Posters will be organized by BFN project number (from 9-98) on the poster boards. Please use this list in order to locate the posters you wish to visit.

BioFuelNet Project Number: 9

Submitting author: Simerjeet Kaur, McGill University

Poster title: Status of cellulose synthase genes in small grain cereals

Abstract: Cellulose is the most abundant polymer on the earth and plays major role in providing physical strength and innate immunity to plants. Cell walls of plants are mainly made up of cellulose along with different proportions of lignin and hemicelluloses. Cellulosic biomass has enormous potential and an important target for synthesis of biofuels and other bioproducts. Cellulose in the primary and secondary cell walls of plants is mainly synthesized by a family of genes called cellulose synthase (CesA) genes. Most of the research about the structure and function of these genes has been performed in model species such as Arabidopsis, rice and barley. In barley, cellulose synthase gene family has eight members encoding the catalytic subunits for cellulose biosynthesis. However, this gene family has not well understood in wheat. Using barley genes as reference, we have explored currently available wheat genomic resources including ESTs. Wheat, being a hexaploid, twenty four genes are expected, eight (TaCesA1 to TaCesA8) of which have been unambiguously identified through comparative genomic analysis. Putative conserved domains were confirmed for all these genes.

BioFuelNet Project Number: 11

Submitting author: David Mochulski, University of British Columbia

Poster title: Unique characterization of biomass gasification suitability via analysis of reaction kinetics

Abstract: The determination of unique kinetic parameters from experimental data has long been a challenge. The way in which the pre-exponential factor and activation energy compensate for one another often leads to inconsistent parameter estimates; each of which may fit the data equally well. This is a particular problem in biomass systems due to the composite nature of the material.

What will be presented is a workflow which sequentially constrains the problem parameters, solving for each in turn, until arriving at a unique solution which is globally consistent across all measured data sets.
This procedure can be summarized in brevity:

1. Uniquely estimating the activation energy through the advanced isoconversional technique laid out by Vyazovkin. This provides a model-free and Arrhenius factor uninfluenced estimate of the activation energy.

2. Using this unbiased activation energy, the kinetic compensation effect is then used to estimate the Arrhenius constant.

3. With the Activation energy and Arrhenius factor known, the reaction mechanism is estimated on a per experiment basis.

4. Finally a global reconciliation of all experimental data is performed. This filters any results which are purely mathematical, leaving only physically consistent models.

This leads to a unique estimate of the kinetic triplet - activation energy, pre-exponential factor, and reaction mechanism.

**BioFuelNet Project Number: 12**

Submitting author: Dave Barchyn, University of Manitoba

Poster title: Pretreatment of lignocellulosic biomass with superheated steam generated by solar energy

Abstract: Pretreatment of lignocellulosic biomass is the necessary step in the production of lignocellulosic biofuels but it is also a very energy intensive step substantially affecting the economy of large-scale production of lignocellulosic biofuels.

Research at the University of Manitoba has explored pretreatment of wheat straw using superheated steam at 180°C and measured its effects on the digestibility by the cellulolytic bacterium *Clostridium thermocellum*. X-ray diffraction analysis was performed on the treated and untreated samples, and showed that pretreatment with water boiling at 1.2 bar and superheated steam (severity factor of 5.27) resulted in a decrease in cellulose crystallinity, indicating a potential increase in digestibility. Fermentation experiments with *C. thermocellum* confirmed that this pretreatment method increased the generation of hydrogen, carbon dioxide by 25 and 35%, respectively, and ethanol production doubled when compared to the control material.

To further improve the energy balance of pretreatment, integration of solar thermal technology was considered for implementation into the generation of superheated steam. Using the ambient conditions in June, 2013 in Winnipeg, Manitoba, it was shown that evacuated tube solar collectors (ETSC) could produce superheated steam at 178°C at an overall efficiency of 9%. While generation of superheated steam of a quality suitable for pretreatment is possible, further experimentation demonstrated that continual operation of ETSC at high temperatures results in component failure,
implying material fatigue due to repeated loading. Further study will examine the implementation of parabolic trough reflectors, which have been shown to operate at high efficiency and at high temperatures.

**BioFuelNet Project Number: 12**

Submitting author: Valery Agbor, University of Manitoba

Poster title: Enhance bioprocessing of lignocellulosic biomass: the contribution of amorphous cellulose and effect of exogenous glycosyl hydrolases

Abstract: Improved access to cellulose chains will improve rates and yields attained during direct microbial conversion of cellulosic biomass for biofuels and co-products production to compete against current industrial alternatives. Regarding biological hydrogen, ethanol and co-products, we have identified the effect of a novel factor called; the contribution of amorphous (CAC) cellulose and have investigated the effect of employing glycosyl hydrolases to enhanced consolidated bioprocessing of agricultural residues using Celluclast and NS50012 from Novozyme Inc. Hydrolysis dosing was between 1.5-5 % w/w (g enzyme / g cellulose) at cellulose concentrations raging from 2 g/L – 10 g/L. Our initial fermentation experiments show increased yield of end products in samples with a higher CAC and a rapid doubling of the amount of hydrogen produced in the test compared to the control within 24 hours when hydrolytic enzymes are employed. These results indicate that improving the CAC and ‘clearing the way and fractionation of cellulose chains’ by a minimal dosage of exogenous enzyme action on lignocellulosic biomass for direct fermentation is a potential strategy for a low cost, rapid route for bioethanol, biohydroden and co-products derived from fermentation of rich cellulosic feedstocks.

**BioFuelNet Project Number: 13**

Submitting author: Jasmin Blanchard, Université de Sherbrooke

Poster title: A NiAl₂O₄ – YSZ catalyst for dry and steam reforming of CH₄

Abstract: Methane sources like biogas contain large amount of CO₂ and chemical synthesis require syngas with a ratio H₂/CO of 2. The main goal of this project is to provide a catalyst that reforms CH₄ with a mix of CO₂ and (a minimal amount of) H₂O in order to produce the required syngas without losing activity due to carbon deposition and/or sintering.

The NiAl₂O₄-YSZ catalyst prevents the deposition of carbon even under dry (CO₂) only reforming conditions with reactants close to stoichiometric ratio. The conversion of methane is close to equilibrium at 97% after a 275h period on stream at a temperature of 850 °C, gas hourly space velocity of 7150ml / (h*g) (STP) and molar CH₄/CO₂/Ar ratio of 1/1.15/0.5.
This work shows that, when H2O is used along with CO2, above a certain water/CO2 ratio the catalyst loses its activity; nevertheless, this activity can be regained entirely after a short period of time, through a catalyst reactivation when the water is withdrawn from feed gas.

This catalytic behaviour can be explained by a mechanism involving the establishment of a dynamic equilibrium at the catalyst surface involving a partial reduction of the spinel NiAl2O4 into metallic nickel and alumina and a re-oxidation of the nano-particles of Ni into spinel depending on the oxidation potency of the reagents.

**BioFuelNet Project Number: 20**

Submitting author: Yanai Tadanori, University of Windsor

Poster title: Engine Efficiency Improvement for Biofuels in Low Temperature Combustion Application

Abstract: The increasing concerns of energy crisis and global warming demand an increase in the use of bio-fuels in internal combustion engines. It can be expected that use of ethanol and n-butanol produced from biomass sources can contribute not only to the above issues but also to the improvement of the exhaust emissions of a diesel engine. The clean diesel engine research group at the University of Windsor has investigated effects of ethanol and n-butanol on the exhaust emission characteristics of a diesel engine. The tests were conducted on a single cylinder water-cooled four-stroke direct injection diesel engine. A dual fuel technique which combines the port injection of ethanol with direct injection of diesel was applied since ethanol has low ignitability and when blended with diesel fuel, has phase separation issue. The engine test indicated that ultra-low NOx and soot emissions were achieved simultaneously even at very high engine loads, which has always been the most severe challenge for diesel engines. n-Butanol is a promising alternative fuel to ethanol for the future since it has many superior properties as a fuel. Neat n-butanol was applied in the direct injection into a diesel engine without major modifications to the fuel injection system since it had better ignitability than ethanol. From the test results, certain operating conditions for the stable combustion were found, and the NOx and soot emissions at these conditions were very low. This work has demonstrated that both ethanol and n-butanol are promising alternative fuels for clean combustion diesel engines.

**BioFuelNet Project Number: 22**

Submitting author: Richard Chandra, University of British Columbia

Poster title: Multi-stage Pretreatments Improve the Fractionation and Enzymatic Hydrolysis of Woody Biomass

Abstract: Similar to oil refineries, additional value-added co-products will have to be produced as part of a biomass-to-fuels process to make them economically attractive. One way to ensure maximum utilization of the biomass components is through the effective separation/recovery/use of
most of the lignin, hemicellulose and cellulose after biomass pretreatment. An ideal pretreatment process would yield three clean fractions containing cellulose, hemicellulose and lignin. However, the cellulose, hemicellulose and lignin components that comprise the majority of lignocellulosic materials have varying physical and chemical properties, reflecting their differing recalcitrance in terms of their ease of fractionation and recovery. For example, hemicellulose, which can act as a hydrophilic “filler” material in the cell wall, is an amorphous low molecular weight polymer which can be readily hydrolyzed and extracted when compared to cellulose and lignin. In addition, there are also significant differences in the structure and recalcitrance between agricultural, hardwood and softwood biomass. For example, softwoods contain highly refractory cross-linked guaiacyl rich lignin compared to the syringyl rich lignin in hardwoods. These differences in recalcitrance between biomass components and types can confound fractionation strategies. Most of the current pretreatment processes that are being pursued commercially use compromise conditions that maximize the ease of hydrolysis of cellulose while sacrificing hemicellulose recovery or the recovery of lignin with potential value added applications. The presented work will describe a multi-stage pretreatment that provided good recovery of the hemicellulose and lignin fractions while enhancing the enzymatic hydrolysis of the cellulose component, resulting in a recovery of more than 85% of the original carbohydrate from the hemicellulose and cellulose fractions while using enzyme loadings as low as 7 mg protein (3 FPU/g glucan) to hydrolyze the cellulose.

BioFuelNet Project Number: 26

Submitting author: Miloslav Sailer, University of Manitoba

Poster title: Are polyhydroxyalkanoates made by Pseudomonas Putida a viable source of biofuel additives? Using the Reformatsky reaction to generate potential target molecules for combustion testing

Abstract: Pseudomonas Putida LS46 can produce polyhydroxyalkanoates, as a form of carbon storage, from a large variety of downstream by-products, including by-products from biodiesel production. The biodegradable polyesters can be cost-efficiently converted into their monomeric ester forms (i.e. β-hydroxyesters) in a manner similar to the production of fatty acid esters (i.e. biodiesel) from oils. In order to set targets for the optimization of strain selection and fermentation conditions, these β-hydroxyesters were mainly synthesized using the Reformatsky reaction. Furthermore, new synthetic procedures for the reaction were developed for the scale-up of the exothermic reaction. These β-hydroxyesters were tested for their effectiveness as biofuels and as biodiesel blends, and showed a reduction in the cloud point, flashpoint, and oxidative stability when blended with biodiesel derived from canola. Further investigations include the cost-efficient conversion of the polyhydroxyalkanoates into 1,3 diols of varying carbon chain length and their effectiveness as biofuels or biofuel blends.

BioFuelNet Project Number: 28
Abstract: Heterotrophic and mixotrophic micro-algae productions require usually external carbon sources, other than CO2, to maintain and increase productivity. For algae derived fuels, a massive amount of lipid rich micro-algae biomass is needed to achieve profitability. Heterotrophic and mixotrophic production systems can achieve such amount if they are feed with sufficient and low cost carbon sources. Securing cheap carbon sources in a short area could also be a problem. The quest for sources of bio-waste, rich in sugars or organic carbon, abundant and locally available is on. Some cheap sugars or organic carbon sources could be supplied by agri-industries, but may not be sufficient in most areas (e.g. cheese whey). Cellulosic residues, available at high quantities in most regions, could be a solution, but need primary and secondary transformations that may increase their cost. The goal of this project is to assess the possibility of feeding algae heterotrophic or mixotrophic production systems with different cheap carbon sources hydrolysates, especially cellulosic hydrolysates obtained after enzymatic hydrolysis of pretreated lignocellulosic residues and studying the effect on productivity (biomass, lipids). Use of silage juice hydrolysates and starchy wastewater hydrolysates as cheap carbon sources are also explored.

BioFuelNet Project Number: 29

Submitting author: Majid Soleimani, University of Saskatchewan

Poster title: Saccharification of pelletized biomass for the biofuel industry

Abstract: The hydrolysis of biomass densified using a continuous pilot-scale pelletizer was investigated as the source of carbohydrates for bioethanol production. Wood and wheat straw biomass were separately pelletized using carbohydrate-based binders and with the application of four lubricants namely mineral oil, canola oil, crude glycerol, and pure glycerol at effective lubrication levels. The impacts of the binding agent, loading level of the binder, and the lubricant were investigated on the kinetics of enzymatic biomass hydrolysis.

BioFuelNet Project Number: 29

Submitting author: Kingsley Iroba, University of Saskatchewan

Poster title: Application of Fourier transform infrared-photoacoustic spectroscopy for the compositional analysis of radio frequency alkaline pretreated and non-treated barley straw

Abstract: Lignocellulosic biomass has been identified as a potential feedstock for the biofuel industry. The individual quantifications of biomass components (lignin, cellulose, and hemicellulose) are often performed using the traditional acid hydrolysis followed by gravimetric determination. This approach
is complicated and time consuming, and could lead to destruction of some components due to the
harsh acidic medium of analysis. In this study, barley straw grind was pretreated using radio
frequency alkaline approach. The effect of the pretreatment was first evaluated using the traditional
acid hydrolysis. Then, Fourier transform infrared-photoacoustic spectroscopy (FTIR-PAS) was used in
light of the need for rapid and easy quantification of biomass components. The effect of the radio
frequency alkaline pretreatment conditions on biomass sample was measured on the basis of their
photoacoustic (PA) spectra. In order to develop a predictive model that will be swiftly used for the
quantitative prediction of the chemical composition contained in the biomass, reference materials:
pure cellulose (microcrystalline powder), hemicellulose (xylan from birch wood), and lignin
(hydrolytic) powders were mixed in different proportions with known concentrations. These were
used as standard spectra to determine the relationship between the respective quantity in the
mixture and the representative biomass sample FTIR spectra. The FTIR wavenumber-dependent
instrumental effects were corrected by using carbon black reference spectrum. Multiple linear
regression models for cellulose, hemicellulose, and lignin were developed based on the generated
regression parameters/coefficients. The results obtained show that the PA infrared spectra can be
rapidly used for biofuel feedstock identification and analysis of the chemical composition with no
degradation unlike the traditional approach.

BioFuelNet Project Number: 29

Submitting author: Edris Madadian, McGill University

Poster title: Switchgrass Evolution, Genetic Improvement and Cultivar Development

BioFuelNet Project Number: 29

Submitting author: Edris Madadian, McGill University

Poster title: Harvest and Post-Harvest Densification

Abstract: Agricultural and forest biomasses are the primary renewable feedstocks for making bio-
fuels and bio-products. However, the development of conversion and end-use technologies will be in
vain if the needed feedstock do not meet quantity and quality criteria throughout the value chain.
This research project has been investigating feedstock from field harvest through to densification to
the final conversion and energy extraction, with a strong focus on the logistics of this pathway. One
of the major components of this project has been the research of a down draft gasifier to produce
bioenergy from different types of biomass feedstock. The results showed that the shape and size of
the feedstock can significantly influence the flowability of the material inside the reactor creating
operational issues. Our results have shown that the bigger and bulkier material result in less
operational challenges, while low density material and smaller sized feedstock results in bridging of
the feedstock. Through the selection of the proper feedstock, size and shape, we can avoid the
limitations of the gasifier and optimize its operation. Testing of new wheat stem varieties both with
solid stem and hollow stem to look into the variation of mechanical properties (tensile, shear, cutting,
compression) that affect the harvesting and handling of agricultural residues is performed. Moisture
content of stem is the main factor that affects these mechanical properties. Densification studies of wheat straw and other agricultural residues are undertaken to determine whether binders are needed in forming pellets. Crop straws are known to be difficult to pelletize and we have identified binders that can be used in the pelletizing process. Optimization of binders and other variables during pelletizing process are being undertaken. Work on biomass compositional analysis through FTIR-PAS was also undertaken.

