



## **POSTER SESSION**

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# SOLID STATE FERMENTATION KINETICS AND REACTOR DESIGN FOR THE PRODUCTION OF CELLULASE

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Corn is predominantly the feedstock used for the production of over 98% of all ethanol presently produced in the U.S. Most of the corn-based ethanol is produced by dry grind processes. In a dry grind process, for every bushel of corn going into the plant about 2.65 gallons of ethanol and 16-18 pounds of dry distiller's grain (DG) are produced. Recent research has shown DG to be suitable substrates for solid state fermentation (SSF) in lab scale batch experiments. SSF is the process which microbes grow on solid substrates with little or no free water and produce fermentation products such as cellulase enzymes. The development of a reliable bioreactor is required before solid state fermentations are considered industrially viable. In this research cellulase enzymes were produced from wet DG using *Trichoderma Reesei* NRRL 11460. Experiments were performed in multiple small diameter columns at constant temperature under forced aeration. The production of carbon dioxide was monitored online to relate cell growth and metabolic heat evolution to enzyme production. An empirical approach was applied to quantify the effects of environmental variables such as temperature, moisture, and aeration rate on cell growth and cellulase production. Kinetic experiments were performed at 27.5, 30.0, and 32.5°C. The initial moisture contents of the substrate were 40, 50, and 60%. Substrates with an initial moisture content of 50% and at 30°C resulted in the highest cellulase yield of 22 FPU/g dry substrate. Cellulase production occurred primarily during the idiophase under these conditions with a CO<sub>2</sub> evolution rate of 0.11 mg CO<sub>2</sub>/min/g substrate. Currently, a kinetic sub-model is being developed from experimental results. Future research will explore the integration of the developed kinetic sub-model into an intermittently mixed spouted bed reactor model to estimate local conditions within a reactor as a function of time and position and to compare the reactor model with a pilot scale reactor. The model provides a tool for design, scale up, and predictive process control.

# PROCESS DEVELOPMENT FOR MANUFACTURING PROPYLENE CARBONATE AND POLY(PROPYLENE CARBONATE) FROM PROPYLENE OXIDE AND CARBON DIOXIDE

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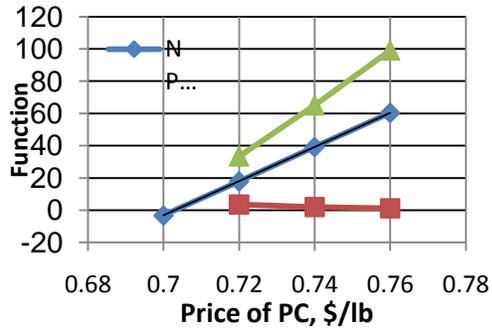
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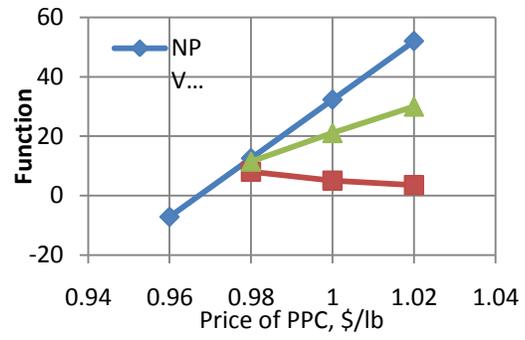
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Reusing existing CO<sub>2</sub> as a feedstock of carbon for producing chemicals such as cyclic propylene carbonate (PC) and poly(propylene carbonate) (PPC) has received much attention recently. Synthesis of cyclic carbonates and polycarbonates from CO<sub>2</sub> needs a reactive cyclic ether such as propylene oxide (PO) and an efficient and stable catalyst. PC is an important intermediate as a replacement of highly toxic phosgene as carbonylating agent in the production of isocyanates or polycarbonates. PC can be used in high energy cells and condensers, as a solvent and plasticizer for polymers as well as an absorbent in the removal of CO<sub>2</sub> and H<sub>2</sub>S from natural gas and petroleum cracking gas. It is an aprotic polar solvent and used extensively as intermediate in the production of drugs and pesticides. It is frequently used as a high-permittivity component of electrolytes in lithium batteries, usually together with a low-viscosity solvent. The process for manufacturing PC uses ionic liquid-1-n-ethyl-3-methylimidazolium chloride (EMImCl) as catalyst. The reaction takes place at 212 °F and 114.7 psi in a stirred tank. Presently, the industrially important polycarbonate is synthesized from bisphenol A and diphenyl carbonate. Concerns about the use of bisphenol A in consumer products were regularly reported. Because of environmental and safety issues the development of feasible alternatives for manufacturing selected polycarbonates has been highly attractive. PPC has many potential applications in industry as binder resins, substitutes of thermoplastic polymers (e.g., polyethylene and polystyrene), and hydrolytically and/or biologically degradable polymers. The process for manufacturing PPC uses the (salen)Cr(III)Cl complex as catalyst, where H<sub>2</sub>salen = N,N-bis(3,5-di-tert-butylsalicylidene)-1,2-cyclohexenediimine. The reaction takes place at 850 psi and 86 °F. When the reaction is performed at elevated temperatures, propylene carbonate is the dominant product as a result of a backbiting mechanism. The manufacturing of PC requires 9990.2 lb/hr CO<sub>2</sub>, while PPC requires 12762.8 lb/hr CO<sub>2</sub>.

