerships amongst biomanufacturers, community colleges, four year colleges, universities and high schools and help to develop local career pathways in biomanufacturing. The NBC2 started by working with Lonza Biologics, a local company at the Portsmouth International Tradeport in Portsmouth, New Hampshire, to provide education and training and internships and apprenticeships utilizing the tools, processes and regulatory structure of the biopharmaceutical manufacturing industry. Skills developed in biopharmaceutical manufacturing work well in the crossover industries of the bioeconomy such as biofuels and the renewable chemicals industry. The bioeconomy currently represents 2% of the annual US GDP and is growing. The success of such companies and the growth of the bioeconomy is dependent of the hands-on skills of technicians that produce and analyze its bioproducts. This panel will follow technician education from the birth of the bioeconomy with the manufacture of human insulin in 1982. Dr. Wallman will talk about the early development of biopharmaceutical manufacturing education throughout the northeast and nation. Katrice Jalbert, Technical Writer, Lonza Biologics, Portsmouth, NH will provide the perspective of a student at a community college who received such training right after high school. Katrice will describe her career pathway from high school to apprentice and many positions at Lonza Biologics, a community college partner since 1994. Mike Fino, Director of Biotechnology at MiraCosta College, Oceanside, CA will trace the origin of his program and its support from Idec, now Genentech, in Oceanside and a few feet from his biomanufacturing education and training facility that is part of EDGE (Educating and Developing Workers for the Green Economy) to
train technicians and professionals in biofuels and industrial biotechnology to support the strong and many pronged bioeconomy in San Diego, CA. Ikenna Nedosa, Operations Manager at Promethean Biofuels in Temecula, CA came into the community college biotechnology and EDGE biofuels certificate program with graduate degrees. He is now using the knowledge, technical and occupational skills set he learned at MiraCosta College as Operations Manager at Promethean Biofuels. Daniel Kainer, Director of the Lonestar Biotechnology Institute will describe innovative education and training methods adopted at Lonestar College-Montgomery, The Woodlands, TX that include internal internships devised through partnerships with local industry and though nurturing student interest.

Assessing the Influence of Cellulose Accessibility on Cellulase Binding and Catalysis

Dong Yang, Cornell University

At the most fundamental level, saccharification occurs when cell wall degrading enzymes (CWDEs) diffuse, bind to and react on readily accessible cellulose fibrils. Thus, the study of the diffusive behavior of solutes into and out of cellulosic substrates is important for understanding how biomass pore size distribution affects enzyme transport, binding and catalysis. Fluorescently labeled dextrans with molecular weights of 20, 70 and 150 kDa were used as probes to assess their diffusion into the porous structure of filter paper. Fluorescence microscopy in combination with high numerical aperture objectives was used to generate high temporal and spatial resolution datasets of probe concentrations versus time. A pore grouping diffusion model was developed and used to estimate the micro-pore diffusion coefficient that described the inherently porous structure of plant-derived cellulose. Given known enzyme sizes, the method developed can help assess the proportion of accessible pore volume available for fast diffusion without any significant pore hindrance. The study of the biomass porous structure accommodating enzyme movement is also very important for understanding the interactions between CWDEs and biomass. The key goal of the second research was to design a size exclusion system that would allow for high precision and large scale measurements of accessible pore volume in pretreated lignocellulosic biomass. A solute exclusion column packed with different sizes of raw and pretreated mixed hardwood and switchgrass was developed to determine the pore volumes and surface area distributions. A group of polyethylene glycol (PEG) probes whose molecular weights ranging from 1000 to 35000 Da were used in the system and the elution curve dispersions were studied. The structural changes of the size reduced and pretreated lignocellulosic biomass were assessed. The need to reduce the particle size of the pretreated material was evaluated as a means to address column packing challenges with the larger particle sizes that are more realistic for a commercial biorefinery plant. The pore size changing mechanisms resulting from size reduction and pretreatment were proposed. This method developed can be
applied to assess the porous structure of raw and pretreated biomass, evaluate biomass morphology changes during hydrolysis and build relationship between pore structure changes and hydrolysis rate/extent.