**BioFuelNet Project Number: 30**

Submitting author: James Aluha, Université de Sherbrooke

Poster title: Carbon-supported iron and cobalt catalysts for Fischer-Tropsch Synthesis

Abstract: Three methods were used to prepare catalysts based on iron and cobalt for the production of diesel in the Fischer-Tropsch synthesis. Catalysts were synthesised by plasma spray, by impregnation or by precipitation with a targeted 40% of the metal on carbon. The catalysts were tested for their activity and selectivity for Fischer-Tropsch synthesis in a batch reactor for 24 hours using about 2.5 g of catalyst at 30-bar pressure and 493K in 150ml of hexadecane solvent (C16). The reaction products were analysed by both Gas Chromatography (GC) and High Performance Liquid Chromatography (HPLC). Fresh catalysts were characterised by Scanning Electron Microscopy (SEM) in conjunction with Energy Dispersive X-ray Spectroscopy (EDX), which (semi-quantitatively) indicated that catalysts prepared by either the plasma spray technique or by impregnation method had about 30%(±5) metal loading while the precipitation approach indicated about 17%(±1) metal loading.

**BioFuelNet Project Number: 31**

Submitting author: Garret Munch, University of Manitoba

Poster title: Microbial Conversion of glycerol into triacylglycerides for biodiesel production using oleaginous yeasts

Abstract: Current methods for biodiesel production utilize vegetable oils as a source of lipids or triacylglycerides (TAGs). Oleaginous yeasts (able to store >20% their cell mass as lipids) are of interest due to their ability to utilize a wide range of waste carbon sources, high cell densities achieved in a short amount of time, and high TAG production in proper conditions. This study compares the growth and lipid production of three oleaginous yeasts (*Rhodosporidium babjevae*, *Rhodosporidium diobovatum*, and *Yarrowia lipolytica*) when grown with glycerol (a biodiesel industry by-product which is abundantly available) as the carbon source. Culture conditions were arranged so that a nitrogen-limiting environment was achieved through natural consumption of the available nitrogen during growth, driving lipid production via nutrient deprivation. Following 120 hours of growth, the final biomass concentration (in g/L) of *R. babjevae*, *R. diobovatum*, and *Y. lipolytica* were 9.4 ± 0.80, 12.0 ± 0.82, and 8.6 ± 0.67, respectively. Final lipid amounts (in % dry cell weight) for *R. babjevae*, *R. diobovatum*, and *Y. lipolytica* were 34.9 ± 3.0%, 63.7 ± 4.5 %, and 28.8 ± 2.7%,
respectively. The chain length of these lipids were found to be mostly C16 and C18 moieties, which are commonly found in vegetable-derived biodiesel. *R. diobovatum* was able to produce 7.64 g/L TAGs, a 3.1 fold increase over *Y. lipolytica* and a 2.4 fold increase over *R. babjevae*. As well, 63.5% of the carbon taken up was used for TAGs or biomass in *R. diobovatum*. These results show that oleaginous yeasts could be used as an alternative to vegetable-derived oils for biodiesel production. Future work will explore scale-up and alternate culture methods to increase the yield, while alternate carbon substrates will be assayed to reduce production costs.

**BioFuelNet Project Number: 31**

Submitting author: Umesh Sharma, University of Manitoba

Poster title: Effects of media sterility and carbon loading on production of Medium-Chain Length Polyhydroxyalkanoates (Bio-plastics) by *Pseudomonas putida* LS46 using biodiesel waste glycerol

Abstract: The market for bio-products has been rapidly growing, and is largely attributed to widespread concerns over the environment. Numerous bio-products have been introduced, one of which includes Polyhydroxyalkanoates (PHAs), commonly known as bio-plastics. PHAs are a class of linear polyesters produced and stored within intracellular granules of some bacteria, genetically modified microorganisms, and transgenic plants in high carbon environments and specific nutrient limiting conditions, primarily nitrogen. Currently, sustainable and cost-effective fermentation strategies are being developed for microbial production of PHAs by incorporating various industrial waste streams as rich carbon source. Nonetheless, the need for understanding conditions most optimal for high cell densities and PHA accumulation is necessary. In this study, a comparative analysis was carried out using a novel mcl-PHA-producing strain of the gram-negative bacterium *Pseudomonas putida*, LS46. Effects of media sterility and increased carbon loading on overall cell biomass and mcl-PHA accumulation were investigated. LS46 was grown in two separate flask-batch conditions, i.e. sterile versus non-sterile media. Both conditions contained 1g/L of nitrogen. Varying concentrations of waste glycerol (WG) obtained from a biodiesel production plant were provided at concentrations of 1, 3, 6, 9, and 12% v/v over a 72 hour period. Variable carbon loading examined the possibility of selective pressure for increased PHA accumulation exclusively by LS46, particularly at higher WG concentrations in non-sterile media. However, contrarily both cell biomass and PHA accumulation by LS46 declined with increased WG within the non-sterile media. Furthermore, media sterilization was deemed necessary to maintain a PHA producing culture, as growth of competing bacteria was observed in non-sterile conditions. Cell biomass in non-sterile media with 12% WG resulted in ~10g/L, while PHA accumulation occurred below 0.5% indicating low LS46 densities. Conversely, sterile media conditions exhibited highest LS46 densities in 1% WG at 3.94g/L with PHA accumulation at 15% of cell dry weight; cellular activity of LS46 was impeded in high WG concentrations, which implied probable inhibitory effects in such conditions. All in all, sterile media and low WG concentrations were essential for LS46 in flask-batch given the high cell density and PHA accumulated.
BioFuelNet Project Number: 32

Submitting author: Benedicte Gelebart, Université de Sherbrooke

Poster title: Ultrasound-induced simultaneous extraction of algal biomass’ metabolites using a green solvent as process analytical tool for 3rd generation biofuels

Abstract: The depletion of non-renewable sources of energy has a dire impact in the field of liquid transportation fuel in Canada. Increasing prices at the pump combined with the alarming accumulation of greenhouse gas emissions has drawn many companies toward finding new solutions to displace part of the 40BL of gasoline and 17BL of diesel being consumed annually in Canada. Algal biomass is being considered around the world including in northern countries, this biomass has the advantage of growing significantly faster than lignocellulosic biomass thus providing a unique opportunity to assimilate large amounts of CO2 whilst producing an energy-rich biomass. However, independently of where this biomass would be grown in the world (which in Canada is another significant factor) the industrial scale production of biofuels from microalgae faces some technological challenges including the necessity of dewatering the algal biomass from the culture medium prior to conversion. This project reports on a process allowing the extraction of targeted metabolites of interest from microalgae whilst avoiding the energy-consuming dewatering step. In order to do so, a green solvent is added to the algal biomass directly in the culture medium after which the combined mixture is sonicated. The ultrasounds act as a cavitation unit inducing device thus provoking lysis of the microalgae cell wall. As well as destructuring the micro-organisms, the ultrasounds as well create a solvent/water emulsion allowing sequestration of the hydrophilic compounds for the hydrophobic ones. The characteristics of the ultrasounds, the time of exposure and the concentration of microalgae will be determined to reach the optimal yield of extraction using a minimum amount of solvent. After sonication, the emulsion is broken and separation of aqueous and organic phases is done to isolate the extracts. Whilst the aqueous extracts (mostly composed of carbohydrates) could be used for ethanol production, the non-polar extracts (essentially composed of lipids) could be used for the production of derived products, including but not limited to biodiesel.

BioFuelNet Project Number: 32

Submitting author: Joey Labranche, Université de Sherbrooke

Poster title: Production of 2nd and 3rd generation bioethanol using a synergetic steam treatment

Abstract: Production of algal biomass at an industrial scale is constrained by many technological challenges and especially in Canada where the climate is also to be considered. Amongst the common problems that can be related to algae and more specifically to their conversion, dewatering and lipids or sugars extraction remains a technological barrier across the planet even in tropical climate. In this work, a novel dewatering process will convert the algal biomass where first the algae
are concentrated by flocculation to decrease the total volume to be filtered and to increase the average size of the algae agglomerates (thus facilitating the filtration step). The flocculation agents used are not toxic to the algae so that the remaining solution can be used again as a growth medium to provide minimal water and nutrients requirements. The next step is to filtrate the concentrated solution on a filter medium composed of lignocellulosic fibers. These fibers and the algae are then simultaneously converted using the feedstock impregnation rapid and sequential steam treatment (FIRSSST). Following this process, the filtrating medium can be separated to lignin and cellulose and the algae can be broken, giving access to sugars, lipids and proteins. Cellulosic glucose (post hydrolyze treatment) is then combined to the sugars and proteins from the algae are then fermented to ethanol. Lipids, recuperated as a non-miscible upper phase, can be used for the production of added-value compounds including, but not limited to biodiesel, via transesterification.

BioFuelNet Project Number: 33

Submitting author: Jilagamazhi Fu, University of Manitoba

Poster title: Metabolic response of Pseudomonas putida LS46 producing medium chain length polyhydroxyalkanoates under three selected industrial “waste” streams through systematic “omics” analyses

Abstract: Pseudomonas putida LS46, isolated from wastewater treatment plant, was able to metabolite industrial wastes as carbon into medium chain length polyhydroxyalkanoates (mcl-PHA): bioplastic. P. putida LS46 displayed reduced metabolic activity and decreased polymer content (17.4 wt% vs 15.3 wt%) when grown on biodiesel-derived glycerol (REG-80) compared with chemical reagent grade glycerol (30 g/L). These phenotypes may be partially explained by decreased expression of enzymes in three metabolic networks: the glycerol uptake system, energy production, and mcl-PHA biosynthesis. Proteomic and RNASeq analyses were conducted to survey possible cellular responses to impurities in REG-80 and showed up-regulation of gene products involved with copper resistance. Furthermore, up-regulation of a sodium/proton exchanger may suggest that P. putida cells experience stress from the high salt concentration in REG-80. Biodiesel-derived free fatty acids (1% v/v) and used (“waste”) canola fryer oil were also tested as alternative inexpensive carbon sources for mcl-PHA production by P. putida LS46. In contrast to glycerol-based cultures, mcl-PHA polymers were synthesized during exponential phase from these substrates, and accumulated to 28.8% of dry cell mass (wt%) and 17.7 wt%, respectively, during stationary phase. No significant differences in the expression levels of mcl-PHA synthesis genes or gene products were observed during exponential phase of cells cultured with free fatty acids or used canola oil versus glycerol-based culture. However, the analyses revealed that P. putida LS46 cultured with free fatty acids or vegetable oils was likely to use fatty acid beta-oxidation rather than de novo synthesis for mcl-PHA production during exponential growth. The synthesis of mcl-PHAs during exponential cell growth may associate with altered and/or unique gene expression patterns in the fatty acid oxidation pathway. Linking cellular metabolism and physiology to carbon source and growth phase with
comparative ‘omics can assist with further metabolic engineering work to optimize mcl-PHA production from industrial waste streams.

**BioFuelNet Project Number: 33**

Submitting author: Rumana Islam, University of Manitoba

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

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

**BioFuelNet Project Number: 33**

Submitting author: Ryan Sestric, University of Manitoba

Poster title: Substrate Utilization by the Oleaginous Yeast, *Yarrowia lipolytica*, for Single Cell Oil Production

Abstract: Triacylglyceride (TAG) production in the oleaginous yeast, *Yarrowia lipolytica*, has potential for biodiesel synthesis. Growth and lipid production by *Y. lipolytica* were compared for cells cultured in nitrogen-complete medium containing glycerol, dextrose, canola oil, or waste glycerol derived from biodiesel production as the carbon sources. Growth and lipid production were also analyzed for *Y. lipolytica* cells cultured in nitrogen-limited media containing pure glycerol and mixed substrates containing glycerol and dextrose. *Y. lipolytica* displayed biphasic growth and basal levels of TAGs when cultured in rich media. Significantly greater amounts of TAGs were synthesized by *Y. lipolytica* cultured in minimal media compared to rich media (approximately 3 fold on dry weight basis when grown on glycerol). Cultures in minimal medium containing glycerol yielded 31% TAGs on a dry cell weight (dcw) basis, while cultures in minimal medium containing glycerol and dextrose produced 38% TAGs (dcw), with glycerol consumption favored over dextrose consumption.
BioFuelNet Project Number: 34

Submitting author: Madjid Birouk, University of Manitoba

Poster title: Improving biodiesel cold flow properties

Abstract: The use of biodiesel as a replacement/supplementary fuel for compression ignition engines requires improving its cold flow properties (e.g., cloud point, pour point, viscosity, etc.) for practical usage in ambient conditions that correspond to below freezing. There are several attempts in the literature in which a variety of additives and blending were tested. The most common methods used are blending biodiesel with petroleum diesel fuel, use of additives and modification of the biodiesel feedstock. The addition of diesel fuel to biodiesel was found to improve cold flow properties. Metallic additives (e.g., Mg, Mn and ferric chloride) were reported to improve cold flow properties of biodiesel. Also, oxygenated additives (e.g., ethanol, methanol, butanol, etc.) have been reported to improve both cold flow properties and emissions of biodiesel. The present study examined canola biodiesel blended with 5%, 10%, 15% and 20% (per volume) of C4-Esters (3-hydroxy fatty acid methyl esters (3-OH FAMEs or 3-OH Mes) and 3-hydroxy fatty acid ethyl esters (3-OH FAEEs or 3-OH Ets). Single droplet gasification experiments of canola biodiesel (B100) blended with C4-OET have shown to accelerate the vaporization rate of B100. Tests revealed that about 25% to 37% of the fuel total mass recorded an increase in the gasification rate from about 40% to 80% (where the lower end corresponds to 5% C4-OET in the blend and the higher end corresponds to the 20%C 4-OET in the blend). Similar trends were observed when blending B100 with similar percentages of C4-OME. However, the blending with C4-OME resulted in a slight increase in the gasification rate in comparison to the blending with C4-OET.

BioFuelNet Project Number: 36

Submitting author: Nguyen Nguyen-Tran, University of Manitoba

Poster title: Increasing efficiency and reducing economic barriers in biological conversion

Abstract: Recently, there has been a growing interest in producing fuels and bioproducts from non-food biomass feedstock, such as wheat straw, mainly due to the increased concerns about climate change and the need to reduce carbon emissions. However, lignin, which is an integral part of the plant cell walls, limits the accessibility of plant cell wall polysaccharides to chemical, enzymatic and microbial digestion. Thus, the conversion of biomass to biofuels requires the removal of lignin through costly pre-treatment processes. Genetic reduction of biomass lignin content has been considered as one of the viable alternatives to overcome this limitation. Although, the molecular basis of lignin biosynthesis is well studied in dicot species, most cereal crops such as wheat are much less studied with this respect. In this study, we profiled the lignin content and analyzed the spatial expression patterns of several lignin biosynthesis genes (4CL, C3H, C4H, CAD, CCoAOMT, CCR, COM, F5H, HCT and PAL) and their family members in different tissues of wheat. The lignin content of the leaf and internode tissues was found to be 4.5% and 9.1% of their dry weight, respectively. Our data
show that the lignin biosynthetic genes exhibit tissue specific expression patterns. When compared across tissues, most of the genes are highly expressed in the internodes than in the leaf, except that CCR1, CAD2-4 and CCoAOMT2 showed either higher expression in the leaf or similar expression in both tissues. Comparison of gene expression in each tissue revealed that CAD2, CCR1 and COM2 genes are predominant in the leaf while 4CL1, CCoAOMT1, COM2, and PAL8 are highly expressed in the internode. These results suggest that COM2 regulates lignin formation and accumulation in both tissues while the other predominant genes have a tissue specific contribution.

BioFuelNet Project Number: 37

Submitting author: Naveenji Arun, University of Saskatchewan

Poster title: Evaluation of transition metallic nitride catalysts for the hydrodeoxygenation of oleic acid

Abstract: Hydroprocessing of vegetable oils to produce diesel fuel substitutes is a promising route for the production of third-generation alternate fuels to meet the present energy demands. Nitrides of NiMo supported on Al2O3, SBA-15 and HMS were evaluated for their hydrodeoxygenation activity in terms of extent of oxygen removal and selectivity towards paraffin products especially octadecane (n-C18H38). All the catalysts were prepared by incipient wetness impregnation method with same metal loading (12 wt% Mo and 4 wt% Ni). Physio-chemical characterization of the catalysts were performed using XRD, BET, NH3-TPD, CO-chemisorption and XANES techniques. Characterization results indicate that nitride of NiMo/SBA-15 had the highest surface area (484 m2/g) among the other nitride catalysts and average pore diameter of 9.4 nm. Above temperature 400°C, cracking was observed and selectivity towards paraffin especially n-octadecane decreased even though the conversion was high (>75%), hence, 390°C was chosen as optimum temperature. Ni-Mo supported over γ-Al2O3 gave maximum oxygen removal of 77% and alkane (C14-C18) selectivity of 42% at a reaction temperature of 390°C, agitation speed maintained at 500 rpm in a reaction time of 8h proving its superiority over the other catalysts that were evaluated. Acidity of the support material is proven to play a very crucial role in the hydrodeoxygenation reaction. Higher acidity of γ-Al2O3 favors the oleic acid conversion in comparison to the other support materials. Kinetic model for this process was developed to fit the experimental data and the activation energy for the conversion of oleic acid to n-octadecane was determined.