This study presents engineering and economic analyses of industrial scale processes to manufacture around 200 million lb/year pure cyclic propylene carbonate and around 200 million lb/year pure poly(propylene carbonate) (PPC) from CO<sub>2</sub>, supplied by a bioethanol plant and purchased PO. The simulation package of Aspen Plus is used to design and simulate these processes with known kinetics data and catalysts to obtain the material and energy balances, the stream tables, list of the major equipment with operating conditions, and the utilities necessary. The economic analyses provided the discounted cash flow diagrams for the processes with net present value, payback period, and rate of return at the end of a 10-year of operation. As Figure 1 shows, when the selling price of PC is \$0.72/lb the values of NPV, PBP, and ROR are \$17.97 10<sup>6</sup>, 3.5 years, and 33 %, respectively. Therefore, the process is feasible under the current economic data, available technology, and catalyst utilized. Figure 2 shows how the values of NPV, PBP, and ROR vary with the selling price of PPC. The selling price of PPC should be at least \$0.97/lb or higher for the process to be feasible.



**Figure 1.** Change of NPV, PBP, and ROR with the selling price of PC.



**Figure 2.** Change of NPV, PBP, and ROR with the price of PPC.

## SOLVENT FRACTIONATION PRETREATMENT OF SWITCHGRASS FOR BIOFUELS

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Interest in the biorefinery production of fuels from lignocellulosic biomass has grown steadily over the last few years, particularly because the pretreatment step in lignocellulose processing can generate valuable chemical co-products in addition to high-purity cellulose for bioethanol production. Switchgrass (*Panicum virgatum*) is a promising lignocellulosic source for bioethanol fuel. It is a versatile and adaptable plant and can grow under variable conditions. Its highly available cellulose content makes it a good candidate for solvent fractionation, a type of organosolv process that isolates the required cellulose from biomass. It is a process of choice for pretreatment because it is suitable for use with several different biomass feedstocks that give favorable separations. Other features of the process include easy isolation of products after fractionation and high recovery of each component. Co-products hemicellulose and lignin are also recovered, which are precursors to valuable chemicals such as paper additives, biodiesel fuel, biodegradable polymers, computer boards, and epoxies. Lignin is also burned as an energy source in biorefineries. In this work, fractionation is carried out by adding switchgrass, a ternary solvent mixture, and sulfuric acid as a promoter to a 3.5 L Hastelloy flow-through reactor, controlled via LabVIEW, and operated at three temperatures: 120 °C, 140 °C, and 160 °C. Products of the reaction are nearly clean cellulose and black liquor, which is then subjected to phase separation giving an organic phase containing lignin and other solvent soluble components. An aqueous phase containing hemicellulose is also recovered. Cellulose was obtained at a yield of 30.0% w/w after being stripped of the majority of lignin and sugars. Lignin and additional solids, which could be sugars and their decomposition products, were obtained at a maximum yield of 27.9% w/w. A modified separation method using sodium chloride gave lignin yields of 10.9-16.5%, improving the purity of lignin. Hemicellulose sugars were obtained at a maximum yield of 22.9% w/w. Most of these yields are consistent with switchgrass feedstock compositions previously reported. Qualitatively, 2D-NMR results using the HMQC technique show that switchgrass lignin has many similarities to pine lignin, although it generally contains only small amounts of the syringyl moiety. From these fractionation experiments, we conclude that this laboratory reaction system is viable and worthy of further study to obtain switchgrass components at higher levels of purity.

