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Satellite Tobacco Mosaic Virus RNA Secondary Structure Determination: A Mutagenesis Approach

Satellite Tobacco Mosaic Virus (STMV) is an ssRNA virus with a genome of 1058 nucleotides that requires tobamoviruses for its replication. The x-ray crystallography structure has been solved for the icosahedral T=1 virus to 1.8Å, showing 30 helices of at least nine base pairs long. Current RNA secondary structure prediction algorithms do not predict structures consistent with the crystallography data. In RNA secondary structure folding algorithms, covariation can be used as an experimental restraint. Site directed mutagenesis is used to look for covariation in the STMV sequence. Traditional random mutagenesis approaches are utilized at the transcriptional level. Varying concentration of Mn^{2+} to Mg^{2+} transcription buffers are used to cause misinsertions of nucleotides during the transcription with T7 RNA polymerase. This is tested with mutations in restriction enzyme sites in a plasmid. Mutated restriction enzyme sites are no longer cleavable. This same technique is now being used to create STMV mutants used in the search for covarying nucleotides.

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Effect of the Reducing Agent Dithiothreitol on Ethanol Production by *Clostridium* Strain P11 using Syngas

The effect of dithiothreitol (DTT) on ethanol production by *Clostridium* strain P11 using commercial and producer syngas (obtained from gasifying switchgrass) was examined in 250-ml serum bottles. Reducing agents can enhance the regeneration of NADH/NAD(P)H from NAD+ /NAD(P)+, which are utilized in the production of alcohols. Various concentrations of DTT were added to yeast extract (YE) and corn steep liquor (CSL) media. Results showed an over fourfold increase in ethanol production in 1 g/L YE media that contained at least 7.5 g/L of DTT after 360 h of fermentation compared to the control medium (without DTT) with commercial syngas (20% CO, 15% CO_2, 5% H_2, and 60% N_2). A 35% increase in ethanol production was noticed in 10 g/L CSL media in the presence of 2.5 and 5.0 g/L of DTT compared to control medium. No significant enhancement of ethanol production in either YE or CSL media were noticed with producer syngas (14.5% CO, 14.3% CO_2, 8.5% H_2, 3% methane, 0.4% acetone, and 59.3% N_2). The acetone in producer syngas was reduced to isopropanol by strain P11. DTT could have been used in the production of isopropanol instead of ethanol, which could explain why DTT was not effective when producer syngas was used in the fermentation.

Keywords: syngas, fermentation, ethanol, clostridium, reducing agent

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Hydrodeoxygenation of M-Cresol on Copper Catalysts

Phenolic compounds which are commonly found in bio-oil can be upgraded to improve thermal and chemical stability by oxygen removal. In this work, hydrodeoxygenation of m-cresol over copper catalysts on different supports has been studied using trickle bed reactor and GC-FID. The reaction was performed at 500 psig and 425 degree C. under the presence of hydrogen. In the present work, hydrogenation of m-cresol followed by dehydration was observed. After that, hydrogenation and dehydrogenation of methyl-cyclohexene was occurred. The selectivity of methyl-cyclohexane on copper-chromite (Engelhard-Cu-1886P) was more than on 10 wt% Cu/SiO_2 which selectively produced toluene. The support was changed to gamma-alumina, and it was found that the acidity of alumina could perform the radical addition-elimination to phenol and aromatic hydrogen substitution to xylenol. The rate of reaction was also studied on the catalysts. Temperature-programmed reduction and XPS of the catalysts was studied. It was found that dispersion of copper on the support had an important role on product distribution. It is obviously seen that copper based catalyst can catalyze the reaction to convert bio-oil model compound to fuel compounds such as toluene and methyl-cyclohexane.

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Expression of Select Hydrogenase Genes in Clostridium Carboxidivorans P7 during Growth on Syngas

A finite supply of oil has lead to research in renewable fuels such as ethanol. In order to meet the government mandates for ethanol production, a cellulosic ethanol production process is required. There are several new and emerging technologies for the production of cellulosic ethanol. One of these technologies is syngas fermentation, where biomass is gasified and the gasses (a mixture of CO, CO_2 and H_2) are converted to ethanol using a microbial catalyst. *Clostridium carboxidivorans* P7 is capable of converting syngas into ethanol and other products. A key gene for this process is the hydrogenase gene, where as the bacteria uses hydrogen as the electron source. Analysis of the genome sequence for *C. carboidivorans* revealed multiple punitive hydrogenase genes. Six primer sets were designed using genome sequence data, and checked for specificity using public data bases. *C. carboidivorans* was grown on minimum media with syngas gas the only carbon and electron source. Samples were taken on a daily basis and used for RNA extraction. The resulting RNA was used in reverse transcription reaction to form cDNA. Real-time PCR was preformed for each primer set using the same cDNA template. One hydrogenase gene was found to be expressed during early growth phase and at low level during solvent production, 2 genes were expressed during the mid to stationary phase (where the shift from acetate to ethanol production occurs) then the expression decreased, one gene was expressed at approximately the same level throughout the experiment, and the remaining 2 genes were not expressed at anytime. Different hydrogenase genes are expressed at different times during the growth and product formation of *C. carboidivorans.* None of the genes in this study is responsible for the utilization of hydrogen as an electron source during the solvent phase, indicating there is another hydrogenase gene responsible.

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Conversion of Lauric Oils to Fungible Transportation Fuels on Supported Platinum Catalysts

Vegetable oil conversion to green diesel by catalytic deoxygenation has advantages over the traditional route of transesterification to fatty acid methyl esters. This work focuses on the deoxygenation of model triglycerides and natural lauric oils over supported platinum catalysts. Pt/SiO_2 and Pt-Sn-K/SiO_2 catalysts were used in a 300 ml Parr reactor at 300-350 psi and 320^{o}C in batch and semi-batch modes. Catalyst studies have shown that trimetallic Pt-Sn-K/SiO_2 has higher activity for triglyceride conversion in comparison to Pt/SiO_2. Also, the conversion of different triglycerides displays similar kinetic fitting parameters and overall product distributions. Products include primarily hydrocarbons in a useable fuel range, along with oxygenated intermediates and condensation reaction products.