BioFuelNet Project Number: 37

Submitting author: Naveenji Arun, University of Saskatchewan

Poster title: Green diesel production from biomass: Characterization of the biomass and transition metallic nitride catalysts for hydrodeoxygenation reactions

Abstract: Production of third generation fuels are mainly dependent on environment friendly, renewable and cost effective feedstocks such as biomass. Biochar and bio-oil were obtained by the slow and fast pyrolysis of biomass (pinewood, timothy grass and wheat straw). The fast pyrolysis of
pinewood, timothy grass and wheat straw resulted in the yield of 40-48 wt. % bio-oils, 21-24 wt. % biochars and 17-24 wt. % gases. The slow pyrolysis of the three biomasses produced 18-24 wt. % bio-oils, 42-44 wt. % biochar and 24-27 wt. % gases. Pinewood is a promising feedstock as the yield of bio-oil was high (48 wt. %) in comparison to other feedstocks (Timothy grass – 40 wt. %; Wheat straw – 37 wt. %). Transition metallic nitride catalysts such as NiMo supported on γ-Al2O3, SBA-15 and hexagonal mesoporous silica (HMS) were synthesized using incipient wetness impregnation method to perform the hydrodeoxygenation of bio-oils obtained from biomass pyrolysis. All the catalysts were prepared by impregnation method with the same metal loading (12 wt. % Mo and 4 wt. % Ni). Synthesized catalysts were extensively characterized using XRD, BET, NH3-TPD, CO-chemisorption and XANES techniques to understand their physico-chemical properties. Characterization results indicate that nitride of NiMo/SBA-15 had the highest surface area (484 m2/g) among the other nitride catalysts and average pore diameter of 9.4 nm. NH3-TPD results indicate that γ-Al2O3 exhibited higher acidity in comparison to SBA-15 and HMS. Catalysts with moderate acidity are anticipated to favor hydrodeoxygenation reactions in comparison to less acidic catalyst materials with better bio-oil upgrading abilities.

**BioFuelNet Project Number:** 37

**Submitting author:** Sonil Nanda, York University

**Poster title:** Biochemical characterization of agricultural and forestry biomass

**BioFuelNet Project Number:** 39

**Submitting author:** Kayla Nemr, University of Toronto

**Poster title:** A Novel Computational Model-Based Metabolic Engineering Strategy for Biofuel and Biochemical Production in *Escherichia coli*

**Abstract:** Due to the inevitable depletion of fossil fuels and their negative environmental impact, there is an increased focus on producing biofuels and biochemicals using sustainable processes that utilize renewable-based feedstocks. One promising approach is whole cell biocatalysis, in which organisms are used to convert sugar-based resources to a desired compound. However, microorganisms have not evolved to maximize the production of certain chemicals, but require fine-tuning at the genetic level for enhanced chemical production and tolerance. Systems metabolic engineering coupled with computational modelling are invaluable tools for enhancing biofuel and biochemical production in microorganisms. A novel computational method has been shown to yield a minimal number of gene knockouts required to produce a compound of interest at high yields regardless of growth rate. We therefore developed a model-based engineering strategy, using this method, to engineer *Escherichia coli* for enhanced ethanol production. Based on this strategy, ethanol is hypothesized to be produced at a minimum yield of 1.4 moles of ethanol per mole of glucose consumed regardless of growth rate. Ethanol production in the engineered *E. coli* strain was chosen as a proof of concept, since the chemical is naturally produced in *E. coli*, which can be easily
genetically modified. First, we engineered the strain and characterized it to validate the computational strategy. The next step will be to further engineer the platform strain for the production of other valuable chemicals such as diols, which require similar precursors as ethanol. Once the strategy is validated in *E. coli*, the computational method can then be extended to design similar engineering strategies for enhancing biofuel and biochemical production in yeast, which is a robust organism with a relatively high tolerance to alcohols and acids.

**BioFuelNet Project Number: 39**

Submitting author: Naveen Venayak, University of Toronto

Poster title: Synthetic Biology Applications for Dynamic Metabolic Engineering

Abstract: The current use of oil reserves is unsustainable and has negative environmental impacts. By harnessing the diverse metabolism of microorganisms, we can produce current petroleum-derived chemicals and fuels using renewable resources, thus reducing our reliance on petroleum. Metabolic engineering aims to genetically modify these organisms to improve their ability to produce important chemicals. Classically, metabolic engineering focused primarily on the improvement of yield, with improvements in productivity only recently being discussed. While an improvement of yield is critical to reduce operating expenditures, productivity improvements can drastically reduce capital expenditures. In vivo and in silico metabolic engineering strategies to improve productivity are limited, and have only been recently discussed. Using in silico metabolic models, it has been shown that the optimal strain design strategy for yield will result in suboptimal productivity, necessitating new algorithms which can account for this inherent trade-off. These algorithms determine static metabolic engineering strategies, and they do not exhibit dynamic behavior through the course of a batch fermentation. Conversely, dynamic metabolic control can be applied to greatly improve productivity by dynamically regulating metabolism. Recent progress in synthetic biological circuits ease their adoption in metabolic engineering to allow for dynamic control. In this work, we have shown that the optimal productivity for a static engineered strain can be improved two-fold through implementation of dynamic metabolic control. We also propose an implementation of the genetic toggle switch, a seminal work of synthetic biology, to control this system. Furthermore, dynamic metabolic control allows the application of new computational algorithms to design strains which are not based on the commonly used growth-coupled production strategies. Successful implementation and characterization of dynamic metabolic control is essential for streamlined adoption in metabolic engineering, and has the potential to greatly improve the industrial viability of engineered strains and related bioprocesses.

**BioFuelNet Project Number: 40**

Submitting author: Lucas Dooley, Memorial University

Poster title: The influence of age on the chemical properties of fast pyrolysis products from sawmill and forest residues
Abstract: The forestry industry is under increasing pressure to diversify due to a decrease in world demand for paper products. One challenge is to utilize the forest/mill residue (i.e., hog fuel and bark from P&I mills; sawdust and bark from sawmills; forest residue from tree harvesting) and convert them to useful biofuels/products in regions of remoteness, lack of infrastructure, while having a diverse age of feedstock. One feedstock of particular interest is the large heritage piles of conifer bark and sawdust that is accumulating at sawmill sites from lack of active P&P mills. Our study involved the development of useful profiling methods of forest feedstocks using 1) Py-GC/MS to predict the quality of bio-oil and 2) oxidative TGA to determine the bulk compositional changes with age and type of feedstock. The bio-oil and biochar were produced using a 10 g scale tube furnace. The oils were characterized by GC/MS, water content by Karl Fisher titration, ash and residue content were also measured. Biochars were characterized by CEC and GAC. Oxidative TGA results showed significant decrease in hemicellulose fraction with age for sawdust and bark, while bark also showed a loss of ash content. Py-GC/MS profiles of feedstock showed good correlation with resulting GC/MS analysis of bio-oil products. Interesting the age of sawdust (fresh to 5 years) did not affect the yields of bio-oil. There was a marked decrease in pH and organic acids in bio-oil samples with age of both sawdust and bark indicating that the hemicellulose fraction containing acetyl groups was being removed during long-term storage. Further work will focus on optimum storage of forest wastes/residues and scale-up of fast pyrolysis using a 2 kg/hr auger pyrolysis unit.

**BioFuelNet Project Number: 42**

Submitting author: Craig Slepicka, University of Alberta

Poster title: Characterization of microbial biofuels for high efficiency HCCI engines

Abstract: HCCI (homogeneous charge compression ignition) has a premixed charge like a spark-ignition engine except the ignition is triggered by the chemical kinetics similar to a diesel engine. This combustion process allows HCCI engines to run on a variety of fuels making it a very promising high efficiency engine that can burn a variety of biofuels. Controlling combustion timing is the main challenge of HCCI combustion and requires that the combustion properties of the fuel be understood. The proposed project will characterize fundamental microbial biofuel combustion properties and use reduced order chemical kinetic modeling of these fuels for ignition timing control. Concurrently the fuels will be appropriately blended with known fuel ratios and the combustion properties in an engine running both in SI and HCCI modes will be measured. Experimentally characterizing the basic fuel combustion properties and the combustion properties in the engine will be performed to study the potential of these biofuels for future high efficiency and environmentally friendly combustion engines.

**BioFuelNet Project Number: 45**

Submitting author: Erin Dul, University of Alberta
Poster title: Use of combinations of antimicrobials to mitigate microbial contamination in the bioethanol industry

Abstract: Bioethanol is frequently produced by fermentation of grain or lignocellulosic substrates using the yeast *Saccharomyces cerevisiae*. A major inefficiency in bioethanol production is microbial contamination of the bioreactor with lactic acid bacteria. Lactic acid bacteria compete with yeast for the fermentation substrate, lowering fermentation yields. In addition, lactic acid bacteria produce compounds that are toxic to yeast. Several methods are currently employed in the bioethanol industry in order to minimize losses to contaminating bacteria. The primary method used to reduce contamination is the addition of conventional antibiotics. One of the downsides to conventional antibiotic use is the potential for low levels of antibiotics to end up in the dried distillers grains and solubles, which are used as animal feed. Antibiotic use in animal feed breeds resistant bacteria.

An alternative to the use of conventional antibiotics is the use of bacteriocins to mitigate contamination by lactic acid bacteria. Bacteriocins are small peptides normally secreted by lactic acid bacteria that kill closely related lactic acid bacteria. Unlike conventional antibiotics, bacteriocins are approved for use in the food supply. A second alternative is the use of hops or hop extracts to reduce the contaminants.

This study examines the use of bacteriocins, hop compounds, and conventional antibiotics both alone and in combination to control fermentation spoilage organisms.

**BioFuelNet Project Number: 45**

Submitting author: Esma Ines Achouri, Université de Sherbrooke

Abstract: A recently patented Ni-Al spinel supported on alumina (Al2O3) and Y2O3-stabilized ZrO2 (YSZ) catalyst "NiAl2O4/Al2O3-YSZ" demonstrated high activity and high resistance to coke deposition when used in commercial diesel and biodiesel steam reforming.

In previous publications it has been shown that spinel (NiAl2O4) is deposited preferentially on the alumina phase. Nevertheless, the presence of YSZ had proven instrumental in terms of catalyst efficiency and stability, mainly associated with a decrease of surface carbon formation. In order to explain the YSZ role in the spinel catalyst performance, a wet-impregnation-based protocol was used to prepare catalyst formulations with different Al2O3/YSZ mass ratios as well as YSZ with different Y2O3/ZrO2 molar ratios.

In all tested formulations the Ni load was kept constant at 5% w/w. The morphology, the metal dispersion and crystalline phases were examined by scanning electron microscopy coupled with Energy-Dispersive-X-Ray Spectroscopy (SEM-EDXS) and by scanning electron microscopy (SEM) (in transmission mode) and X-ray diffraction (XRD). Thermogravimetric analysis (TGA) and temperature-programmed-reduction/Oxidation (TPO and TPD) were been used to analyze fresh and used catalytic formulations to evaluate the role of YSZ as an atomic oxygen-vehicle during the reaction.
The experimental runs were performed in a differential fixed-bed reactor set-up. A proprietary diesel-water emulsion mixture, at a C/H2O molar ratio of 2 was vaporized in the preheating area of the set-up before entering the steam reforming reaction zone operated at the relatively low temperature - for steam reforming- of 730°C and mass space velocity of 25 000cm3h-1g-1. Gaseous products were analyzed using on-line Gas Chromatography.

The results indicated that diesel conversion and products selectivity were influenced by the presence of YSZ and its Y (as yttria) content. Moreover, catalyst efficiency over time-on-stream were also affected. Details regarding the mechanism of these phenomena will be presented and were used to optimize the catalyst formulation.

**BioFuelNet Project Number: 45**

Submitting author: Yiqiong Jin, University of Alberta

Poster title: Co-fermentation of wheat and whey permeate for ethanol production and effect of lactic acid bacteria in whey permeate on ethanol yield.

Abstract: Lactic acid bacteria are a major concern to the ethanol industry. Contamination by them in industrial fermentations is a well-documented source of efficiency loss. Despite this, there are increasing interests to use more and cheaper sources of fermentable sugars as feedstocks. Whey is a by-product of the cheese industry, in which about 55 % of milk nutrients are retained. The protein in whey is usually recovered, resulting in a lactose-rich stream so-called whey permeate. Lactose in whey/whey permeate is a potential cheap carbon source for ethanol production. However, the natural lactic acid bacteria present in whey/whey permeate is the major concern to the ethanol production. Therefore, the aim of this study is to use whey permeate as a co-substrate to wheat for ethanol production, as well as to investigate the effect of natural lactic acid bacteria in whey permeate on the co-fermentation.

Whey permeate was blended with wheat to prepare the co-fermentation media. Simultaneous lactose hydrolysis during fermentations was carried out by supplying external β-galactosidase at the onset of the fermentation in 5-L bioreactors. The glucose, lactose, galactose, lactic acid, acetic acid, and ethanol concentrations were monitored during the co-fermentation. The production of lactic acid was compared to that obtained by the fermentation without whey permeate. The production of lactic acid in the co-fermentation was comparable to that of the fermentation without whey permeate, indicating that the natural lactic acid bacteria in whey permeate did not cause contamination problems.

**BioFuelNet Project Number: 47**

Submitting author: Mohammad Reza Kholghy, University of Toronto
Poster title: A study of the effects if the ester moiety on soot formation in a laminar coflow diffusion flame of a surrogate for B100 biodiesel.

Abstract: To better understand the effects of ester functional group on soot formation and further expand the applicability of detailed soot models to more complex fuels, a numerical and experimental study is performed to investigate soot formation in an atmospheric laminar coflow diffusion flame of a surrogate for a B100 Biodiesel. The surrogate is a mixture of 50% n-decane and 50% methyl-octanoate to represent methyl-oleate. The combustion chemistry and soot formation are solved using a detailed chemical kinetic mechanism with 288 species and 2073 reactions and a detailed sectional soot model, respectively. The mechanism is obtained by combining the detailed biodiesel oxidation mechanism with a subsection of a C1-C2 fuel oxidation mechanism that has an enhanced PAH formation scheme and can predict PAH growth up to benzopyrene. Soot volume fraction and temperature profiles are compared to experimentally measured values for biodiesel. In addition, the effects of ester functional group on soot formation are studied by numerically comparing the biodiesel surrogate flame with a pure Decane flame. The model could successfully predict the temperature profiles in the biodiesel flame. The maximum value of soot volume fraction is also reproduced in the flame. The biodiesel flame shows slightly reduced soot volume fraction as the ester functional group diverts carbon atom from the pool of soot forming carbons. Around 50% of the oxygen in ester group directly produce CO2 and the other half produce CO and CH2O.

**BioFuelNet Project Number: 47**

Submitting author: Yashar Afarin, University of Toronto

Poster title: Study on Fuel Properties and Combustion of Wood Derived Fast Pyrolysis Liquid (Bio-oil)

Abstract: Most biomass is composed of cellulose but converting it into high quality transportation fuels is challenging. Fast pyrolysis can convert solid biomass into a liquid fuel (fast pyrolysis liquid or bio-oil) at a low cost and with minimal fossil inputs. However the fuel contains oxygen, nitrogen and ash, has a low energy density and is acidic and very viscous. Nevertheless, bio-oil is proposed for use in stationary heat and power applications. To better understand the combustion behavior and emissions of bio-oil, a 10 kW spray burner was designed and constructed. The effect of swirl, atomization quality, ignition source (pilot)energy, air/fuel preheat and equivalence ratio on the stability and emissions of bio-oil spray flames was investigated.