## ADVANCEMENTS IN SWITCHGRASS BIOTECHNOLOGY TO IMPROVE CELL WALL COMPOSITION FOR THE BIOREFINERY

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Switchgrass (*Panicum virgatum*) is a leading candidate feedstock for biofuels in the U.S. and is a crucial model feedstock component of the BioEnergy Science Center (BESC). Biotechnology of switchgrass is important in screening potential cell wall biosynthesis genes with the ultimate goal of generating designer feedstocks with improved traits for the biorefinery, and this work is being performed multi-collaboratively among six laboratories in three institutions within BESC. Within our lab, biotechnological approaches towards enhancing switchgrass traits include improving tissue culture and transformation systems, isolating novel switchgrass promoters, developing a versatile DNA vector set for transformation, and subsequently altering lignin, cellulose and hemicellulose content within switchgrass. Switchgrass cell suspension cultures have been produced and characterized for mutant selection, mass propagation, gene transfer experiments via protoplast isolation and cell biology in view of cell wall trait assessment. In order to coordinate switchgrass transformation within BESC and to facilitate rapid screening of genes of interest, we have developed a Gateway-compatible monocot transformation vector set (pANIC) for overexpression and RNAi-mediated knockdown with visual and selectable markers. Overall, BESC has facilitated the coordination of scientific expertise and research in switchgrass biotechnology that would have been otherwise impossible by one investigator with funding under a traditional grant.

## CATALYTIC PYROLYSIS MEDIATED CONVERSION OF BIOMASS TO BIOHYDROGEN

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Subcritical aqueous pyrolysis of lignocellulosic materials using novel homogeneous and heterogeneous catalysts was investigated as a means of producing hydrogen, bio-oil and hydrocarbons. The proposed process involves two-steps: 1) complete conversion of the biomass in aqueous environment in presence of a homogeneous catalyst, and 2) selective conversion of bio-oil into hydrocarbons in presence of a metal supported mesoporous catalyst. In the first phase, the subcritical pyrolysis of biomass led to the formation of three product phases: a gas phase containing lighter gases and hydrocarbons ( $H_2$ , CO,  $CO_2$ , and  $CH_4$ ); a liquid bio-oil slurry containing alkanes, alkenes, aldehydes and carboxylic acids; and a solid residue (bio-char). The second step involves selective reduction of bio-oil into alkanes utilizing a mesoporous catalyst (pore size of 3.8-4.2 nm) for hydrocarbon enrichment. Subcritical aqueous phase liquefaction of lignocellulosic materials was carried out at 150-275°C and 850 psi in presence of 2-5 wt% Ni-catalyst. It was found that a homogenous  $Ni^{2+}$  catalyst produced more hydrogen and a bifunctional Ni-impregnated mesoporous silica catalyst generated higher hydrocarbons whereas the amount of bio-oil and biochar increased with the increase in the biomass loadings. Further enrichment of hydrogen and methane appeared feasible using the mesoporous catalyst. Preparation of the mesoporous catalyst was performed using the surfactant assisted sol-gel synthesis route involving generation of an ordered mesoporous silicate structure with controllability of pore size and porosity. The mesoporous gels were functionalized with poly (4-vinyl) pyridine and treated with metal ions to establish binding with the mesoporous silicate network. Functionalization of the catalyst surface was confirmed using FTIR, and the metal concentration was determined using energy dispersive spectroscopy. Results on the subcritical reforming of biomass will be presented and discussed. Complete conversion of waste paper was observed at 250°C suggesting that the use of subcritical conditions could result in significant energy savings over the process of fast pyrolysis at 500°C.