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Screening Switchgrass for Water Stress Tolerance

Under marginal and semi-arid conditions, water stress can have a major effect on biomass production. Screening the performance of switchgrass genotypes under water stress will enable to identify traits needed for improving tolerance to water stress. The objective of this study is to evaluate growth and physiological parameters and identify switchgrass traits that can contribute to increased water use efficiency. Thirteen genotypes were studied; three lowland (Carthage, Alamo, and Kanlow) and ten upland (Southlow, Cave-in-Rock, Forestburg, Blackwell, Nebraska 28, Shelter, Shawnee, Dacotah, Sunbrust, and WI Ecotype). Plants were grown in 12 L pots, with pure sand medium, in a greenhouse facility at OSU in Summer 2009. Water stress was applied, after 74 days after emergence, through automatic drip irrigation system by withdrawing water in two levels of water stress (60%WW and 20%WW) of control (well watered plants (WW)). During the stress time, physiological parameters measured included photosynthesis, fluorescence, electron transport rate and stomatal conductance using LI-6400 portable photosynthesis measurement system. Multivariate analysis was carried out to identify the tolerant genotypes and traits contributing to tolerance. Total biomass and biomass components of all genotypes were measured at final harvest. Results showed that genotypes responded differently to water stress treatments. Photosynthesis decreased with increase in water stress and the decrease was steep in 20%WW compared with 60%WW. Genotypes Carthage (lowland) and Forestburg (upland) had the least decrease in photosynthesis at both 60%WW and 20%WW conditions. In general, biomass and its components decreased with increase in water stress. Exceptions for this included upland genotypes Forestburg, Blackwell, Shelter, Sunburst and WI ecotype. These genotypes exhibited either an increase or minimum decrease in biomass under water stress conditions. These genotypes can be used in breeding programs to improve switchgrass performance under water stress conditions.

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Silica-Based Janus Nanoparticles Simulated at the Water-Decane Interface: Evidence of Emergent Behavior

This work stems from a recent paradigm-changing proof-of-concept result reported by the group of Daniel Resasco at the University of Oklahoma [Science 327 (2010) 68]. The experimental results showed that it is possible to perform *in-situ* upgrade of bio-oil (the pyrolisis product of lignocellulosic biomass) when solid particles are used to both stabilize water-in-oil emulsions and support heterogeneous catalysts. The solid particles used were hybrid materials obtained by fusing silica particles on carbon nanotubes.

To generalize this proof of concept to large-scale industrial applications it is necessary to design simpler and cheaper particles that stabilize oil-in-water emulsions and support the catalysts. It is necessary to understand how the molecular-level features characterizing the solid particles determine macroscopic properties such as drop size and shape, as well as the mechanism of droplets coalescence. It is also desirable that the particles can be recovered after the bio-oil upgrade is complete.

It is well known that solid particles adsorb at water-oil interfaces to reduce the contact area between the two immiscible phases. Stable emulsions are obtained when the particles strongly adsorb at the interfaces. It is plausible that by adding appropriate surface-active compounds the particles can be easily released from the interfaces. Once in the continuous phase, the particles tend to agglomerate, facilitating their recovery. Quantification of these qualitative expectations will transform the bio-energy field.

We report herein our first all atom molecular dynamics simulation results for silica-based nanoparticles functionalized with hydrophobic moieties at the decane-water interface. The simulation results are quantified in terms of contact angle at the water-nanoparticle-decane interface, mobility of the nanoparticles, association of multiple nanoparticles at the interface, and free-energy landscapes that dictate the nanoparticle adsorption at the interface. The results are quantified based on the chemical features of the nanoparticle surface.

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Nanohybrid Particles that Catalyze Biofuel Upgrade Reactions at the Interface of Water/Oil Emulsions

The pyrolysis of biomass is one of the most technically and economically feasible process for the production of alternative fuels. The pyrolysis process converts solid biomass into gases, char and liquid (Bio-oil). The latter is an unstable biphasic mixture of oxygenated molecules (furfurals, guaiacols, phenols, small acids and aldehydes) with water. In this context, a very convenient strategy for the refining of bio-oil could be the utilization of a recoverable and recyclable solid catalyst that naturally segregates to the water-oil interface. The aim of this project is to perform catalytic upgrading reactions (condensation and hydrodeoxygenation) at the water-oil interface of emulsions, using transition metals (Pd) supported on solid nanohybrid particles based on carbon nanotubes fused to metal-oxides (SiO2 and MgO).

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Development of Bacterial Enrichment Cultures that Degrade Lignin as the Sole Source of Carbon

Biomass represents one of the world's most abundant renewable energy sources. However, utilization of this resource for biofuel is difficult due to the presence of significant amounts of lignin, a polymer that hinders the digestion of cell wall polysaccharides to simple sugars intended for fermentation. This recalcitrance to bioconversion requires the use of chemical or physical pretreatment to break the lignin seal to allow polysaccharides accessible to microbial attack. However, these techniques are costly and result in the loss of significant amount of polysaccharides. Therefore, there is growing interest in developing microbe-based technologies that are safe and cost-effective. To date, not much is known about bacterial ability to degrade lignin. The goal of this project was to develop bacterial consortia that degrade lignin in plant biomass. We have enriched several lignin-utilizing cultures from naturally decaying wood, rumen fluid, and termite gut. These cultures were obtained by repeatedly sub-culturing mixed cultures derived from the above sources in mineral medium (MM) with lignin as the sole source of carbon. Measurements showed a steady increase in cell protein for the first 3 - 4 weeks followed by a drop when grown on lignin as the sole carbon source. This drop could be due to the production of toxic phenolic intermediates. Enrichments also grew well on lignin model compounds including ferulic acid, veratric acid, vanillin or *p*-anisoin as the sole carbon source. Analysis of clone libraries generated from DNA isolated from the enrichments showed the presence of a large percentage of clones belonging organisms known to degrade aromatic rings. In addition, ring-cleaving genes including catechol 1, 2- dioxygenase, catechol 2, 3-dioxygenase, and protocatechuate 3, 4 -dioxygenase were amplified using degenerate primers and genomic DNA isolated from our cultures. **Conclusions:** enrichments grew well on lignin or lignin-model compounds indicating lignin-degradation ability. Detection of ring-cleavage genes indicates the enrichment's genetic ability to degrade aromatic structures.