Implementing crude bio-oil in some current combustion systems can degrade combustion performance and emissions. Optimizing the fuel properties to improve combustion is one way to solve this problem. The relationship between fuel properties and combustion performance has been studied: (1) Varying the solids, ash and water content of bio-oil; (2) Ethanol blending; (3) Comparison of bio-oil with light and heavy fuel oil; and (4) The effect of fuel aging (i.e. storage time and temperature). Preliminary results of a bio-oil fueled Stirling Engine are presented and other engine tests are discussed.
**BioFuelNet Project Number: 48**

Submitting author: Valerie Orr, University of Western Ontario

Poster title: Method development for efficient optimization of oil and starch production in microalgae

Abstract: High throughput methodology (HTP) for the optimization of lipid and starch production in microalgae has been developed through the combination of a significantly improved HTP lipid assay and design of experiments. The commonly used HTP assay for volumetric lipid content in microalgae; the Nile Red assay, is currently a poor indicator of gravimetric lipid content in microalgae. Improvements to the assay include bleaching cells prior to dry cell weight analysis as well and the inclusion of a new standard, milk fat. These two simple steps markedly increase the accuracy and precision of the Nile Red assay allowing it to become a reproducible indicator for response surface assessment in microtiter plate media optimization.

**BioFuelNet Project Number: 49**

Submitting author: Julie Barrette, Natural Resources Canada

Poster title: Wood characterisation in salvaged trees affected by fire for the production of bioenergy

Abstract: Forest biomass from trees killed by natural disturbances is an abundant feedstock source for bioenergy production in Canada. Although well developed in Western Canada due to the Mountain Pine Beetle, this potential is virtually untapped elsewhere in the country. Following natural disturbances, foresters aim to rapidly salvage affected stands for the production of traditional products (lumber and pulp&paper). After a few years, insect attacks and fungal infections alter fibre quality; this restricts the possibility to conduct salvage operations for such end-uses, but represent an opportunity for the bioenergy sector. This study investigates the potential for the production of bioenergy from trees killed by forest fires and insects along that of traditional forest end-products, in the boreal forest of eastern Canada. Critical parameters for the production of energy is wood density, which influences the biomass recovery potential from a given site, and chemical composition of the biomass, which influences the type and quality of bioenergy carrier that can be produced. Wood fuels from salvaged trees vary by type of disturbance, time since disturbance, species and site characteristics, which indicates that understanding the physical and chemical structure of biomass from salvaged trees is extremely important in order to choose the right bioenergy pathway.

In the summer of 2013, a field sampling study was designed to characterise woody biomass of fire killed-trees and spruce budworm killed-trees. Wooden discs from these types of trees were collected at different locations in the north of Québec (Canada), to cover a gradient of disturbances, site conditions, species and wood degradation status. Part of these wooden discs have now been grinded into fine powder to characterize their chemical and thermal properties, while others have been used
to measure their physical properties. This characterisation shall provide the necessary information to assess the potential of this wood for various bioenergy pathways.

**BioFuelNet Project Number: 49**

Submitting author: Larissa Sage, University of Toronto

Poster title: The effects of biomass harvesting on carbon cycling and ecological substantiality in the boreal forest

Abstract: Climate change and global demand for bioenergy continue to raise concerns over the resilience of boreal forest ecosystems to natural disturbances and harvesting events. Sustainable forest management policies must be based on sound understanding of post-disturbance carbon dynamics, given these growing pressures. The purpose of my research is to evaluate effects of insect infestations and harvesting on carbon cycling in eastern boreal balsam fir – white birch forest ecosystems over time. I will use empirical field data to test hypotheses related to carbon fluxes and the validity of model parameters, in order to improve the accuracy with which we predict post-disturbance carbon dynamics.

Forêt Montmorency, Quebec, provides an opportunity to use a chronosequence experimental design to compare the carbon recovery trajectories for several alternative-harvesting scenarios against a stem-only harvest chronosequence established in stands harvested from 1933 to 2010. I will also sample carbon pools in stands that have been disturbed and/or harvested as follows: whole-tree harvesting, intensive harvesting, and insect infestation events with/without salvage harvesting. Quantifying forest carbon pools over time will enable me to test and refine, as appropriate, the assumptions of the Carbon Budget Model of the Canadian Forest Sector (CBM-CFS3) for predicting carbon dynamics in eastern boreal forests (Kurz et al. 2009).

I hypothesize that recovery trajectories for forest carbon pools will vary with the method and intensity of biomass removal, and ecosystem resilience may become unlikely beyond certain biomass removal thresholds. In particular, I predict that salvage harvesting after natural disturbance will result in net carbon emissions, potentially reducing total ecosystem carbon by the end of the next 70-year rotation. My null hypothesis is that the CBM-CFS3 will be able to accurately predict the observed stem-only harvesting carbon recovery trajectory, and that the alternative disturbance model projections will fall within the range of error observed empirically.

**BioFuelNet Project Number: 49**

Submitting author: Nicolas Mansuy, Université Laval

Poster title: Harvesting sustainable feedstock after forest fires: Where, when, and how much volume is profitable economically and ecologically? A case study in Eastern Canada – NRCan
Abstract: Forest biomass harvesting from naturally disturbed forests has been recognized by the Intergovernmental Panel on Climate Change (IPCC) as a promising resource for the deployment of the bioenergy market at a global scale. Forest fire is an important natural disturbance in Canada’s boreal forest. Hundreds of thousands of hectares of forest burn annually in Canada, producing greater volumes of salvageable woody biomass than traditional clearcut residues. In this case study, we evaluated the potential wood volumes available and the harvesting costs associated with both post-harvest residues and post-fire salvage wood in the North Shore area of Quebec. Recent standardized grids of biomass forestry inventory data, soil properties and fire distribution, at 250 m resolution for the Canadian commercial forest were used to estimate the biomass yearly flow from both sources between 2001 and 2010. Using the decision support system Logilab, we built different biomass harvesting scenarios under various logistical (distance, machinery efficiency) and environmental (topography, distance to the water bodies, soil fertility) constraints. Optimization models were run to provide optimal solution for decisions related to the harvesting cost, feedstock choice, supply areas, plant size and location, and logistical options. Despite temporal and spatial variability of fires, salvage logging following years with intense fire activity (4 out of 10 years in our study) could offer more than twice the volume available from harvest residues, or between 40 % and 70 % of the total biomass volume available for those years. Accounting for the fact that fire-killed stands remain salvageable for bioenergy for many years after disturbance, post-fire salvage wood could therefore represent a substantial feedstock source for bioenergy at the regional scale. Because of the drastic increase in volume available after fire, preliminary results suggest also a decrease in the harvesting cost when fires occur near clearcut harvested areas. In a climate change context, increase in the number and extent of fires within the commercial forest are expected; salvage logging for bioenergy production (for which requirements in terms of wood quality are much lower than for lumber or pulp) represents an opportunity to adapt forest management and industrial network to this new context. A spatially-explicit decision-making platform is required that quantitatively and qualitatively accounts for wood for traditional forest products and biomass for bioenergy production sourced from both harvesting of mature healthy stands and salvage logging of fire-killed stands.

BioFuelNet Project Number: 49

Submitting author: Stéphanie Jean, École Polytechnique de Montréal

Poster title: Sustainable Opportunities in the Bio-Economy Using Naturally-Disturbed Wood from the Boreal Forest as Feedstock

Abstract: Canadian forests represent a large sustainable feedstock. Indeed, more than 40% of Canada is covered by forest, and our forest products sector is in decline. More and more, we read about wood pellets being sold to Europe to fuel their need for green feedstocks, and as Chemical Engineers, we wonder if this the best use of our forest resource. At the same time, Canadian Forest Products companies seek to improve their competitiveness by diversifying to new markets, and the forest-based biorefinery could be an important opportunity. Also, Forest biomass impacted by
natural disturbances such as epidemic diseases, insect invasion or fire hazards could be a good potential biomass feedstock for these biorefineries. In British Colombia, Ontario and Quebec there are vast boreal forests that undergo these natural disturbances each year, and leave a huge quantity of dead forest biomass to decompose.

The principal goal of this project is to investigate the potential of using forest biomass from natural disturbance in thermochemical and biochemical biorefinery context by performing environmental impacts analysis using life-cycle assessment (LCA), techno-economic analysis and MCDM (Multi-Criteria Decision Making) analysis. The concepts and models should be applicable to the Canadian boreal forest landbase. The best LCA methodology will be selected in order to analyze the net carbon balance and environmental impacts associated with forest biomass from insect epidemic and wildfire along the full supply chain. The LCA will also permit the comparisons with other comparable product. Finally, the techno-economics studies and the MCDM will allow us to identify in which conditions a natural disturbed wood site has a good potential to be used in biorefineries processes.

**BioFuelNet Project Number: 49**

Submitting author: Stéphanie Jean, École Polytechnique de Montréal

Poster title: Establishing sustainable biomass supply chains from natural disturbances in canadian boreal forest

Abstract: The aim of the project is to develop a conceptual framework and tools to predict the sustainable supply of forest biomass and energy at the local level from salvage logging of insect epidemic and wildfire - killed stands. This project will determine how this supply can be integrated with the more predictable feedstock sources that are associated with logging residues, low quality/value roundwood and unused annual allowable cut, as well as residues from primary manufacturing of forest products, given environmental, technical, social and economic constraints.

**BioFuelNet Project Number: 49**

Submitting author: Tasseda Boukherroub, Université Laval

Poster title: A Sustainable Allocation Decision Process for Maximizing the Sustainability Benefits of Bioproduct Value Chains based on Biomass Supplied from Naturally Disturbed Forests

Abstract: We address the problem of the sustainable allocation of forest feedstock supplied from disturbed and public owned forests (fire, epidemics, etc.) to forestry companies manufacturing bio-economy-based products (e.g. bioenergy, advanced biofuels, etc.). Our objective is to optimize the potential economic, environmental, and social benefits of the bio-product value chains to be established, in their upstream stages (i.e. sourcing process). As any value chain starts in the forest and the quantity and the quality of the biomass are uncertain in the context of disturbed forest, upstream decisions related to biomass allocation are of primary importance. We propose an
approach combining multi-criteria decision making techniques such as Analytical Hierarchy Process (AHP) and Analytical Network Process (ANP) with mathematical programming (e.g. linear programming) and/or simulation techniques to produce optimal allocation plans (timber volumes to allocate, forest patches to assign, etc.) that maximizes the sustainability benefits of bio-products value chains.

BioFuelNet Project Number: 50

Submitting author: Alexa Bagdasarian, École Polytechnique de Montréal

Poster title: Production of butanol from wood biomass

Abstract: The production of biofuels from lignocellulosic biomass has gained much interest in recent years due to the increasing demands of the market, the instability of oil prices, and growing environmental concerns. Biomass-based butanol is a very promising biofuel and/or biofuel additive, demonstrating a number of advantages, such as higher energy content, when compared to ethanol.

Among the types of biomass used for the production of industrial chemicals, wood presents a profitable alternative to consumable lignocellulosic sources, such as corn or sugarcane. The production of butanol from wood biomass has thus attracted attention in biorefineries.

While previous research focused on utilizing hemicelluloses pre-hydrolysate as the starting feedstock for biobutanol production, the present study is aimed at producing the chemical directly from hardwood biomass. By doing so, the entirety of the sugars contained in the wood will go through fermentation in order to yield butanol, acetone and ethanol. Concerning the pretreatment of wood, an ionic liquid solution is used in order to dilute the components (cellulose, hemicellulose and lignin) prior to enzymatic hydrolysis and fermentation. The use of ionic liquids as a pretreatment method is a novel approach, which ensures less degradation of monosaccharides than conventional techniques, and allows for the conversion of sugars in a shorter processing time period. Furthermore, ionic liquids minimize the formation of inhibitors for downstream fermentation, thus eliminating the need for a detoxification step. Fermentation is done with the Clostridia beijerinckii strain of bacteria, which are known for their hyper-butanol producing properties. Based on work done by other authors, it has been demonstrated that solvent production in a reactor terminates when butanol concentration reaches about 13 g/L. For this reason, gas stripping is integrated in the system as an in situ product recovery technique. Gas stripping has low energy requirements and decreases butanol toxicity and inhibition, therefore enhancing the production of the biofuel during the fermentation process. As part of the product separation step, a triple phase flash decanter coupled with two distillation columns separates butanol from water. Additionally, a series of three distillation columns yield acetone and ethanol. An evaluation of the technical and economic feasibility of the process is performed based on an Aspen simulation model.

BioFuelNet Project Number: 50
Poster title: Fermentative butanol production by Clostridium Acetobutylicum from a combination of a wood hydrolysate and an alfalfa juice

Abstract: Butanol is considered to be a superior liquid fuel with many applications due to its several advantages compared to other fuels. Its biological production by fermentation from renewable lignocellulosic feedstock is recognized as an effective potential process.

The objective of this study is to investigate the development of a new fermentation process for butanol production based on the combination of a detoxified wood hydrolysate and an alfalfa juice.

Alfalfa (Medicago sativa, L) or “Queen of forages” is the most important forage crop species in the world. In the green crop drying industry it is used for the production of fodder pellets, this process also generates large volumes of plant juice very rich in proteins nutrients and vitamins. Three different cuts (June, July and August) of early-bloom Canadian alfalfa have been pressed, analyzed and compared. Based on the composition of the juice extracted from each sample in term of C/N P/N and P/C ratios the most suitable juice has been selected to be used for the fermentation experiments.

The carbon source used was wood pre-hydrolysate, hydrolysed with sulfuric acid, which has been subsequently detoxified using nanomembranes. Results have demonstrated that through nanofiltration weak organic acids (acetic acid, formic acid), furfural and HMF, as well as an important amount of phenolic compounds can be removed.

Butanol has been produced using the proposed combined fermentation medium by the Clostridium Acetobutylicum ATCC824 strain.

In conclusion, the success of using raw materials to replace chemical processes in the production of butanol by fermentation depends largely on the method of detoxification used as well as the efficient conversion of sugars contained in these materials to solvents.

BioFuelNet Project Number: 50

Submitting author: Francois Zasieczny, École Polytechnique de Montréal

Poster title: Biobased chemicals: potential collaboration between the forestry sector and the chemical industry

Abstract: The Canadian Pulp and Paper sector has been undergoing economic struggles for more than a decade. At the opposite, the development of the Canadian bioeconomy and the use of biobased chemicals sound very promising in the near future. Collaboration between both these sectors would help the first one to recover from its difficulties while it would enable the second one to develop opportunities for the production of biochemicals through integrated biorefinery.
However, given that the variety of wood-derived chemicals, sensed choices have to be made. A selection has to be done so that the most suitable chemicals can be produced in pulping processes and in integrated biorefineries.

This work deals in a first time with the methodology development for the choice of biobased chemicals and its application. Several criteria are applied in a specific order:

- Ability of the biobased product to replace its petrochemical equivalent,
- Economic criterion (Size of the targeted market, derivative from oil or gas)
- Technical criterion (Is the chosen technology mature enough? Is it possible to integrate it in an existing pulp and paper process? How economically viable is it?)
- Is it better to deliver the selected product to a “final user” or to a chemical company which will refine it?

In a second time, this methodology will be applied to determine the top 3/5 biobased chemicals are the based in order to be produced in n integrated biorefinery in a shorter term (within five years).

**BioFuelNet Project Number: 50**

Submitting author: Hana Lee, École Polytechnique de Montréal

Poster title: Techno-economic analysis of levulinic acid production from biomass

Abstract: The biorefinery concept which consists in valorizing biomass feedstock through conversion processes and equipment to produce fuels, power, heat, and value-added chemicals is considered as a promising solution to develop a sustainable chemical and energy industry. Despite the several advantages of the lignocellulosic biorefinery concept, it is still a long way before large scale commercial plant will be build across the world. Process and product design issues still need to be assessed.

The purpose of this work is to evaluate the technical and economic feasibility of lignocellulosic biomass valorization into levullinic acid (LA). Due to its various potential utilisations, LA has been chosen as one of the top 12 value-added chemicals from sugars and syngas by the US Department of Energy. Different process designs configuration have been considered for LA production and simulated on ASPEN Plus platform. Each process design has been evaluated from a technical and economic point of view.

The preliminary result demonstrates that LA could be cost effectively produced from biomass to be used either as end product or as precursor for production of value added-chemicals.

**BioFuelNet Project Number: 50**

Submitting author: Miyuru Kannangara, École Polytechnique de Montréal
Poster title: Extraction and primary treatment of lignin from Kraft pulping mills by acid precipitation. A combined process design and integration approach

Abstract: Lignin, which is currently burnt in the Kraft pulp mill recovery boiler, can be recovered and converted to a broad spectrum of bio-based products using integrated biorefinery processes. Extraction of lignin can also be used to debottleneck an overloaded recovery boiler thus allowing mills to increase pulp production. Acid precipitation is the most commercially developed lignin extraction process. In this process, a part of the black liquor is diverted from the evaporators and treated with CO2 to precipitate the lignin. The precipitated lignin is washed with H2SO4 and water to remove impurities. Lignin lean black liquor after filtration and wash filtrates are returned to the recovery cycle of the mill to recover the remaining chemicals.