## A COMBINED BIOLOGICAL AND CHEMICAL APPROACH TO THE CONVERSION OF LIGNIN TO HYDROCARBON BIOFUELS AND VALUE-ADDED CHEMICALS

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Lignin is the second most abundant component of cellulosic biomass, comprising as much as 25% of the dry mass. Ongoing development of new biomass fractionation processes assures that lignin will be a low cost and readily available source of renewable carbon. Current biorefinery conditions mainly target the sugar components of vascular biomass, cellulose and hemicellulose, but burn the residual lignin component. While this method is successful in utilizing the fermentable sugars from plant cell walls, it fails to exploit the energy-rich bonds within lignin's heteropolymeric network of monolignols. Many of the aromatic constituents within lignin may serve as valuable building blocks for industrial chemicals and hydrocarbon fuels. While development of selective conversion processes for lignin is a well-recognized need for the biorefinery, progress has been hindered by lignin's high level of structural heterogeneity. By focusing the conversion reaction on the single unifying structural feature of lignin, its network of aromatic rings, we anticipate that predictable, high yield changes will be introduced into an otherwise low-value residue.

One promising route to this type of ring targeting may be accomplished through the activity of a class of microbial enzymes known as ring hydroxylating dioxygenases (RHDs). These aerobic enzyme systems have been reported to selectively disrupt the stable aromatic structure of a wide range of arenes by adding a vicinal pair of hydroxyl groups to the ring, forming a reactive cis-dihydrodiol. Here we report results on the dihydroxylation of several lignin models using a recombinant bacterial toluene dioxygenase, and development of optimal growth and product monitoring assays for the intermediate dihydroxylated arenes. Ongoing work includes analysis of the conversion efficiencies of previously characterized RHDs as well as efforts to isolate novel RHDs. Ultimately, catalytic hydrogenation or reforming will be used to convert the dihydroxylated intermediate into a hydrocarbon or oxygenated hydrocarbon, suitable for direct blending with existing fuel supplies or chemical conversion to other useful bioproducts.

## THERMAL ANALYSIS OF ORGANOSOLV FRACTIONATED LIGNIN

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A selected group of biomass derived lignins isolated using clean fractionation, an Organosolv process, was characterized using FT-IR spectral and thermal property analysis coupled with multivariate analysis. PCA indicated significant variations between the hardwood, softwood, and grass lignins due to differences in syringyl and guaiacyl content, also the processing temperature and time used to extract the lignins. PLS revealed that the concentration of syringyl units is the foremost factor in the variation of glass transition temperature (T<sub>g</sub>) for each lignin sample. Understanding the influence of feedstock and pulping conditions on rheological and thermal properties of lignin is fundamental for its successful use in polymer melts and solution processing. Therefore, the thermal and rheological characteristics of different lignin samples were studied by measuring the glass transition temperature, complex viscosity, dynamic moduli, and transient viscosity. Master curves were generated for complex viscosity and dynamic moduli by using several viscosity models to fit experimental data. From the Arrhenius plots of the shift factors with respect to temperature, the activation energies for shear flow were determined. The complex viscosity curves showed typical and atypical shear thinning behavior indicating that the lignin samples had a wide range of rheological properties. It was concluded that the structural variations resulting from altering the processing time and temperature and the lignin species, directly affect the thermal properties of the lignin.