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The Tertiary Structure Contributes to the Prohead RNA Self-assembly

Prohead RNA (pRNA) is an essential component of a powerful biomolecular nanomotor that packages bacteriophage genomes into viral capsids. The pRNA sequences in bacteriophage phi29, GA1, M2 and SF5 have only 12 % sequence conservation, but share a conserved secondary structure and function. The 4 pRNAs self-assemble into dimers, trimers, and multimers in a magnesium ion, temperature, and concentration dependent manner. The dissociation equilibrium constant for dimer formation are quantitatively measured by gel binding experiments. The predicted intermolecular Watson-Crick base pairs in the four pRNA sequences vary, but the free energies of dimer self-assembly are all 7-8 kcal/mol. Mutagenesis experiments in the interlocking loop sequences shows that pRNA sequences form dimer even if only GU noncanonical base pairs may form in the interlocking loops. Thus, in addition to the intermolecular base pairs, the tertiary structure around the loops contributes to the energetic stability of the pRNA multimers. This study contributes to understanding how the pRNA nanomotor assembles and will also enable future design of RNA nanoparticles.

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NMR Structure of Prohead RNA E-loop Hairpin

The *Bacillus subtilis* phage phi29 packaging motor requires prohead RNA for genome encapsidation. The NMR structure of the prohead RNA E-loop hairpin, r(5'AUUGAGUU), and MC-SYM predictions for prohead RNA E-loops provide a basis for comparative analysis of hairpin structures in prohead and ribosomal RNA. All the hairpins contain a U-turn motif but differ in the first noncanonical pair and backbone orientation. These structures provide benchmarks for further improvements in RNA structure predictions from sequence.

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Characterization of Microbial Strains Important in Biofuels Production and Biomass Conversion

Genomic sequencing of 20+ clostridia strains related to biofuels production and biomass conversion were sequenced, including multiple strains from Cluster III thermophilic and mesophilic cellulolytic *Clostridium* species and multiple strains of saccharolytic *Thermoanaerobacter* species. This dataset represents a significant improvement in the genomic knowledge base of bacteria important to biofuels production. The genomes of three *Thermoanaerobacter* strains from this group, *T. pseudethanolicus* 39E, *Thermoanaerobacter* sp. X514 and *T. italicus* Ab9, have been finished and comparative genomics analysis has been conducted. Comparative analysis of the four complete genomes provides the most detailed view to date of the dynamics of *Thermoanaerobacter* genome evolution. Strains derived from hot springs environments show a conserved genome structure whereas the single subsurface strain X514 shows a highly dynamic genome that suggests possible roles of environment on shaping genomic architecture. Further analyses of the metabolic profiles of the strains resulted in testable hypotheses regarding the relative carbon uptake and usage profiles of the strains. These hypotheses were experimentally tested using metabolics and molecular biology techniques to identify physiological traits relevant to ethanol fermentation and biomass degradation in co-culture with select *Clostridium thermocellum* strains. In particular, it was observed that the three strains employ distinct lineage-specific xylose metabolism and transport systems and that X514 shows a significantly increase absolute rate of carbon flux from xylose compared to 39E. Furthermore, it was show that the ability of X514 to synthesize vitamin B_{12} *de novo* alleviates the need to supplement ethanol-producing cultures with that vitamin, whereas ethanol yields from 39E cultures are very sensitive to B_{12} concentrations due to the inability of 39E to synthesize that vitamin. The transcriptional profiles of *Thermoanaerobacter* sp. X514 grown on different carbon substrates was also determined. Experimental studies have shown that X514 is able to metabolize hexose (glucose, fructose, galactose) and pentose (xylose and ribose) monosaccharides as well as some complex carbohydrates (cellobiose, starch and sucrose). When X514 is grown on a given substrate (ie xylose), the corresponding metabolism genes are highly expressed as expected. X514 employs both the Embden-Meyerhof-Parnas (EMP) and pentose phosphate (PPP) pathways for sugar metabolism and encodes carbohydrate active enzymes specific to fructose, xylose and cellobiose. In contrast to glucose metabolism, growth on xylose, fructose or cellobiose results in a shift in the carbon flux towards ribose, suggestions increased production of substrates for nucleotide and amino acid biosynthesis. Experimental evidence shows higher rates of energy metabolism when X514 is grown on fructose and higher yields of acetate, ethanol and lactate. Furthermore, V-type ATPase genes and a large number of genes involved in inorganic ion transport and metabolism (i.e. Na-translocating decarboxylase, Na+/H+ antiporters, etc.) are significantly upregulated, suggesting increased energy metabolism and ATP production during grown on fructose. All conserved *Thermoanaerobacter* alcohol dehydrogenase genes are expressed at similar levels on all substrates, but adh genes specific to X514 showed differential expression under different growth conditions. Finally, the accumulated genomic knowledge base of ~140 clostridia genomes was used to conduct a phylogenomics analysis of the clostridia to identify genomic relationships and properties of the clade. Analysis provides the first genomic rationale for classification of *Lachnospiraceae*, confirms previous classifications of *Clostridium sensu stricto* and strengthens a previous genomic association between Class III cellulolytic *Clostridium* and Class V saccharolytic *Thermoanaerbacteraceae* species.