The market for lignin is still developing. Currently, the lignin extraction projects in Kraft mills are justified by the economics of potential pulp production increase and internal use of lignin as a fuel. Therefore, lignin extraction projects should have minimum capital and operating costs as possible. This project aims to increase the profitability of lignin extraction process by a combined process design and integration approach, which accounts for interactions between lignin extraction process and Kraft mill. The methodology contains following steps. 1. Preliminary assessment (Precipitation trials and economic assessment). 2. Plant sizing, flow sheet development and process simulation. 3. Energy and chemical integration 4. Experimental validation and improvement of the process proposals. 5. Techno-economic assessments. An integrated lignin extraction process design is proposed. Several important contributions in the areas of lignin extraction impacts- limits interaction analysis and lignin filterability are identified. Process improvements are proposed based on the contribution.

BioFuelNet Project Number: 50

Submitting author: Xinhe Zhao, École Polytechnique de Montréal

Poster title: Metabolic engineering of acetone-butanol-ethanol (ABE) fermentation

Abstract: ABE fermentation is a promising method for efficient butanol production. In this project, hemicellulose hydrolysates containing xylose, from water wastes of pulp and paper industry, are valorized as substrates to obtain butanol. Since xylose is the main component of hemicellulose hydrolysate, it is studied as the unique carbon source in microbial fermentation process.

A kinetic metabolic model, combined with Metabolic Flux Analysis (MFA) and Metabolic Control Analysis (MCA), is being developed to describe central and nitrogen sources metabolic pathways of Clostridium acetobutylicum (ATCC 824). Based on Michaelis-Menten kinetics, intracellular mass balance, the model will carry out a comprehensive dynamic mathematical model, which can describe cell behavior with time. Model parameters (Vmax and Km value) will be identified using experimental data for providing a robust tool to optimize ABE fermentation. The model describes PPP (Pentose Phosphate Pathway), EMP (Glycolytic Pathway), TCA (Tricarboxylic Acid Cycle), amino acid and
butanol biosynthesis pathway. Preliminary simulations of the culture behaviour, as well as experimental data, will be shown.

**BioFuelNet Project Number: 54**

Submitting author: Alex Campbell, Concordia University

Abstract: Terpenoid biosynthesis is a crucial metabolic process that produces a wide variety of valuable compounds that have applications in everything from anti-cancer indole alkaloids to cosmetics and biofuels. Engineering this pathway in *Saccharomyces cerevisiae* is somewhat challenging due to the fact that several of the metabolites in the process strongly inhibit upstream enzymes or participate in side reactions. To address this problem we are working to scaffold several enzymes in this pathway to a synthetic peptide backbone using modular binding domains. Protein scaffolds have been used previously in *E. coli* to improve product yields up to 70 fold. The substrate channeling effect provided by scaffolding these reactions is intended to reduce the impact of feedback inhibition and side reactions in order to maximize production of geraniol, a simple monoterpenoid. This case should serve as an example for the viability of improving metabolic flux through pathways in *Saccharomyces cerevisiae* with synthetic protein scaffolds.

**BioFuelNet Project Number: 54**

Submitting author: Marshall Timmermans, Concordia University

Abstract: Conversion of carbohydrate feedstocks into value-added chemicals via expression of heterologous metabolic pathways in an industrial host is frequently inefficient. One method of improving the flux of carbon from feedstock to product is by combinatorial optimization of the transcription and translation levels of each enzyme in the heterologous pathway independently. This is done by putting each enzyme in a pathway under the power of one of multiple promoters with a wide range of expression levels, to create a library of unique genetic constructs with different combinations of promoter strengths for each enzyme. Genetic constructs of combinatorially optimized pathways can be assembled in a rapid and unbiased manner, however screening for the desired phenotype (i.e. improved product titers) using chemical analytics can be extremely low-throughput. My research is focused around creating functional genomics tools to improve the throughput of combinatorial optimization experiments via the construction of removable fluorescent protein tags fused to heterologous metabolic enzymes. Genetic constructs can be altered at the DNA level to remove these fluorescent protein tags by the action of the DNA integrase from the BXB bacteriophage. In this way libraries of industrial microorganisms can be subjected to high-throughput fluorometric assays to determine enzyme expression levels in vivo, as well as to chemically and metabolically characterize the same strain once the fluorescent tag has been removed. In this way, it may be possible to create a rapid, high throughput method to optimize the production of heterologous pathways independent of the sequence of the enzymes used in these pathways. My work has been to characterize the function of the BXB integrase in the yeast
**Poster title: Optimizing cis,cis-Muconic acid production in *Saccharomyces cerevisiae***

Abstract: Aromatic chemicals are essential feed-stocks in the chemical industry with applications in polymers and preservatives to name a few. The major source of aromatics is benzene and toluene from the petrochemicals and natural gas. The rapid decrease in availability of petrochemical sources, environmental concerns and an increase in demand of aromatics will generate a need for a biological aromatic production process. Metabolic engineering could offer an economical and efficient alternative to synthesizing products using heterologous biochemical pathways reconstituted in robust production organisms. This project outlines the development of a *Saccharomyces cerevisiae* strain that could be used to produce high levels of aromatic compounds. It involves deregulation of the central aromatic biosynthesis pathway and introduction of a heterologous pathway. The strain will primarily be used to produce cis, cis-Muconic acid, a precursor to adipic acid used in Nylon 6,6 production. However, such reconstituted pathways usually show low product yields due to interference with native pathways, loss of intermediates and precursors and accumulation of toxic intermediates. Thus, competing side reactions will be deleted to concentrate flux down the cis, cis muconic acid pathway. Initial results show a 10X increase in amounts of precursor, 3-Dehydroshikimate, available to be channeled to the pathway.

**Poster title: Model driven increases in tyrosine in *Saccharomyces cerevisiae* evaluated by targeted metabolomics**

Abstract: A targeted metabolomics approach was used to evaluate metabolic engineering strategies to increase production of L-tyrosine in the yeast *Saccharomyces cerevisiae* CEN.PK. Our engineering strategies combined localized pathway engineering with global engineering of central metabolism, facilitated by both steady-state and kinetic modelling. Deregulation of key control points was revisited, namely tyrosine feedback resistant versions of 3-deoxy-D-arabino-heptulosonate-7-phosphate synthase and chorismate mutase. Additionally, overexpression of the pentafunctional arom protein Aro1p, prephenate dehydrogenase Tyr1p and cyclohexadienyl dehydrogenase TyrCp from *Zymomonas mobilis* were tested. Loss of aromatic carbon was limited by eliminating phenylpyruvate decarboxylase Aro10p. The TAL gene from Rhodobacter sphaeroides was used to produce coumarate as a simple test case of a heterologous byproduct of tyrosine. Multiple strategies for engineering global metabolism to promote tyrosine production were evaluated using metabolic
modeling. The ΔCDC19T21E point mutant variant of pyruvate kinase was hypothesized to slow the conversion of phosphoenolpyruvate to pyruvate and accumulate the former as precursor to the shikimate pathway. The ZWF1 gene coding for glucose-6-phosphate dehydrogenase was deleted to create an NADPH deficiency designed to force the cell to couple its growth to tyrosine production via overexpressed NADP+-dependent prephenate dehydrogenase Tyr1p. Although the attempted coupling of tyrosine production to redox balance produced our highest recorded tyrosine level at 520 µmol•g DCW−1 or 192 mM, this did not translate to improved overall coumarate, possibly due to previously unknown regulatory functions.

BioFuelNet Project Number: 55

Submitting author: Mehdi Dashtban, University of Guelph

Poster title: Role of hexo(gluco)kinases in glucose repression in the pentose-fermenting yeast Scheffersomyces stipitis

Abstract: One of major problems in fermenting the pentoses in lignocellulosic substrates is the presence of glucose and mannose which inhibit xylose utilization. Yeasts typically prefer these hexose sugars as substrates for ATP regeneration and formation of metabolic precursors. The yeast Scheffersomyces stipitis has four genes encoding enzymes that catalyze the phosphorylation of glucose to glucose 6-phosphate: hxk1, nag5, glk1 and glk2. We hypothesize that one of the hexokinases may be associated with hexose repression in S. stipitis. To test this hypothesis, we have begun to disrupt each of the 4 hexo(gluco)kinases in S. stipitis and then assess the effect of the gene disruption on mixed glucose-xylose utilization. Towards this goal, the hxk1 in S. stipitis NRRL Y-7124 was disrupted using a disruption cassette containing the upstream and downstream sequences of hxk1 gene and coding sequence for aureobasidin A resistance gene. Both the parental strain and hxk1 disrupted mutant (Δhxk1) showed similar trends for glucose and xylose utilization when each sugar was supplied as the sole carbon source. However, different trends were observed when a mixture of xylose and glucose was provided. In the parental strain, xylose utilization began only after all the glucose was depleted. In contrast, the Δhxk1 mutant showed partial de-repression in that it started to utilize xylose even when considerable glucose remained in the medium. This suggests that hxk1 might be involved, at least in part, in glucose repression in S. stipitis. Efforts are under way to disrupt each of the other 3 hexo(gluco)kinases and assess the mixed sugar utilization patterns of the disruptants.

BioFuelNet Project Number: 58

Submitting author: Can Tao & Merly Xavier, University of Quebec at Trois-Rivieres

Poster title: Hydrogen production from biomass by continuous fluidized bed reactor

Abstract: Biomass is a feedstock that is renewable, carbon neutral, diverse, and widely spread throughout Canada. However, biomass is a very low-energy density feedstock. Many thermochemical
pathways have been developed to increase its energy density by producing hydrogen, methanol, etc. Our research group is actively working on hydrogen production with zero emissions of carbon oxides. Ultra-high pure hydrogen production can be an expensive process that requires considerable use of gas purification systems, but in our one-step alkaline reforming process, the oxides of carbon are sequestered by alkali hydroxide reactant, thus forming alkali carbonate. Unlike other biomass conversion processes, the moisture content in the feedstock does not affect the process as the reaction proceeds under alkaline conditions. The reaction is summarized as follows:

\[(C_6H_{10}O_5)_n + 12n \text{NaOH} + n \text{H}_2\text{O} \rightarrow 6n \text{Na}_2\text{CO}_3 + 12n \text{H}_2\]

We have successfully demonstrated the technology in a batch reactor on a laboratory scale. Mass and energy balance were calculated based on the data obtained. At present, we have designed the fluidized bed reactor and other essential components required to run the reaction in continuous mode. The parts were fabricated and the assembly of the system is currently on progress. The object for the first half of this year is to complete the assembly and optimize the system, thus enabling us to test various feedstocks available across Canada in the latter half of the year. The hydrogen, thus produced not only can be used for hydrogen applications and can also meet the needs of several other BiofuelNet projects which require hydrogen for bio-oil upgrading.

**BioFuelNet Project Number: 59**

Submitting author: Amit Kumar, University of Alberta

Poster title: Techno-economic assessment and process modeling of steam processed lignocellulosic biomass for pellet production

Abstract: Lignocellulosic biomass such as wood, herbaceous and purposely grown crops can be utilized to produce fuels and chemicals. Biomass utilization for energy has two key characteristics obstructing its large scale usage. First one is biomass yield, which is low per unit of area resulting in longer distances and higher costs of biomass transport from the field or forest to an energy conversion facility. The second characteristic is related to energy density (MJ/m3) of "as-received" biomass, which is about 1/8th of that of coal. Biomass conversion to densified form like pellets helps in increasing energy density by seven times. Pellets are currently produced through conventional process where biomass is dried, ground, fractionated and processed. However, steam pretreatment of biomass facilitates its breakdown, resulting in pellets with better characteristics. This study is focused on the detailed techno-economic modelling of steam pretreatment reactor and pellet production equipment using Aspen Plus. The mass and energy balance of the pellet production from steam pretreated Douglas fir estimated through the model was used for the cost assessment of the production pathway. The heat input required for grinding of untreated biomass (1.31MJ/kg) was found to be higher than that for steam pretreated biomass (0.82 MJ/Kg). The calorific value (HHV based) of untreated ground biomass, 18.61 MJ/kg, was lower than that of steam pretreated ground biomass, 21.16 MJ/kg, which further increased to 22.2 MJ/Kg after pelletization. The net energy ratio
of the pelletization process decreased from 4.07 (untreated pellet) to 2.41 (treated pellet) due to the addition of heat input in the form of steam. The energy input for steam pretreatment was 4.30 MJ/Kg. Further techno-economic model development for pellet production from steam pretreated biomass is in progress and would help in production of cost-effective pellets for the production of fuels and chemicals thus boosting the biorefinery industry in Canada.

BioFuelNet Project Number: 60

Submitting author: Alan Froese, University of Manitoba

Poster title: Enhanced degradation of wheat straw and other lignocellulosics by co-cultures of anaerobic clostridia and assessment of population dynamics using cpn60-based quantitative PCR

Abstract: Consolidated bioprocessing (CBP) simplifies production of second-generation biofuels by combining cellulase production, lignocellulose hydrolysis and fermentation in a single process. However, low rates of hydrolysis in CBP remain a challenge that can potentially be solved using co-cultures of micro-organisms with complementary lignocellulose degradation pathways. Few previous co-culture studies have determined dynamics of co-culture sub-populations using molecular techniques. Therefore, we combined three clostridial thermophiles whose characteristics indicate potential synergy in cocultures and optimized a qPCR assay based on the universal chaperonin-60 (cpn60) gene to track all three organisms simultaneously. Clostridium thermocellum DSM1237, C. stercorarium DSM8532, and Thermoanaerobacter thermohydrosulfuricus strain WC1 (monocultures), dual cultures comprising all 3 possible pairings and tri-culture were grown on milled wheat straw, provided by Dr. Belay Ayele, at 62°C for six days, followed by quantification of gas and liquid end products. Hydrogen production was highest for the tri-culture at all time points, followed by dual cultures. Ethanol production was highest for the tri-culture and the C. thermocellum/C. stercorarium co-culture. Xylanolytic T. thermohydrosulfuricus grew poorly on its own but enhanced end-product formation in co-cultures. Total end-products were approximately twice as high in all co-cultures compared to mono-cultures. Optimized qPCR confirmed that all three organisms grow differently when in co-culture compared to monoculture. A survey using various raw and pre-treated lignocellulosic substrates, namely wheat straw, hemp, and cattail, was also conducted, with twin-screw extrusion being performed by Dr. Simon Barnabé’s lab and super-heated steam treatment being performed by Dr. Stefan Cenkowski’s lab. While raw substrate fermentation was observed, the survey showed increased degradation of the pre-treated materials compared to their raw counterparts. The results of this study provide insight into population dynamics that may help to explain why co-cultures more effectively degrade and utilize lignocellulosic material for production of biofuels by CBP.

BioFuelNet Project Number: 60

Submitting author: Charushi Panditharatne, University of Manitoba
Poster title: Fed Batch fermentation of Clostridium Thermocellum DSM 1237 with high cellulose concentrations

Abstract: Increased substrate concentration during direct bioconversion of cellulose can lead to higher economic gains by increasing product concentrations, reducing unit sizes, and reducing downstream processing costs. Clostridium Thermocellum DSM 1237 was grown in an optimized medium containing 25 g/L cellulose. The fermentation was initially carried out in batch cultures in a pH and temperature controlled reactor with a working volume of 4L. With an objective to operate with higher solid concentration, three types of fed-batch fermentation were investigated: variable volume (VV) fed batch, fixed volume (FV) fed batch, and sequencing (SQ) fed batch. In variable volume fed batch, the fermentation was started at an initial volume of 2 L and the volume was increased following a predetermined feeding profile of medium and substrate. In the fixed volume fed-batch, the fermentation was initiated with 25 g/L of cellulose and 4 L working volume, and the cellulose concentration was maintained at around 25 g/L by intermittent feeding with cellulose. In the sequencing fed batch, the fermentation was carried out as a batch reactor and the culture was replenished in every 24 hours with new medium by cellulose settling followed by decanting of the supernatant. The maximum ethanol concentration obtained in batch, VV, FV and SQ are 138 mmol/L, 80.8 mmol/L, 91.3 mmol/L and 53.7 mmol/L, respectively. In the same order, the hydrogen concentrations obtained were 94 mmol/L, 73.3 mmol/L, 46.6 mmol/L, and 140 mmol/L. These differences are most likely due to substrate inhibitions caused by excessive cellulose accumulation, differences in dissolved gas concentrations and changes in metabolic pathways that allowed more lactic acid formation.