## STRUCTURAL TRANSFORMATION OF CARBONACEOUS SOURCES IN LIGNOCELLULOSIC MATERIALS BY PYROLYSIS PROCESS

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Switchgrass-derived biochars produced by auger pyrolysis process at various conditions (450, 600 and 800 °C for 30s of residence time) were characterized by using approximate analysis, thermogravimetric analysis (TGA) and spectroscopic techniques. These assessments will help to determine if biochars can be used as a carbon sequester and as a fertilizer for bioenergy crop production. Fixed carbon and ash contents in the biochars increased with higher pyrolysis temperatures. In particular, ash contents increased significantly up to 13 and 22 % at 450 and 800 °C, respectively. Thermograms obtained by combustion of biochars using TGA showed that mass loss curves of the biochars shifted to higher temperatures with biochars produced at elevated temperatures. Derivative TG identified sharp peaks associated with autocatalytic reaction of cellulose-derived chars. These sharp peaks followed by broader peaks of lignin-derived biochars shifted to higher temperatures with thermal treatment. This result indicates the presence of various cellulose- and lignin- derived carbon structures in the biochars. FT-IR spectra of biochars produced at 450 and 600 °C contained surface functionality derived from switchgrass. Additionally, the increase of the intensity ratio of *D* (1350 cm<sup>-1</sup>) and *G* peaks (1590 cm<sup>-1</sup>) from Raman spectra suggests that the amount of aromatic carbon rings in the biochars augments with thermal treatment. These results imply that biochars produced at higher pyrolytic temperature possess carbon structures with a more recalcitrant form.

## CELLULOSE MICROFIBRILS – SOURCE, PREPARATION, DISPERSION, CHEMICAL MODIFICATION AND SOME USES

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Cellulose microfibrils obtained by the acid hydrolysis of cellulose fibers are used in the food, drug and separation industries. The hydrolyzed microfibrils serve as fillers, viscosity altering agents, dietary fiber, and vehicles for releasing materials or as purification media. However, the microfibrils are not isolated and are produced commercially as microcrystalline cellulose; these are compact aggregates of cellulose microfibrils, sometimes only partially hydrolyzed, that cannot be re-dispersed to form the original microfibrils. The usual process for drying the fibrils is by spray drying; the particular conditions used determine the final size of the aggregates.

Microfibrils derived from cotton fiber have dimensions on the order of 5nm square by 400nm long and are thus strong crystal rods with a very high L to D ratio. This implies that they may be highly suitable entities for the reinforcement of fibers, films and other materials. To recover the fibrils as a dispersible solid, work has been done to modify the surface of the fibrils so that they will not aggregate during the drying process and so they have a greater compatibility with other polymers. However, the literature contains little work pertaining to the use of these to reinforce materials and is in the main concerned with the introduction of the fibrils from the aqueous dispersion recovered from hydrolysis directly into water-soluble polymers. It is reported that the inclusion of these fibrils enhances the properties of the composites greatly even at low levels of addition.

This preliminary study first demonstrates the recovery of cellulose microfibrils as a solid and of their re-dispersion into water and other solvents through a proprietary method. Secondly, the fibrils are chemically modified to have an azide group at C6 as a vehicle towards further chemical modification for crosslinking in composites through the thermal generation of 6-nitreno-6-deoxycellulose or for conversion into an amine to enable further functionalization.

## A WEB-BASED BIOMASS SITE ASSessment Tool (BIOSAT)

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Key to ensuring long-term, sustainable cellulose supply is the assessment of the economic availability of woody and agricultural-derived biomass feedstocks. The genesis for any emerging or existing industry is the ability to successfully secure commercial business loans with a defensible business plan for the expansion of existing plants or development of new manufacturing facilities. A key component of any business plan must include a profitable strategy for the geographic location of a cellulose using facility. This study addresses the problem with the development of web-based system for optimal siting of cellulose using mills called the Biomass Site Assessment Tool (BioSAT). The project integrates contemporary web-based information technology (e.g., Virtual Earth and Microsoft SQL) with existing U.S. Forest Service FIA data, agricultural data, harvesting, and transportation models. BioSAT has real-time database update capabilities. Transportation networks of truck and truck/rail combinations are presented for least cost solutions by zip code tabulation areas for a potential demand site. Least cost sites are selected for 33 Eastern United States using logistic regression models. Producers' marginal cost curves are presented for least cost site locations.

**Keywords.** Biomass, economic availability, siting model, BioSAT, wood feedstocks, agricultural residue feedstocks.