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Interfacial Water Structure on Silica Surface: Force Field Effects

Molecular dynamics simulations were used to study how the model implemented to simulate silica affects the predicted properties for interfacial water. Two silica surfaces, with low and high hydroxyl density, respectively, were simulated implementing four force fields. These force fields yield different orientation and flexibility of surface hydrogen atoms, and also different interaction potentials with water molecules. The properties for interfacial water were quantified by calculating atomic density profiles, surface density distributions, hydrogen bond density profiles, and residence times for water near the solid substrates. We found that the position and intensity of peaks observed from oxygen and hydrogen atomic density profiles are quite different when different force fields are implemented. Particularly, when the CLAYFF force field is used the first water layer is closer to the surface than when the Bródka and Zerda force field is implemented. It was found that the surface density distributions for water strongly depend on the orientation of surface hydrogen atoms. In all cases, we found an elevated number of hydrogen bonds formed between interfacial water molecules. The hydrogen bond density profile does not depend strongly on the force filed implemented to simulate the substrate, suggesting that interfacial water assumes the necessary orientation to maximize the number of water-water hydrogen bonds irrespectively of surface properties. On the contrary, the residence time for water molecules near the interface strongly depends on the force field. Our results show that when CLAYFF force field is used, the residence time of water molecules at contact with the solid is longer than that predicted using any of the other force fields considered herein. These results should be considered when comparisons between simulations and experimental data are attempted.

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Understanding Switchgrass Nitrogen Use Efficiency

Nitrogen (N) is an important input for biomass production. The amount of nitrogen used can significantly alter the sustainability of bioenergy production systems. Therefore reducing the nitrogen use or increasing nitrogen use efficiency of bioenergy crop species is of vital importance. A study was conducted in summer 2009 to evaluate growth and physiological parameters and identify switchgrass traits that can contribute to increased nitrogen use efficiency (NUE). Thirteen genotypes were studied; three lowland (Carthage, Alamo, and Kanlow) and ten upland (Southlow, Cave-in-Rock, Forestburg, Blackwell, Nebraska 28, Shelter, Shawnee, Dacotah, Sunbrust, and WI Ecotype). Plants were grown in 12 L pots, with pure sand medium, and were supplied with Hoagland's nutrient solution. Three nitrogen treatments 100%N (control), 20%N and 0%N were imposed starting at 74 days after sowing. During the stress period, growth and physiological parameters measured included plant height tiller number, node number, photosynthesis, fluorescence, electron transport rate and stomatal conductance using LI-6400 portable photosynthesis measurement system. Principle component analysis was carried out to identify the genotypes with, and traits contributing to higher nitrogen use efficiency. Total biomass and biomass components of all genotypes were measured at final harvest. Results showed that genotypes differed significantly in response to nitrogen treatments. A significant ($P<0.05$) genotype x nitrogen interaction was recorded for physiological, growth and biomass parameters. Genotypes that showed no decrease in biomass or increase in biomass under low N treatments had significantly higher root biomass than under control conditions. Kanlow among the lowland genotypes, and Cave-In-Rock and Blackwell among upland genotypes, exhibited significant increase in root biomass under low N compared to that under control conditions. Correlation analysis indicated plant height and root biomass as major indicators of NUE in switchgrass genotypes.

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Response to Temperature of Upland and Lowland Genotypes of Switchgrass

The objectives of this study were (a) to quantify the effect of temperature on growth, development, partitioning, photosynthesis and feedstock quality of switchgrass genotypes, (b) to determine if there is ecotype and/or genotype difference in upland and lowland switchgrass in response to growth temperature and (c) to screen switchgrass genotypes for traits for improved biomass production in present and future climates. Two lowland (Alamo and Kanlow) and two upland (Caddo and Cave-in-Rock) genotypes of switchgrass were grown in pots filled with pure, fine sand. Growth chambers at the Controlled Environment Research Laboratory (CERL) facility at Oklahoma State University were used for this study and four different day/night temperature treatments of 23/15, 28/20, 33/25, and 38/30°C with 14 hours daylight and 10 hours night condition was imposed at four leaf stages. High temperature significantly decreased the biomass yield across all cultivars but effect varied for other traits among genotypes. Stem elongation rate (SER) and leaf elongation rate (LER) decreased at the highest temperature treatment but lowland genotypes had significantly higher SER and LER across the temperature treatments. Upland genotypes produced higher number of tillers across the temperature treatment. Both shoot/root and leaf/stem ratios increased at the highest temperature condition across all genotypes but upland genotypes partitioned more biomass to the leaf and root in higher temperature. Net photosynthesis rate (P_n), photochemical efficiency of leaves (F_v'/F_m'), and rate of electron transport (ETR) increased in the highest temperature in Alamo and Cave-in-Rock genotypes. Among lowland genotypes, Alamo genotype showed wider adaptation to temperature gradient compared to Kanlow genotype. Among upland types, Cave-in-Rock genotype was more tolerant to higher temperature than Caddo genotype. Caddo was the most tolerant genotype and Kanlow was the most sensitive genotype to cold temperature.

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Effect of Vacuum Packaging on Storage Losses and Packing Density of Chopped Sorghum

Production of fuel ethanol from domestic sources can enhance the agriculture sector, decrease oil import and reduce green house gas emissions. Cellulosic feed stocks like switchgrass, sorghum, and corn stover etc are widely studied these days as potential biofuel feed stocks. Some of the major handling issues of biomass crops are their high transportation costs and losses during storage. Conventional biomass transport and storage uses square and round bales which have an average density varying from 7 to 10 lb/ft^3. Continuous operation of a mid-size biofuel production facility would require thousands of bales per day at this density which can cause a stress on already saturated road networks. In this study, a vacuum packaging technique was used as an alternative to reduce the packaging size and minimize losses during storage. Chopped forage sorghum was stored for six months under vacuum conditions at moisture content of 15% (Wb). Vacuum pressure of 9 mm Hg was maintained during this storage period. The effect of vacuum on variation in chemical composition was evaluated after every month for 6 months and its effect on packing density was evaluated.