BioFuelNet Project Number: 61

Submitting author: Letitia Da Ros, University of British Columbia

Poster title: TREES: Tools for Remediation and Efficient Energy Supply

Abstract: Bioenergy is considered crucial in achieving present day climate goals and economic objectives. It constitutes an alternative form of energy that has the potential to lower carbon emissions, decrease soil and water pollution, while acting as a closed-loop renewable resource. The feedstock required to sustain such a system should have several distinct physiological characteristics, including efficient nutrient uptake, high growth rates and productivity, ease of propagation and the ability to be coppiced. In Canada, trees belonging to the family Salicaceae such as willow, poplar and poplar hybrids inherently possess these characteristics and more importantly inhabit a diverse geographical range that stretches across the continent. As a consequence of this significant distribution, attributes related to biomass production such as growth traits and cell wall characteristics are highly variable among genotypes, allowing poplar and willow to be used for a wide range of environmental applications. This comprises of their use in agroforestry to prevent soil erosion, reclaim marginal lands and act as vegetative filters that prevent environmental contaminants from entering surface water runoff. In Canadian agricultural systems, phosphorus has become a
contaminant of concern as industry, agriculture and urban discharge has exponentially increased phosphorus concentrations in surface water runoff, causing increased incidents of eutrophication and declining water quality. Here we assess the performance of a variety of willow and poplar genotypes under excess phosphorus conditions, in an attempt to identify genotypes that could serve the dual purpose of remediating phosphorus rich-soils and being the feedstock for bioenergy production. The resulting information could then be used by plant breeders for further tailoring of feedstocks for growth on an array of marginal lands while allowing producers to achieve maximum productivity.

**BioFuelNet Project Number: 63**

Submitting author: Houman Fei, Saint Mary's University

Poster title: Improving productivity of biomass feedstocks via application of beneficial soil microbes and plant supplements

Abstract: Purpose-grown biomass crops are one of several renewable feedstocks that can be used to produce “second-generation” biofuels. Fast-growing hybrid poplars, grown as short-rotation crops, and high yielding switchgrass show great promising as biomass feedstocks in North America. Both crops have the advantage of being able to grow on agriculturally marginal lands, thereby not competing with high-value food crops. It may be possible to improve productivity, decrease fertilizer and pesticide inputs, and reduce the greenhouse gas emissions of such biomass crops by the application of beneficial soil microbes and plant supplements. In the present work, three clones of hybrid poplars (Populus x cv. ‘Walker’; Populus x jackii Sarg. cv. ‘Northwest’ and P. x ‘Okanese’) and two cultivars of switchgrass (*Panicum virgatum* L. and *P. virgatum* L. cv. Cave-In-Rock) were inoculated with *Penicillium bilaii*, *Azospirillum brasilense* N8, *Variovorax paradoxus* JM63, or a seaweed (i.e. *Ascophyllum nodosum*) extract. The experiments have been conducted under both greenhouse and field growth conditions. Plant and soil samples have been collected from materials grown in greenhouse and field, separately. The biomass yield, plant nitrogen and phosphorus content, as well as microbe profiles in rhizospheric soil are currently being analyzed.

**BioFuelNet Project Number: 65**

Submitting author: Linoj Kumar, University of British Columbia

Poster title: Steam conditioned pellets as a tradable biomass commodity for biorefinery applications

Abstract: Transition from a global “fossil fuel based economy” to a “bioeconomy” will likely require large volumes of biomass to be traded as a commodity. Due to the low bulk density and high moisture content of most biomass feedstocks, densification processes such as pelletisation will be required to transport biomass over long distances and to benefit from the economies of scale of most bioconversion processes such as fermentation and pyrolysis. However, despite an increase in the bulk density, wood pellets are susceptible to humidity and mechanical stress and are often
fragmented during transport and handling. Poor stability at high humid conditions is particularly challenging to the Canadian biomass pellet sector, which depends on ocean transport to export most of their pellets. The presentation will describe a novel approach, using “steam treatment” prior to pelletisation to improve the strength and durability of pellets and to see if the resulting pellets could be used as a feedstock for a range of biorefinery applications. Steam conditioning substantially reduced the energy requirement for grinding step prior to pelletisation. In addition, the energy required to make pellets from steam conditioned material was also lower than the energy required to densify the untreated raw material. More importantly, the resulting steam conditioned pellets had a higher density and substantially higher mechanical strength compared to regular pellets. It appears that various mechanisms such as, redistributed lignin on the fibre surface, the presence of water soluble sugar molecules and the smaller particle size, all enhanced the binding characteristics resulting from densification. Unexpectedly it was found that pelleting did significantly reduce the amount of carbohydrates that could be recovered after steaming and pelleting. We also showed that the cellulosic component could be as readily enzymatically hydrolysed as the non-pelletized substrate. Thus the lower moisture content, enhanced friability and ease of handling of steam conditioned pellets indicates that they should prove to be an excellent feedstock for biorefineries, based on either biochemical or thermochemical modes of processing.

BioFuelNet Project Number: 67

Submitting author: Charles Xu, University of Western Ontario

Poster title: Development of inexpensive catalysts for bio-oil upgrading by hydrodeoxygenation

Abstract: Catalytic hydro-de-oxygenation (HDO) has been considered an effective technical route to upgrade fast pyrolysis oil to liquid transportation fuels, in particular when using expensive catalysts such as Ru/C. The intent of the research was to explore inexpensive supported metal oxides catalysts for converting the fast pyrolysis oil into advanced drop-in hydrocarbon liquid fuels by HDO. Our previous work using phenol as a model compound has found that the coke formation could be suppressed by addition of a small amount of phosphorus to Mo-based catalysts, and conversion of phenol into hydrocarbon products such as benzene and cyclohexyl-aromatics was promoted when using MgO-supported catalysts. This study is to investigate the effects of the addition of phosphorous and MgO promoters to Mo-based catalysts on the HDO of real pyrolysis oil. Some novel inexpensive catalysts such as Mo/C, Mo/Al2O3 and Mo/MgAl2O4 catalysts with and without phosphorus as a catalyst promoter were prepared by incipient wetness impregnation method. The HDO activity of those catalysts were compared against commercial Ru/C catalyst in a 100 mL bench-scale reactor system using a wood-derived pyrolysis oil at the temperatures of 250-400 °C and initial hydrogen pressure of 5 MPa.

BioFuelNet Project Number: 69

Submitting author: Anastasia Colomba, University of Western Ontario
Poster title: Development of a new reactor for the pyrolysis and torrefaction of biomass

Abstract: A new reactor has been developed for the pyrolysis and torrefaction of biomass. The reactor is a horizontal cylinder (0.33 m long, 0.20 m id) with a volume of 8.5 litres, which is mixed with a paddle impeller. Biomass is fed at one end of the cylinder with a cooled screw conveyor and the product char is removed at the opposite end by overflow and a cooled screw conveyor. The screw feeder has a capacity of 3 kg/h. The particle residence time is controlled by the overflow located at a height calculated to ensure 60 % of the reactor volume is filled with biomass to achieve good heat transfer from the walls, which are heated using band heaters. Due to the particular geometry of the impeller, this reactor allows for back-mixing between char and fresh biomass, as proven by preliminary mixing tests during the developing phase. This provides fast heating of the biomass, which is a major advantage over traditional auger reactors.

Results were obtained with this reactor during the pyrolysis and torrefaction of different types of biomass, such as crop residues, energy crops, energy crop seeds and milling residues. They are compared with other available technologies, such as a mechanically fluidized reactor and jiggled bed reactor.

BioFuelNet Project Number: 69

Submitting author: Francisco Sanchez, Institute for Chemicals and Fuels from Alternative Resources (ICFAR)

Poster title: Pyrolysis of biomass in a large-scale mechanically fluidized reactor

Abstract: The yield of organic liquids from biomass pyrolysis can be improved by maximizing the biomass heating rate and minimizing the residence time of the product vapors inside the reactor. Fast pyrolysis is traditionally performed with fluidized bed reactors, which require high capital and operating costs and yield product vapors that are highly diluted by fluidization gases, which increases the cost of the condensation train. Pyrolytic auger reactors can be used to avoid product dilution but cannot achieve high heating rates and require mixing of the biomass with a recirculated hot solid carrier such as sand. With both auger and fluidized bed reactors, the product char is contaminated with the inert bed material or solid carrier.

This research presents a novel large mechanically fluidized reactor that can operate under fast pyrolysis conditions without the limitations of auger or fluidized bed reactors. Uncontaminated char is continuously withdrawn and cooled. Hot organic gases are filtered within the reactor and fractionally condensed into heavy waxes, lighter organics, and aqueous fractions. The remaining non-condensable gases are sent to a flare system.

Pyrolysis was performed in the mechanically fluidized reactor with biomass such as woodchips and willow. Products yields and compositions were similar to those obtained with a fluidized bed.
BioFuelNet Project Number: 69

Submitting author: Valentina Lago, University of Western Ontario

Poster title: Mixing and operability characteristics of mechanically fluidized reactors for the pyrolysis of biomass

Abstract: Mixing characteristics of a novel Mechanically Fluidized Reactor (MFR), a continuous vertical convective mixer developed in the ICFAR laboratory, have been investigated with the specific objective of selecting the optimal stirrer geometry for fast pyrolysis of biomass. The MFR does not use any fluidization gas and the stirrer provides the required mixing between the injected biomass and the bed material while effectively breaking any possible agglomerate. In addition, the mixing is the key parameter influencing the heat transfer characteristics between the heating system and the bed.

In conventional fluidized beds, it has been shown that the torque required to mix the bed decreases as the superficial velocity of a fluidization gas increases, becoming constant above a critical superficial velocity and that this behaviour depends on the stirrer characteristics. Similarly, a method has been developed to monitor the torque required to mechanically mix a bed of low density particles in the MFR as a function of the bed aeration resulting from the formation of vapors during pyrolysis.

In this study, three different shaped stirrers were first compared by artificially aerating the bed with nitrogen at different superficial velocities and rotational speeds. Furthermore, different fluidization gases were used in order to simulate the characteristics of vapors produced during pyrolysis and, specifically, to evaluate the effect of their density and viscosity. The stirrers showed to be characterized by a different critical aeration at which the torque becomes constant.

The second part of this study focused on actual pyrolysis tests. The reactor was first aerated with nitrogen above the critical superficial velocity. Then, the nitrogen was shut off and wood pellets were fed into the reactor. The formation of pyrolysis gases and vapors decreased the torque required to mix the bed and this was found to be dependent on the type of stirrer used.

Finally, the stirrers were ranked in terms of their performance in enhancing the wall to bed heat transfer.

The findings of this project have provided the foundations for the optimal design of Mechanically Fluidized Reactors for the pyrolytic processing of biomass materials.

BioFuelNet Project Number: 69, 70

Submitting author: Charles Greenhalf, ICFAR, University of Western Ontario
Poster title: Fractional condensation of pyrolysis vapors from sewage sludge using a mechanically fluidized reactor

The research is concerned with the utilization of sewage sludge as a possible source of energy, chemicals, fertilizers or activated carbon. The aim of this study is to investigate fractional separation and condensation of pyrolysis vapors produced from sewage sludge, which has been obtained from a local wastewater treatment plant, using a bench-scale mechanically fluidized reactor (MFR) developed at ICFAR.

Two types of fractionation schemes have been explored for this type of batch pyrolysis, firstly, physical fractionation, isolating vapors using a lever valve system at different reaction temperatures in a staged sequence. Secondly, the separation has been carried out in a condensation train setup in series with varied trap temperatures.

The results show notable differences in the bio-oil yield and properties, such as calorific value and water content, when comparing the fractionation schemes and the different fractions of each scheme. Through optimization of the second fractionation scheme, by adjustment of the trap temperatures, a bio-oil with a calorific value of ~35 MJ/Kg and water content of <2 % was produced. The effect of the final pyrolysis temperature on the pyrolysis products yields and characteristics have also been investigated.

**BioFuelNet Project Number: 71**

Submitting author: Claudio Lira, University of Western Ontario

Poster title: Distillation and purification of pyrolytic bio-oil

Abstract: The utilization of pyrolytic bio-oils is challenging due to its diversified chemical composition. Batch distillation is a potential unit operation for the separation of small quantities of high-value-added biochemicals. Traditional distillation cannot be used with bio-oils, due to their thermal instability. Therefore, a new lab-scale batch distillation system for the purification of pyrolytic bio-oils has been built; it will use residual oil from fossil crude oil to stabilize the pyrolytic bio-oil.

Model compounds of water, ethylene glycol and glycerol were used to validate and optimize this batch distillation system. Results show that the distillation column was able to recover 100 wt. % of the water and ethylene glycol, with up to 97 % purity.

ICFAR pyrolysis technology includes a fractional condensation train that provides an aqueous fraction and a dry oil fraction. Distillation experiments will be performed with both fractions to extract their most valuable components.

The aqueous fraction is modeled with mixtures of isopropyl alcohol, water, acetic acid and phenol. Experiments will, first, be conducted with this model aqueous fraction and, then, with actual aqueous fractions from the pyrolysis of birch bark.
Future experiments will address the recovery of valuable products from the dry oil fraction. Experiments will, first, be conducted with model compounds and, then, with actual dry oil fractions from the pyrolysis of birch bark.

**BioFuelNet Project Number: 71**

Submitting author: Jamie Gregory, University of Western Ontario / Institute for Chemicals and Fuels from Alternative Resources (ICFAR)

Poster title: Production of lamp oil from renewable resources

Abstract: Many areas of the globe are not well-served with electricity, and fuel-based lighting remains a strong and growing market. Current lamp oil is produced from non-renewable crude oil that must often be imported. The high carbon and hydrogen content of crop oils present an opportunity for a safe, cost-effective, and environmentally friendly alternative fuel source. However, the high viscosity of raw vegetable oils prevents their use as lamp oil without further processing, as they demonstrate poor wickability. Visbreaking and thermal cracking were investigated to utilize the fatty acid content of vegetable oils as a hydrocarbon source for lamp fuel production.

Optimization of the process parameters was performed with the objectives of a) improving wickability, b) decreasing viscosity, and c) obtaining desired lamp performance from the liquid product. Thermal cracking was shown to be the optimal process, achieving 97% reduction in viscosity with significant benefits to product wickability. Yields of 87% by weight of original feedstock were achieved. Wicking related liquid properties such as surface tension and wettability were obtained and shown to be similar to those of commercial fuel. Adequacy of the liquid product of thermal cracking as energy source for lamp was demonstrated. Product blending with commercial lamp oil was also tested and shown to result in successful operation during lamp testing.

**BioFuelNet Project Number: 72**

Submitting author: Anil Kumar Jhawar, ICFAR, University of Western Ontario

Poster title: Co-Pyrolysis of Heavy Oil with Birchwood and Synthetic Bio-Oil in a Mechanically Fluidized Reactor

Abstract: The objective of this project is to integrate bio-oil obtained from the pyrolysis of biomass in traditional fossil fuel refineries. The ultimate goal is to replace some of the residual oil processed in Fluid CokersTM with bio-oil or bio-oil heavy residues from renewable resources such as birchwood.

The petroleum heavy oil was co-pyrolyzed with raw birchwood bio-oil or its high-boiling point fraction (> 130 ºC). The co-pyrolysis was performed at temperatures ranging from 480 to 530 ºC in a continuous mechanically fluidized reactor. The proportion of water-free birchwood bio-oil in the liquid feed was varied from 0 to 44 wt%. The vapor residence time was 12 s in the reactor and 5 s in
the hot reactor filter, which was maintained at the same temperature as the reactor. The yield and characteristics of the liquid product, including fuel properties (heating value and water content), flow characteristics (viscosity and density), and elemental composition, were determined. The resulting liquid products were found to have higher carbon content, lower oxygen content and lower pyrolysis water than when pyrolyzing the feedstocks separately. The co-pyrolysis of the high-boiling point fraction of the bio-oil with the heavy oil resulted in higher valuable liquid products with lower oxygen and water contents when compared to the co-pyrolysis of the raw bio-oil with heavy oil.

BioFuelNet Project Number: 73

Submitting author: Md Zakir Hossain, University of Western Ontario
Poster title: Separation of Valuable Chemicals using Supercritical Water Gasification

Abstract: Supercritical water gasification (SCWG) is a hydrothermal process of converting waste biomass into value-added chemicals including hydrogen or syn-gas. SCWG has several advantages compared to traditional gasification including the direct use of wet biomass feedstocks, a single reactor for biomass hydrolysis and gasification, additional H2 generation through reforming, and a compressed gas product convenient for storage and transportation. The addition of catalyst in SCWG results in high yield of hydrogen and lower carbon monoxide yield via the water-gas shift reaction and accelerates overall gasification efficiency. In this work, metal supported graphene assemblies will be examined as both adsorbents and catalysts during SCWG of biomass. The grapheme metal oxide assemblies will be characterized by using BET, XRD, TPR, Chemisorption, SEM, TEM, Raman and stability and performance tests will be done in batch and continuous process, respectively. Interaction of metal oxide nanostructures with grapheme will also be performed in this work using density functional theory (DFT).