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Characteristics and Compositional Variation in Round and Square Switchgrass Bales under Different Storage Conditions

The net yield of ethanol from biomass depends on various factors. The dry matter loss of biomass during storage is one of the crucial factors that affect the final yield of ethanol. This research evaluates the dry matter loss and changes in chemical composition of round and square switchgrass bales under various storage conditions. Forage switchgrass was harvested and field dried to a moisture content of approximately 11%. The biomass was packaged in square and round bales. Chemical constituent analysis was performed on samples from each bale. The broad class of storage treatments included inside storage and outside storage. The outside storage of bales was further categorized into covered and uncovered storage bales. Bales stored outside were placed on pallets, gravel and on the bare ground whereas those stored inside were placed on concrete. The dry matter loss was monitored by weighing bales initially, then after three and six months of storage. At the end of three and six months in storage, samples from each of the bales were analyzed for variation in chemical constituents, dry matter, and moisture content. This information will assist in the evaluation of logistics models of harvesting, storage and transportation of lignocellulosic biofeedstock.

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Graphene Sheets-Oil Nanocomposites: Equilibrium and Transport Properties from Molecular Simulation

Graphene sheets (GSs), one-atom-thick layers of carbon atoms, are receiving enormous scientific attention because of extraordinary electronic and mechanical properties. These intrinsic properties will lead to innovative nano-composite materials that could be used to produce novel transistors and thermally-conductive polymeric materials.

We conducted molecular dynamics simulations for pristine and functionalized graphene sheets dispersed in liquid linear alkanes (oils) at room conditions. For the first time, our results show that, although pristine graphene sheets agglomerate in the oils considered, graphene sheets functionalized at their edges with short branched alkanes yield stable dispersions.

We demonstrated that excluded-volume effects, generated by the branched architecture of the grafted functional groups, are responsible for the stabilization of small graphene sheets in the organic systems considered here.

As the concentration of GS increases, we observe a possible phase transition from a disordered phase to an ordered phase, possibly columnar.

We conducted non-equilibrium molecular dynamics simulations for GS dispersed in octane to characterize heat-transfer properties. It was found that the resistance to heat transfer offered by the interface between the GS and the oil, the Kapitza Resistance (***K***), is less than that found for carbon nanotubes dispersions.

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Effect of Temperature, pH and Buffer on Producer Gas Fermentation using Clostridium Strain P11

Biomass gasification combined with producer gas fermentation is one of the potential methods for the production of next generation biofuels. Gasification process offers an economically viable approach for the utilization of low cost agricultural raw materials and also ensures greater utilization of recalcitrant lignin fraction of the biomass. Carbon monoxide and hydrogen components of syngas have a low solubility in aqueous broths and solubility decreases with increasing fermentation temperature. Low fermentation pH offers stressed conditions under which cells of Clostridium strain P11 have been shown to preferentially switch from acetogenesis to solventogenesis phase. Morpholinoethanesulfonic acid (MES) added as media buffer during Clostridium strain P11 fermentation has been shown to have significant positive effect on ethanol production and increased lag on ethanol formation. The objective of the present research was to optimize the process parameters of Clostridium strain P11 fermentation. A 3 x 3 central composite experimental design was constructed to evaluate the effect of pH, temperature and buffer on fermentation productivity. Results indicated that there were no significant differences for ethanol and acetate production between 32 and 37°C operating temperatures. At 42°C, no ethanol was produced. Buffer addition and pH did not significantly affect ethanol or acetate production. Comparing individual treatments, it was observed that the treatment with no added buffer at 32°C could provide better process economics by lowering the cost of fermentation media and maintaining a lower fermentation temperature.

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Investigation into the Diversity and Community Structure of Anaerobic Gut Fungi (Phylum Neocallimastigomycota) Reveals Multiple Novel Lineages

The anaerobic gut fungi (Phylum Neocallimastigomycota) have been isolated from the gastrointestinal tract of ruminant and non-ruminant herbivores and are initial colonizers of ingested plant materials. Prolific growth of fungal rhizoids and the production of a wide array of cell-bound and cell-free cellulolytic, hemicellulolytic, glycolytic, and proteolytic enzymes suggests their potential utility in the production of biofuels from various lignocellulosic stocks. With little currently known regarding the diversity of these microorganisms in nature, a culture independent, pyrosequencing approach was used for samples from thirty different herbivore species belonging to 10 separate mammalian and reptilian families. A total of 267, 287 sequences representing all known anaerobic fungal genera were obtained. Sequences affiliated with the genus *Piromyces* were the most abundant, being encountered in 28 different samples, and representing 36% of the sequences obtained. On the other hand, sequences affiliated with the genera *Cyllamyces* and *Orpinomyces* were the least abundant, being encountered in 2, and 8 samples, and representing 0.7, and 1.1% of the total sequences obtained, respectively. Further, 38.3% of the sequences obtained did not cluster with previously identified genera and formed eight phylogenetically distinct novel anaerobic fungal lineages. Some of these novel lineages were widely distributed (e.g. NG1, NG3), while others were animal specific, being encountered in only one or two animals (e.g. NG4, NG6, NG7, and NG8). These results greatly expand the documented global phylogenetic diversity of members of this poorly studied group of fungi that could represent a novel, yet-untapped resource of novel lignocellulytic enzymes for biofuel research.

Keywords: neocallimastigomycota, biofuels, pyrosequencing

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Ethanol and Acetic Acid Production from Syngas using a New Acetogen CP11

Syngas fermentation using a new bacterium CP11 was studied in 250-mL bottles containing 100 ml of yeast extract medium at 37°C and pH 8.0. The new bacterium CP11 was able to convert CO and H_2 present in commercial and producer syngas (obtained from gasifying switchgrass) to ethanol and acetic acid. The commercial syngas was composed of 40% CO, 30% CO_2, and 30% H_2 (by volume). However, producer gas was made of 13.4% CO, 16.4% CO_2, 11.1% H_2, 2.8% methane, 0.4% acetone, and 54.5% N_2 (by volume).

Products formed and conversion efficiencies of syngas components during the fermentation were determined. Preliminary results showed that the new strain CP11 grew faster and produced more ethanol in the medium with commercial syngas compared to producer syngas. However, two times more acetic acid formation was measured in the medium with producer syngas. This showed that the presence of more CO in the syngas favored ethanol formation. The CO and H_2 conversion efficiencies were 28.1% and 6.4%, respectively, with commercial syngas compared to 49.2% and 37.1% with producer syngas. These results showed the potential of the new strain CP11 use in ethanol and acetic acid production from syngas.

Keywords: alkaliphilic bacterium, fermentation, ethanol, yeast extract, syngas, producer gas

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Ethanol Production from Syngas by *Clostridium* Strain P11 using Corn Steep Liquor as a Nutrient Replacement

The objective of this research is to evaluate corn steep liquor (CSL) as an inexpensive nutrient and substitute to yeast extract used in the conversion of syngas to ethanol. CSL is rich in carbohydrates, vitamins, minerals, and trace metals and is much lower in cost compared to yeast extract. Syngas fermentation in yeast extract and CSL media using *Clostridium* strain P11 were studied in 250-mL bottles and 7.5-L fermentor. Yeast extract was used at a concentration of 1 g/L, while two CSL concentrations (10 g/L and 20 g/L) were investigated. The syngas composition was 20% CO, 15% CO_2, 5% H_2, and 60% N_2 (by volume).

After 600 h of fermentation, ethanol concentrations in 250-mL serum bottles with 1 g/L yeast extract, 10 and 20 g/L CSL were 1.3, 1.5, and 2.7 g/L, respectively. Ethanol yields in these media were 72, 79 and 44% of theoretical values based on CO consumed, respectively. In the 7.5-L fermentor, the maximum ethanol concentrations after 360 h of fermentation in 10 g/L and 20 g/L CSL media were 8.6 and 9.6 g/L, respectively. This represent 57 and 60% of the theoretical ethanol yield based on CO consumed. Only about 6.1 g/L of ethanol was obtained in the medium with 1 g/L yeast extract after 360 h, which represents 53% of the theoretical ethanol yield based on CO consumed. These results show corn steep liquor is an acceptable substitute for yeast extract in syngas fermentation, reducing the medium cost by 37%.

Keywords: syngas, fermentation, ethanol, c*lostridium*, corn steep liquor, yeast extract

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Winter Biomass Yield, Year-round Elemental Concentrations of Kanlow Switchgrass, and Associated Soil Nutrients in a Zero Input Environment

Switchgrass (*Panicum virgatum* L.) is a warm-season perennial species native to North America. It was selected by the US Department of Energy (DOE) as the model herbaceous species for the development as a cellulosic feedstock crop for biofuels production. Maximum biomass yields in switchgrass can be harvested with one-cut system by mid-September. However, Information is limited on winter biomass yield, elemental composition of standing cured biomass, and associated soil nutrient status. Therefore, the objectives of this study were to evaluate changes in winter biomass yield, year-round elemental composition of Kanlow switchgrass, and associated year-round soil nutrient dynamics in a zero input environment. An unfertilized Kanlow switchgrass planting established in 1998 was used in the study. The experimental design was a randomized complete block design with 6 replications. The experimental treatment was monthly harvest from November to March in winter and year-round monthly sampling of biomass and soil for chemical analyses. Each replication was on a large plot of 200 m^2. The 2-yr mean dry matter yield of winter harvests was 5.13 t/ha, ranging from 3.88 t/ha in 2007-2008 to 6.38 t/ha in 2008-2009. Biomass yield decreased as winter progressed, statistically significant in first winter but not in the second winter. Concentrations of biomass elements and soil nutrients changed with various degrees over the two years.

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Towards the Development of Multilocus Barcodes for Rust Fungi Infecting Switchgrass and Potential Alternate Hosts

Switchgrass (*Panicum virgatum* L.) biomass production can be significantly reduced by fungal diseases, especially rust. Many uncertainties remain about switchgrass rust etiology. Six species of rust fungi, *Puccinia emaculata*, *P. panici*, *P. pammellii*, *P. huberi*, *P. graminis*, and *Uromyces graminicola*, have been reported to infect switchgrass. It is currently unclear which rust species are the most widespread and which have the greatest impact on switchgrass production in monocultured agricultural systems and native prairie ecosystems. Additionally, switchgrass rust likely requires an alternate host to complete its meiotic life cycle and no such host has been identified irrefutably. Eradication of the alternate host would limit the rust's genetic diversity and thus, its ability to adapt to disease management practices such as resistant cultivars and fungicides. To address some of the ambiguities surrounding switchgrass rust, several collections of switchgrass rust urediniospores were made using a spore vacuum from OSU research plots in Stillwater, OK. In the field rust pustules became apparent later than normal in 2009 (late July), resulting in mild disease severities, overall. DNA isolated from urediniospores has been used to test PCR primers amplifying loci encoding the internally transcribed spacers of ribosomal RNA (ITS-rDNA), beta-tubulin (tub1), translation elongation factor-1 alpha (EF1α) and mitochondrial cytochrome b (cytb). Thus far, only a partial ITS-rDNA sequence has been recovered and was most similar to that of *P. emaculata*. PCR primers and conditions are being optimized.

Switchgrass, maintained in growth chambers, was successfully inoculated with a urediniospore suspension when followed by 24 hours in a dew chamber. Free moisture after inoculation was found to be crucial for establishing infection. This rust strain has been continuously propagated for 7 months, providing a constant supply of rust spores for experiments.

Aeciospores were collected in the spring from the following alternate host candidates, which all commonly grow near switchgrass fields and produce conspicuous aecia: *Conyza canadensis*, *Erigeron strigosus*, *Baptisia australis* and *Asclepias viridis*. However, none of the ITS-rDNA sequences recovered from these aeciospores was similar to *P. emaculata*.