BioFuelNet Project Number: 73

Submitting author: Osariemen Ogbeide, University of Western Ontario
Poster title: Extraction of valuable compounds from bio-oil using supercritical carbon dioxide: experimental approach

Abstract: In this work, supercritical fluid extraction (SFE), a green process, was employed for the isolation of caffeine from pyrolyzed coffee ground bio-oil using pure carbon dioxide as a solvent in a batch extraction. SFE method using supercritical carbon dioxide (SC-CO2) was chosen due to its high throughput of extract recovery, efficient extraction of non-polar compounds and its non-toxic analyte recovery. Coffee ground bio-oil was successfully characterized by gas chromatography with mass spectroscopy (GC/MS), Fourier transform infrared spectroscopy (FT-IR) and high performance liquid chromatography (HPLC) to identify and quantify the important constituents of the bio-oil. The main compounds in the coffee bio-oil were identified as caffeine, 2,4 dihydroxy eicosane, oxirane, n-hexadecanoic acid, and phenol. The isothermal SFE condition was maintained at 50 °C at pressure
ranging from 1.45- 35.2 MPa. Quantification of caffeine and SFE extraction efficiency using HPLC was also investigated. Pyrolysis bio-oil contained about 27 mg/L of caffeine; the compound with highest concentration in the bio-oil matrix. The maximum separation of caffeine from the complex bio-oil matrix was 80 mg/L for the extract phase and 35 mg/L for the raffinate phase with a maximum extraction efficiency of 11% occurring at 35.2 MPa. The future work for this research will encompass the study of other process variables such as effect of temperature, solvent flowrates, and time for caffeine extraction efficiency, solubility of different bio-oils in SC-CO2 and the optimization of caffeine recovery coupled with thermodynamic modeling in a counter-current SC-CO2 extraction using Aspen Plus simulator.

**BioFuelNet Project Number: 73**

Submitting author: Serge Ayissi, University of Western Ontario

Poster title: Application of a DFT method to molecular models relevant to biofuels

**BioFuelNet Project Number: 74**

Submitting author: Christopher Gissane, University of Guelph

Abstract: A Design of Experiments study was undertaken to identify the optimum reaction conditions for the remediation of highly alkaline Red Mud waste as derived from the Bayer process using highly acidic pyrolysis bio-oil as the limiting reagent. Utilizing a factorial design approach enabled identification of the major factors that influenced the neutralization, partial reduction, magnetization and carbonization of the Red Mud. The impact of varying the factors of temperature, reaction time, and most importantly the minimum bio-oil/Red Mud ratio required was analyzed. A central composite design with corresponding response surfaces was then employed to identify the most suitable conditions of the aforementioned factors to optimize properties of the resulting products. This was carried out by evaluating response factors such as minimization of the sodium content and the quantity of the aqueous phase, the maximization of the carbon content, magnetization of the solid neutralized Red Mud phase as well as the stabilization of the pH of both phases close to 7. These optimum conditions can function as the starting point for possible treatment scale-up for large scale viability assessment and opens a possible pathway for the environmental remediation of Red Mud lagoons using a renewable biomass derived resource.

**BioFuelNet Project Number: 75**

Submitting author: Medhavi Gupta, University of Western Ontario

Poster title: Mesophilic Biohydrogen Production and Microbial Community Analyses from Co-fermentation of Glucose, Starch, and Cellulose

Abstract: The aim of this study was to assess the synergistic effects of co-fermentation of glucose, starch, and cellulose using anaerobic digester sludge (ADS) on the biohydrogen production and the
associated microbial communities. Batch studies were conducted with overall substrate concentration of 13.5 gCOD/L and an initial substrate-to-biomass (S/X) ratio of 4 gCODsubstrate/g VSSseed. H2 yields of 1.22, 1.00, and 0.15 mol H2/mol hexose-added was observed in glucose, starch, and cellulose as mono-substrates. The hydrogen yields were greater by 27 ± 4% than expected, which affirmed that co-fermentation of different substrates improved the hydrogen potential. Glucose addition to starch and/or cellulose favored acetate synthesis, while cellulose degradation was associated with the propionate synthesis pathway. Interestingly, hydrogen yield was inversely proportional to the hydrogen production rate. However, hydrogen yields had a linear relationship with the number of observed species. Co-fermentation caused noticeable variation in the microbial community structure. Illumina sequencing technology and bioinformatic analyses revealed operational taxonomic units (OTUs) in the Phyla Bacteroides, Chloroflexi, Firmicutes, Proteobacteria, Spirochaetes, Synergistes, and Thermotogae were common in mono- and co-substrate batches. However, OTUs in the Phyla Acidobacteria, Actinobacteria, and Bacteroidetes were unique to only the co-substrate conditions.

**BioFuelNet Project Number: 75**

Submitting author: Noha Nasr, University of Western Ontario

Poster title: CO2 Sequestration: Effect on biohydrogen production and microbial community in the integrated biohydrogen reactor clarifier system (IBRCS)

Abstract: This study investigated the impact of CO2 removal from the headspace of a novel, continuous flow biohydrogen production system, on the hydrogen yield and microbial community structure. Fermentation reactions were conducted in the Integrated Biohydrogen Reactor Clarifier System (IBRCS) with and without potassium hydroxide (KOH) in the reactor headspace to sequester CO2, using glucose as the carbon source, at low (25.7 gCOD/L.d) and high (51.4 gCOD/L.d) organic loading rates, and a hydraulic retention time of 8 hours. Headspace CO2 sequestration increased the hydrogen yield by 23% to 3.1 ± 0.19 mol/molhexose. The impact of headspace CO2 sequestration was not limited to the improvement in hydrogen yield and gas quality, as it also influenced the metabolic pathway utilization: acetate concentrations increased from 5.8 g/L to 8.5 g/L, butyrate concentrations decreased from 0.78 g/L to 0.38 g/L, and propionate concentrations dropped from 1.00 g/L to below detection limits. Detailed analyses of the microbial community structure in the IBRCS before and after CO2 sequestration revealed that removal of CO2 from the headspace of the bioreactor had a significant impact on the microbial diversity and species distribution. The changes in the microbial community structure rationalize the observed changes in the metabolic pathways.

**BioFuelNet Project Number: 76**

Submitting author: Maryam Tajilrou, University of British Columbia

Poster title: Characterization of wood particles forming a pellet
Abstract: Co-firing biomass with coal can reduce NOx and SOx as well as greenhouse gas (GHG) emissions from coal-fired power plants. Ground wood may be pressed into pellets for improving the logistical handling and combustion processes. For combustion using pulverized coal boilers, the fuel pellets need to be pulverized; otherwise, the material does not burn well as it falls through the boiler’s combustion zones. Therefore it is necessary to obtain data on the size distribution of the particles that form a pellet in order to achieve and manage optimum combustion conditions.

In this study, a wet disintegration method was used for determining the particle size distribution in wood pellets. The method follows a new standard (ISO CD 17830) which is still under development. We investigated the effect of water temperature and stirring on the disintegration of softwood pellets. Experiments were carried out at four different water temperatures: 20, 40, 60 and 95°C, with or without stirring. Hence, the tests involved eight treatments. The particle size distributions of the wood pellets before and after pelletization were measured.

Results showed that particle size distributions under the eight different treatments overlapped in most size ranges. Approximately 30 percent of particles in the disintegrated wood pellets were in the range of 0.5-1.0 mm. The disintegrated bark pellets contained more fines than the white wood pellets. Water temperature at ambient level of 20°C was found to be adequate for a complete disintegration of the wood pellets and stirring was not required. Furthermore, we observed that the particle sizes were reduced during pelletization based on measurements of the particle size distributions of the disintegrated wood pellets versus the ground wood prior to pelletization.

BioFuelNet Project Number: 76

Submitting author: Bahman Ghiasi, University of British Columbia

Poster title: Torrefied pellets as a renewable biocoal: Is it better to torrefy before or after pelletisation?

Torrefied biomass represents a high quality, renewable energy commodity that can be used to substitute fossil fuel feedstocks such as coal. However, densification processes such as pelletisation is necessary to improve the tradability of “low dense” torrefied biomass. In this work, two process pathways were assessed for their overall efficiency in making torrefied pellets from both forest and agricultural biomass. Both the schemes start with wet biomass as the raw material and ends with torrefied pellets as the final product. For pathway I, the raw biomass were dried, torrefied, then ground, and subsequently pelletized. For pathway II, the biomass were first dried, ground, pelletized, and finally torrefied. The process schemes were compared in terms of mass and energy balance as well as the quality of the resulting pellets. Although traditionally the biomass is first torrefied and subsequently densified (Process scheme I), our work showed that it was more energy efficient when following the process route II, which first densify the biomass and subsequently torrefy them. Use of binder was necessary in the conventional process scheme to effectively pelletise the torrefied material with reasonable energy consumption. Despite an increase in durability, use of binder
compromised some critical qualities of the resulting pellets. The pellets produced by process scheme II were found to be superior in terms of their high stability in water, enhanced calorific value, higher carbon content and reduced moisture content. Therefore, “torrefaction subsequent to pelletisation” (Process scheme II) currently appears to be a promising strategy to obtain torrefied pellets, which has reasonable transportable properties with high potential in different end product applications. We are currently comparing the influence of dry torrefaction to that of steam conditioning for their overall energy and mass balance and the quality of the resulting pellets. Results of this comparison will also be presented at the conference.

**BioFuelNet Project Number: 78**

Submitting author: Rachel Backer, McGill University

Poster title: Biochar may serve as a carrier to deliver biocontrol activity of a natural microbial consortium

Abstract: IN-M1 (PTA12383) has demonstrated *in vitro* antagonistic activity against the soil borne, plant pathogen *Fusarium oxysporum*. The goal of this experiment was to determine feasibility of biochar as a carrier for the product to facilitate storage and application to soil. The experiment was conducted by inoculating biochar with IN-M1 and assessing its antagonism against the pathogen. Preliminary results demonstrate that the inoculated biochar maintains full biocontrol activity. Future experiments could be conducted with other biochar materials to determine if this system can be further improved.

**BioFuelNet Project Number: 83**

Submitting author: Julian Cleary, University of Toronto

Poster title: A life cycle assessment of biochar production and application in a northern hardwood forest

**BioFuelNet Project Number: 86**

Submitting author: Nicolas Berthod, Institut de recherche en biologie végétale (IRBV)

Poster title: Wood properties of different willows growing in contrasting environments

Abstract: Previous research has established that SRC willow rapidly produces a large quantity of biomass, thus reducing the footprint on land and minimizing production costs. Willow in SRC therefore has great potential for biofuel and bio-products markets. Here, we describe an experiment in which we investigated how willow wood polymers (cellulose, hemicellulose and lignin) vary under different environmental conditions and between genotypes. In a second experiment, we examined wood extractible substances to identify high-value bio-molecules that could represent a secondary
market for SRC willow. Among these substances, phenolic compounds are of particular interest, since they are already used in several industries. To conduct these two experiments, four sites in the province of Quebec (Saint-Roch-de-l’Achigan, Beloeil, Saint-Siméon and La Pocatière) were selected to represent contrasting environmental conditions. Five willow cultivars (including 5027, SV1, SX61, SX64 and SX67) were planted on each site (400 cuttings/site) and are being maintained following the cultivation practices for SRC willows. According to preliminary data, after two years of growth, the survival rate of willows on most sites is over 90%. The presence of pathogens and insects, as well as growth parameters (diameter and height) have also been monitored throughout this period. During the summer of 2013 (second year of growth), wood samples were collected for analyses. The extractible content, cellulose, hemicellulose and lignin contents of the wood were determined by ASTM D1107-96/84, ASTM D1103-60, TAPPI T-212 and ASTM D1106-56 methods respectively. Using the same samples, 52 phenolic molecules were extracted, analyzed and characterized by LC/MS to identify those that are differentially present according to the various cultivars and environments tested. Preliminary analyses of the results show differences between the cultivars for total extractives and for cellulose content. Analyses are still in progress in order to measure the effects of the growing environments on willow wood properties

**BioFuelNet Project Number: 87**

Submitting author: Kiara Winans, McGill University

Poster title: Biophysical and energy indicators to select land for dedicated bioenergy crops in eastern Canada

Abstract: One of the constraints to increasing renewable fuel production in Canada is the limited supply of low-cost feedstocks that meet the standards for advanced biofuel processing. Dedicated bioenergy crops hold promise as low-cost feedstock, but there are logistical questions about where they should be grown. There are 9.48 million hectares of marginal agricultural land in Canada that could be used for dedicated bioenergy crop production without displacing food crops. Depending on the physiological attributes of the bioenergy crop, land improvements may not be required (i.e. a low-input regime), or agrochemical inputs may be required to achieve yield targets (i.e. a high-input regime). The objectives of the current study were (1) to provide a set of biophysical and energy indicators to evaluate and select land available for switchgrass and corn production in eastern Canada, and (2) to generate an indicators map for two scenarios (a low- and a high-input regime) that could be followed for growing bioenergy crops in eastern Canada ecoregions. Within ecoregions of eastern Canada, the selected biophysical indicators (e.g., soil texture, soil organic carbon, and growing degree days) and energy inputs (e.g., fertilizer and fuel for field operations) were used to create an indicators table per crop, coupled with maps using geographic information system to show the extent of the land base that could be suitable for switchgrass and corn production across eastern Canada, depending on the agricultural regime selected. The methodological approach developed for the current study should be transferable to other crops and ecoregions of Canada.
BioFuelNet Project Number: 90

Submitting author: Kulbir Singh, University of Western Ontario

Poster title: Effect of phytohormones on biofuel production in micro algae

Abstract: Microalgae show unique potential as a biofuel source. They have high oil content (20-70% dry wt), which is easy to convert to biodiesel, high growth and CO2 fixation rates, and can grow on waste streams. Algal biomass also provides raw material to industries such as ethanol, cosmetics and pharmaceuticals manufacturing. Despite the potential, reliability and high production cost involved hampers large scale microalgae production and therefore, it is essential that the algal strains used are robust and able to survive the stresses of industrial scale operations, while also accumulating large amounts of neutral lipids. Studies show that microalgae can produce phytohormones (signal chemicals essential for normal growth and development in higher plants), however their effects on algal growth, stress tolerance and lipid biosynthesis are largely unknown. Phytohormones are important targets for crop improvement and based on evolutionary relations hold potential in microalgae strain improvement. The specific objectives of this research are to: 1) study phytohormones known to enhance plant growth and stress tolerance for their effects on green algae (Chlamydomonas, Chlorella vulgaris and Dunaliella salina); 2) pinpoint key genes through genomic approaches for genetic manipulation of endogenous levels of the phytohormone with most beneficial effects; 3) develop genetically engineered algal strains with increased biomass and stress tolerance; and 4) optimize culture conditions for genetically engineered algal strains for maximal synthesis of neutral lipids. Brassinosteroids (BRs) are a class of plant hormones that promote plant growth and stress tolerance. Our pre-liminary experiments show that epi-brassinolide (EBL) treatment results in an increase in biomass of Chlorella vulgaris relative to the control. Similar experiments accompanied by molecular analysis will be conducted in Chlamydomonas reinhardtii, with a completely sequenced genome, to identify genes involved in BR metabolism. This knowledge will be used in third and fourth objective of the study.

BioFuelNet Project Number: 91

Submitting author: Goretty Dias, University of Waterloo

Poster title: Toward life cycle sustainability assessment of bio-based products and energy systems

Abstract: Canada, with its abundant agricultural land and forests resources, is going to play an important role in the emerging global bio-economy. However, large scale production of biofuel and energy in Canada could result in social and environmental concerns, such as water scarcity and biodiversity loss, offsetting other benefits associated with bio-based product systems, and leading to a spectrum of business risks. Although there have been many life cycle assessment (LCA) studies that
provide insights into greenhouse gas and energy aspects of bio-based product systems, LCA-based tools are not sufficiently developed to consider the full spectrum of sustainability aspects, particularly socio-ecological systems and their dynamic behaviors; therefore a more comprehensive assessment is required that considers various impacts on different social, environmental and economic systems.