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Phenylamine Functionalization of Single-Wall Carbon Nanotubes

Single-wall carbon nanotubes (SWNT) were functionalized with phenylamine using dissolving metal reduction and halophenylamines. A number of different reaction methods and procedures were explored to determine which would be the most efficient and reproducible. Lithium metal was used as the electron donor in all reactions with fluoro-, chloro-, bromo-, or iodoaniline serving as the source of the phenylamine functionality. Analysis of the reaction products revealed significant functionalization and the presence of amine functional groups. The products of these reactions will be incorporated into polyimides and polyamides in future studies.

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Effect of Time of Harvest on Ethanol Production from Switchgrass

Understanding how the entire system of biomass production and conversion is crucial to improving the economics of biofuel production. One important characteristic is how the chemical composition changes as the plant matures, and how the changes affect ethanol production. In this study the effect of the harvest date on the cell wall composition and ethanol production from switchgrass via simultaneous saccharification and fermentation (SSF) was investigated. The harvest dates occurred once a month from July to November in 2008 on a mature stand of Kanlow switchgrass. Results indicate that xylan content decreased, lignin content increased, and glucan content was constant over the growing season. Ethanol production was influenced by the lignin content in the switchgrass before pretreatment. Lignin content in the switchgrass after a liquid, hot water pretreatment did not affect the ethanol production. Samples from August resulted in the greatest ethanol production per ton of dry switchgrass.

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Identification and Expression Analysis of MicroRNAs in Switchgrass

In recent years, several plant species such as switchgrass, *Miscanthus* and *Brachypodium* have been recognized as potential model plant species for cellulosic bioenergy production. Of these, switchgrass has attracted much attention in the United States and worldwide because it can grow well on marginal lands and tolerate frequent drought spells. However, little is known about the basic biology that control biomass production and stress tolerance in switchgrass. Genome-encoded ~21–24-nt microRNAs (miRNAs) have emerged as critical regulators of gene expression important for normal growth and development and adaptation to abiotic stress, including nutrient-deprived conditions. To understand miRNA-guided post-transcriptional gene regulatory networks in switchgrass, we sought to identify miRNAs in switchgrass. By sequencing small RNA libraries from the seedlings, flowers and emerging tillers, we identified ~45 miRNA families. Temporal expression analysis suggested distinct tissue-specific expression of several miRNA families. miR395 or miR399 are induced when sulfate or phosphate levels are limiting in other plant species. However in switchgrass, miR395 and miR399 were detected in plants grown on optimal levels of sulfate or phosphate suggesting the differential regulation of these miRNAs, which can be attributed to the plant's adaptation to marginal soils with low-nutrient availability under natural conditions. These initial findings provide a snapshot of the miRNA component in switchgrass.

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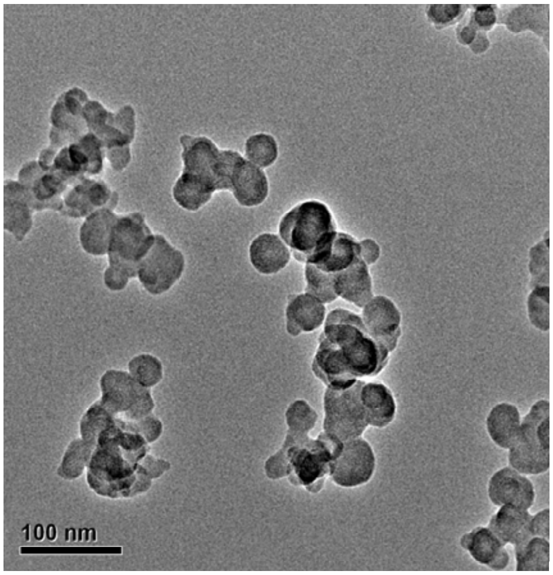
Soot Morphology of Canola Methyl Ester and Diesel Air-Flames

Soot particle formation was studied experimentally in a wick-generated open air flame of Canola Methyl Ester (CME) biofuel and Diesel fuel using a thermophoretic sampling technique and transmission electron microscopy (TEM). Examination of the evolution of soot particles along the axial streamline reveals the existence of two characteristic layers. In both cases, a flame region containing polydisperse precursor particles is located in the lower part of the flame just above the burner's nozzle. The precursor particles undergo carbonization and agglomeration as they are driven through the flame volume forming the layer of mature soot. A comparison study of soot particle evolution (morphology and size) along the axial flame axis for both types of flames is performed. A strong correlation between soot morphology and local flame temperature is observed. Atomic resolution TEM analysis on the young and mature soot collected from both flames shows the presence of highly organized carbon nanostructures.



TEM image of primary soot particles collected from a CME air flame

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Support Effects on the Catalytic Properties of Pt-Au Bimetallic Nanoparticles

Carbon nanotube-supported metal nanoparticles show promise as catalysts for a wide variety of reactions. The catalytic activity of these materials depends on their composition, structure, and the coordination numbers of exposed atoms. We have used all-atom molecular dynamics simulations to investigate the properties of bimetallic nanoparticles deposited on bundles of carbon nanotubes, focusing on nanoparticles composed of platinum and gold. The distribution of the atom types on the surface of the nanoparticle can be tuned by changing the composition of the nanoparticle and the geometry of the support. For example, it is possible to have isolated platinum atoms surrounded by gold, or chains or islands of platinum atoms. We have also used ab initio density functional theory (DFT) calculations to investigate the adsorption of CO on selective sites identified on the adsorbed bimetallic particles. Absorption energies and vibration frequencies are compared to available experimental data. Our results will lead to the design of active and selective materials for heterogeneous catalysis.