We present a conceptual-analytical approach, connecting resilience theory to LCA, and propose a new conceptual framework integrating both environmental engineering and ecological perspectives. In the engineering perspective, complexities are reduced to average values, environmental changes are studied within linear cause-effect chain models, and impacts are assessed as the system deviates from its average steady state. In contrast, in the ecological perspective, environmental systems are seen as dynamic self-organizing systems, and the impacts are envisioned as larger changes to the systems’ threshold. Based on these differences, we can select appropriate tools to analyze the full supply chain, achieving a more comprehensive sustainability analysis of that system.

We have considered the concepts of resilience and socio-ecological systems, and evaluated their importance within life cycle assessment of bio-based production systems. We provide an example of how these two concepts can be used to help build a comprehensive life cycle sustainability assessment framework, which will not only increase the efficiency of those systems, but also improve their resilience.

**BioFuelNet Project Number: 91**

Submitting author: Goretty Dias, University of Waterloo

Poster title: Life cycle assessment of a portable pyrolysis unit for forest residues-process improvement

Abstract: Abri-Tech portable pyrolysis systems have great potential as an alternative solution for supplying liquid energy to remote areas. The bio-oil and char products of pyrolysis systems could potentially be an alternative low-carbon energy source for the aluminum, steel, and cement industries, which currently use large amounts of bunker oil and fall into the class of “large emitters”.

Although ABRI-Tech has developed a relatively simple and efficient pyrolysis system that reduces parasitic energy required to operate the system, when operating in off-grid areas, electricity requirements of the plant may come from diesel generators, thereby reducing the GHG (greenhouse gas) reductions associated with its operation. A complete LCA is required to identify hotspots in the pyrolysis systems so as to reduce the impacts and maximize GHG reductions associated with the system.

The purpose of this study is to compare the environmental profile of the Abri-Tech 50 dtpd (dry tonnes per day) mobile pyrolysis unit with its conventional fossil fuel system counterpart, and to determine how hotspots in the system can be reduced to reduce environmental impacts. It is assumed that this system will be operated at remote locations, without access to grid electricity, and
using forest residues from selective harvesting as the feedstock. The ABRI-Tech pyrolysis system consists of the following activities: forest residue collection and grinding/chipping, transportation to pyrolysis unit, and pyrolysis of biomass into the products of bio-oil, syngas and biochar.

Screening LCA results for various impact categories (GHG, energy, etc.) will be presented, with an emphasis on identifying process "hotspots" in the pyrolysis system, with the goal of improving the energy and GHG profile of the pyrolysis unit. A comparison between the pyrolysis products and conventional fossil-fuel based systems will also be presented.

**BioFuelNet Project Number: 92**

Submitting author: Enas Alhassan, University of Ottawa

Poster title: The characteristics of research output

Abstract: This study addresses the characteristics of biofuel research outputs that render research results useful for biofuel research users from research producers and research users perspectives. It is descriptive nature and its main aim is to explore the characteristics of research results that benefit biofuel research users to meet their requirements and facilitate policy decisions. This will provide biofuel research producers with better understanding of characteristics that are relevant to biofuel research results users.

This study follows qualitative research methodologies using grounded theory analysis. We obtained responses from 22 biofuel research producers and research users, covering a broad range of research areas and research users. In a semi-structured questionnaire, respondents were asked to describe their assessments of the usefulness of research results.

Overall, the responses of research producers and research users show considerable similarities. Producers and users show strong similarities in the description of the characteristics of useful research results, and the usefulness of the different methods of communication. One key dimension of the usefulness of research results is the ability to lead to cost reductions in the biofuel production process. Using questions derived from the Perceived Characteristics of Innovation (PCI) framework (Moore and Benbasat, 1991), we identify a broad range of criteria used in the assessment of research results. For example, the quality of data provided by researchers and ability to replicate results in the user’s environment featured prominently. We also find that academic journals – and particularly the quality of the journals and citation rates – are an important way for research users to assess the quality of the research results.

**BioFuelNet Project Number: 98**

Submitting author: Fiona Tran, University of Toronto

Poster title: Database and web application for biofuels life cycle assessment
Abstract: Life cycle assessment (LCA) is a tool that can be used to identify, quantify, and analyze the environmental impacts of biofuel production and use over the life cycle. Comparing LCA results for different conversion processes can advise the development of future commercially viable and environmentally sustainable biofuels production systems. We have developed a web application to help researchers conduct biofuels LCA. The underlying database allows collaborating BioFuelNet researchers to share their experimental and calculated data. Using this data, the application allows the user to design biofuels production systems by combining different biomass feedstocks, conversion pathways, and utilization options. An algorithm calculates the life cycle inventory, one major component of LCA, based on the selected pathways and options for the production system. The web application has been tested by several BioFuelNet researchers from other institutions, and has undergone revisions based on user feedback. An example study of a wheat straw ethanol product system for the Canadian Prairies has been examined using this web application, and the results are presented and compared with those for a reference gasoline system. Integration of the web application with existing LCA software tools (e.g. SimaPro) will provide comprehensive numerical data for comparing biofuel production systems, and inform the development of more sustainable alternatives to fossil fuels.

BioFuelNet Project Number: 98

Submitting author: Jon Albert Obnamia, University of Toronto

Poster title: Life cycle based analyses of emerging biofuels production pathways

Abstract: The SEES group within BioFuelNet assesses impacts associated with the production of novel biofuels and other bioproducts in the Canadian context using life cycle (LC) based methodologies to develop and assess biorefinery pathways, considering environmental performance. In this study, current developments by other network members on areas relating to feedstock, conversion process, and product utilization are incorporated into biorefinery pathways leading to the intended bioproduct. The objective is to identify key factors affecting environmental and economic performance, and thus identify focal RD&D activities that could improve existing pathways or lead to creation of alternative pathways, including feedstocks, conversion processes, and end uses.

Most conventional LC packages treat the conversion process as a “black box”, when, in reality, there are different process configurations, with different underlying technologies, co-products, resource and energy requirements, and emissions. A starting point in assessing biorefinery pathways compared several LC modeling packages (GREET, GHGenius, and SimaPro). Results were used to identify gaps in data and estimation methods, leading to more in-depth analysis of underlying assumptions and methods in the different modeling platforms. Subsequently, tailored process models were developed using Aspen Plus, and compared with processes built into the LC modeling packages.
Ultimately, the design, assessment, and optimization of biorefinery pathways utilized the customizable Aspen Plus and SimaPro packages. Aspen Plus allows user-designed biofuel production processes utilizing different technologies, while providing data on process inputs, outputs, emissions, and energy use. These data can be incorporated directly to SimaPro, which is useful for quick screening and for detailed impact assessment of biofuels. SimaPro uses its broad database of feedstocks, products, processes, emissions, and waste treatment, along with adaptations or user-based definitions of these items, thereby enabling assessment of biofuel production from various feedstocks, with different underlying technologies, and different co-products.

**BioFuelNet Project Number: 98**

Submitting author: Katherine Rispoli, University of Toronto

Poster title: Life cycle assessment & demand projection of aviation biofuels: potential GHG reduction & land requirement to 2050

Abstract: Aviation biofuels are seen as a crucial mitigation strategy to reduce greenhouse gas (GHG) emissions while maintaining growth in this sector. However, there are important implications that need to be investigated relating to using aviation biofuels on a large scale. This work demonstrates the potential GHG emission reduction and land required to displace various shares of the future jet fuel demand into 2050. First, GHG reductions were evaluated using life cycle assessment (LCA) for the multiple feedstock options (e.g., rapeseed, camelina, agricultural residue) and process options (e.g., gasification or pyrolysis with Fischer-Tropsch, hydrodeoxygenation), which were compared to conventional jet fuel. Other metrics were considered aside from GHG emissions, such as eutrophication, which have not been included in many previous assessments. Second, land requirements for displacing large amounts of jet fuel were estimated. Feedstock and process yields for the various aviation biofuel pathways were combined with regional jet fuel demand projections to 2050. These projections have been completed by combining various parameters, such as GDP, aircraft fuel efficiency, and loading factors. Potential emissions reductions, environmental impacts, and land requirements for 2050 are discussed within regional contexts. These are further compared to land availability in these regions to demonstrate the feasibility of producing aviation biofuel on a large scale and achieving significant GHG reductions.

**BioFuelNet Project Number: 98**

Submitting author: Kelsey Gerbrandt, University of Toronto

Poster title: Xylitol production from hemicellulose residues: process development with life cycle and techno-economic assessment

Abstract: Lignocellulosic biomass is a promising feedstock for the production of liquid biofuels such as cellulosic ethanol, which may be utilized as a low-carbon replacement for conventional transportation fuels. However, the commercial viability of large-scale cellulosic ethanol production
has been restricted by technical constraints leading to elevated production costs. The inclusion of value-added co-products produced concurrently with cellulosic ethanol has the potential to mitigate these risks. Xylitol, a polyol (C₅H₁₂O₅) with sweetening applications, is a prospective co-product option for cellulosic ethanol. The direct precursor to xylitol is a major component of hemicellulose, which constitutes approximately 35% of certain types of lignocellulosic biomass. This study aims to isolate the effects of including xylitol as a co-product of cellulosic ethanol production through cradle-to-gate life cycle assessment. Xylitol is produced via two pathways: conventional hydrogenation and fermentation. Process models for both pathways are developed using Aspen Plus software then subsequently evaluated with life cycle and techno-economic assessments. Results are provided for energy requirements and greenhouse gas emissions associated with individual process stages. Additional life cycle metrics and economic indicators determined include water usage, potential carbon credit values, product value, return on investment, internal rate of return, and capital costs. Results indicate that intensive evaporation requirements during the hydrogenation process constitute a significant fraction of the overall process energy demand. As xylitol is a solid, crystalline product, evaporation is necessary for all process pathways considered, and the advantages of one pathway over another may not be apparent without the completion of detailed life cycle assessments. This study demonstrates the value provided by life cycle assessment and techno-economic analysis to ensure the development of energy efficient, low emission, and economic value-added co-product pathways in a lignocellulosic biorefinery.

BioFuelNet Project Number: 98

Submitting author: Lucas Pereira, University of Toronto

Poster title: The virtual sugarcane biorefinery

Abstract: The Virtual Sugarcane Biorefinery (VSB), developed by the Brazilian Bioethanol Science and Technology Laboratory (CTBE), is a methodological framework that integrates simulation platforms and assessment tools with the purpose of obtaining technical and sustainability indicators of various technological alternatives/routes within sugarcane biorefineries. The concept of ‘biorefinery’ is analogous to an oil refinery: coproduction of biofuels for transportation and high valued bio-based chemicals for the industry. The Virtual Sugarcane Biorefinery accounts for all life cycle stages, from the cropping and harvesting of the feedstock, to the final use of the end-products. In order to simulate agricultural operations and estimate the feedstock cost, an electronic spreadsheet called CanaSoft has been developed; industrial processes are modelled using Aspen Plus. Results for the financial analysis are obtained using a discounted cash flow (DCF) method, whereas environmental assessment is performed using Life Cycle Assessment (LCA), implemented within the SimaPro software. In the VSB, the assessment of the viability of an investigated biorefinery alternative depends upon the comparison of the financial and environmental impacts calculated. The term ‘virtual’ is therefore related to the fact that the tool is able to calculate and predict parameters for industrial scale for the production routes by means of process simulation. The VSB has been applied
to several case studies including the assessment of autonomous and annexed first generation sugarcane distilleries, standalone or integrated with second generation ethanol production and other bio-based product production such as butanol, jetfuel and polylactic acid.

The VSB is under continuing development with recent attention being placed on the operationalization of a method for the evaluation of social impacts based on Social Life Cycle Assessment (S-LCA) and the assessment of uncertainties and risks involved within the financial analysis.

**BioFuelNet Project Number: 98**

Submitting author: Mohammad Pour Bafrani, University of Toronto

Poster title: Impacts of process technology developments and regional factors on life cycle GHG emissions of ethanol production from corn stover

Abstract: This study assesses the impacts of regional factors affecting biomass and conversion process input supply chains and the National Renewable Energy Laboratory’s (NREL’s) technology developments on the life cycle greenhouse gas (GHG) emissions of ethanol production from corn stover in the U.S. We considered ethanol production from corn stover in eight different counties. The life cycle boundary includes corn cultivation, stover harvesting, transportation, preprocessing, conversion of stover to ethanol employing NREL’s technologies, and final combustion of ethanol. The supply of corn stover is found to result in GHG emissions ranging from -6 gCO2eq./MJ ethanol (Macon County, MO) to 13 gCO2eq./MJ ethanol (Hardin County, IA), due to differences in soil carbon and N2O emissions responses to stover removal in the studied counties. Lower life cycle GHG emissions are found in regions where grid electricity is more GHG-intensive, as co-product electricity receives a larger emission credit. Biorefinery emissions, assessed based on the 2011 NREL process design model, are the single greatest contributor to life cycle emissions (18 gCO2eq./MJ ethanol). Emissions for the 2011 NREL model are approximately double those assessed for the 2002 NREL design model, due primarily to the inclusion of GHG-intensive inputs (caustic, ammonia) in the more recent design. Energy demands of on-site enzyme production included in the 2011 design contribute to a reduction of the electricity co-product and associated emissions credit. Total life cycle emissions for corn stover ethanol are found to vary between 1.5 gCO2eq./MJ and 22 gCO2eq./MJ depending on production location, representing reductions of 98% to 77% relative to gasoline. Results indicate that ethanol production in all studied locations would meet the Energy Independence Security Act emissions requirement for cellulosic biofuels; however, regional factors and on-going technology developments significantly influence these results.

**BioFuelNet Project Number: 98**

Submitting author: Pei Lin Chu, University of Toronto

Poster title: Environment and cost assessment of aviation biofuel production
Abstract: Recent advances in aviation biofuel (biojet) production technologies have demonstrated that biojet can be a feasible alternative to conventional petroleum based fuels. Industry attention is beginning to shift from the technical challenges to the financial requirements and the environmental impacts of biojet production and utilization. Strict regulation of the aviation industry has limited the number of suitable biojet production processes for commercial production. The two dominant processes that have successfully produced biojet that has been used in flight tests are hydrodeoxygenation (HDO) and catalytic hydrothermolysis (CH). This study aims to compare the environmental and financial performance of these pathways (using various feedstocks) with reference petroleum-based processes, and to provide guidance to technology developers regarding feedstock and technology selection. Life cycle assessment methodology is applied to evaluate the environmental impacts and life cycle costing is applied to evaluate the financial performance. Both biojet processes convert different sources of triglyceride-based feedstocks into an array of hydrocarbon products, including liquefied petroleum gas (LPG), naphtha, kerosene and diesel. System expansion is used in the impact assessment stage to account for co-product emissions for each process; energy and market value allocation methods are also used for comparison against the system expansion method. To determine the emissions generated during biojet production, an Aspen Plus model was developed and is also used to estimate the capital and operating costs of production at various scales. Modeling results indicate that agricultural inputs during feedstock production and methane use during hydrogen production have the greatest impact upon environmental and financial performance. Sensitivity analysis indicates that targeting improvements in the environmental and financial performance of these two production inputs is critical to advancing the adoption of biojet as an alternative to conventional jet fuels.

BioFuelNet Project Number: 99

Submitting author: Adam Li, Concordia University

Poster title: Xylose Metabolism in Candida albicans

Abstract: Efficient and rapid fermentation of all sugars present in cellulosic hydrolysates is essential for economic conversion of renewable biomass into fuels and chemicals. Because of its role in wood composition, the pentose sugar, xylose, is the second most abundant sugar in nature. The closely-related ascomycetes Candida albicans and Saccharomyces cerevisiae metabolize the xylose very differently; S. cerevisiae fails to grow on xylose while C. albicans can grow well. The two species possess highly similar genes that encode similar xylose reductases and xylitol dehydrogenases. We have created a C. albicans strain deleted for the xylose reductase gene GRE3 and xylitol dehydrogenase gene XYL2 and rescued this with the genes from S. cerevisiae (GRE3 and SOR1), called CA271. This shows that S. cerevisiae has the enzymatic potential to convert xylose to xylulose, but normally fails to use this potential.

Here, numerous metabolic engineering approaches have been performed of CA271 by: EP-PCR, transcriptional reprogramming (in response to xylose adaption) and protein engineering of the
xylose reductase. The growth of these mutants was quantified in xylose by taking growth curves. Metabolomics profiling was performed to see if there were any physiological changes. The key enzymes in xylose metabolism of these mutants were sequenced to see if there were any genetic changes.

Together, our study investigates how we can metabolically improve the key enzymes in *S. cerevisiae* so we can potentially use *S. cerevisiae* for industrial ethanol production using xylose as the sole carbon source.