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Reduction of Toluene from Syngas Using Steam Reforming Catalysts

Formation of tars continues to be a major problem in the process of biomass gasification. For most applications, the syngas produced must be cleaned of tars before its intended final use. Three different commercial catalysts, Cerium Zirconium Platinum, Hifuel R110 and Reformax 250, were investigated for their ability to reduce toluene, a model tar compound, in biomass-generated syngas. Studies were carried out in a fixed bed reactor using a synthetic gas mixture with composition similar to that generated during gasification of switchgrass. During testing, gas flowrate and steam to carbon ratio were held constant, while space-time and catalyst particle size were varied. The highest toluene conversion was achieved with the Hifuel R110 catalyst, and catalyst particle size (pellets vs powder) also had a large effect on toluene conversion. In addition, an increase in H_2 and CO_2 concentration and a decrease in CH_4 and CO concentration were observed during toluene conversion for all three catalysts. Temperature studies were also conducted with the Cerium Zirconium Platinum catalyst in the range of 600-800˚C. Conversion of toluene increased 1.6 times due to this increase in temperature, and the apparent activation energy for steam reforming of toluene with the Cerium Zirconium Platinum catalyst was found to be 64.74 $kJmol^{-1}$.

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GU Pairs are Stable at Helix Ends

RNA interference is a process where a small RNA binds to a messenger RNA and disables gene expression through translational repression or cleavage (Doench and Sharp, 2004). One specific base pairing commonly found in RNAi is guanine and uracil. Data from the UV melting experiments suggests that terminal GU base pairs are more stable than previous data combining internal and terminal base pairs. Single and double terminal GU base pairs are predicted well with the Nearest Neighbor Model, but prediction of the third added GU pair deviates due to non-nearest neighbor effects, and the fourth GU pair adds no additional stability. NMR data show evidence for the resonance of imino protons in the first and second GU pairs, but not the third or fourth GU pair. This suggests that only stacking not hydrogen bonding of the third GU base pair is contributing to the overall stability of the RNA sequence. This information will provide new insight into secondary structure prediction of RNA and can help develop new siRNA theraupetics.

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Analysis of Switchgrass Flower cDNA Library

Switchgrass has emerged as a prime bioenergy crop. Switchgrass is a great candidate for cellulosic ethanol production, since it requires very little care and management. Using switchgrass to make cellulosic ethanol would reduce the need to use food crops such as corn. However, genetic and genomic resources to facilitate development of improved switchgrass varieties are scant. Identifying the genes expressed is an important first step. This research focuses on cataloging genes expressed in switchgrass flowers. Total RNA was extracted from flowers, then mRNA was isolated and purified. The first and second strands were synthesized, digested, and ligated into vectors. Those vectors were then introduced into chemically competent cells by transformation. The cells were then plated and in time over 2,000 colonies will be picked for sequencing. In the future, we plan to use these cDNA clones for constructing microarrays to facilitate detailed gene expression profiling to identify gene networks associated with biomass.

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Alkylation Reaction of Model Small Oxygenate and Phenolic Compounds in Bio-oil Refining

Biooil produced by fast pyrolysis of switchgrass contains various compounds including acids, alcohol, aldehyde, ketones, esters and phenolic compounds etc. Reducing oxygen content and making the compounds more stable and suitable for fuel is crucial in biooil refining. Alkylation reaction should be effective in this process. Here n-propanol and meta-cresol are chosen as model compounds. Acidic zeolite Hβ (Si/Al=19) and HZSM-5(Si/Al=45) are used as catalyst. On HZSM-5 no alkylation was observed because the pore size is too small. On Hβ it is found that the optimal temperature for alkylation of the two compounds is 200℃ and the yield of alkylation product increased with increasing W/F. Propylene was found to be the intermediate of the reaction. Then the reaction between propylene and meta-cresol was also tested and at different W/F and the yield of alkylation product equals to that when using n-propanol and the products are the same. It can be concluded that on Hβ n-propanol first quickly dehydrated to propylene and then alkylated with meta-cresol.

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Method to Assemble Biosynthetic Pathways in Microalgae

Microalgae have garnered increasing interest over the past few years for their ability to produce valuable products ranging from therapeutic proteins to biofuels. In particular, the potential of microalgae-based biofuels is creating a great deal of excitement due to the innate ability of microalgae to synthesize high levels of oils and hydrocarbons. The potential of algae-based products is therefore well recognized, but the full potential of microalgae-based production systems is still unrealized. The genetic engineering of microalgae may be necessary in order to increase the concentrations of target metabolites to acceptable levels. Recent advances in molecular biology and genomics have revolutionized our ability to discover and engineer biosynthetic pathways, yet the expression of multi-gene biosynthetic pathways in microalgae chloroplasts has not been fully achieved and represents a critical step in our ability to tailor microalgae for specific purposes. This poster will summarize the recent advances in our laboratory to engineer multi-gene biosynthetic pathways in the chloroplasts of microalgae.

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Effect of Input Air Preheating on Downdraft Gasification of Forage Sorghum

Use of high temperature gasifying air is considered as one of the economical ways to improve the gasifier performance in terms of gas quality and process efficiencies. One of the on-going gasification research activities at Oklahoma State University is to study the effect of input air temperature on downdraft gasification of a variety of low bulk density biomass materials like switchgrass and sorghum. A unique exploratory downdraft gasifier system has been developed at Oklahoma State University. It has an internal separate combustion section where turbulent, swirling high-temperature combustion flows are generated. Effects of air temperature on composition and lower heating value of the dry producer gas, and gasifier efficiency for forage sorghum are presented in this poster.

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Simultaneous Saccharification and Fermentation of Kanlow Switchgrass by Thermotolerant *Kluyveromyces marxianus* IMB3 at Various Temperatures

A simultaneous saccharification and fermentation (SSF) of pretreated Kanlow switchgrass was performed using a thermotolerant yeast *Kluyveromyces marxianus* IMB3. Pretreatment of switchgrass to improve the accessibility of cellulose to hydrolytic enzymes was done using hydrothermolysis at 200°C for 10 min. The pretreated solids had a glucan content of 58.2%. The SSF process was conducted at three temperatures 37, 41 and 45°C in triplicate, buffered at pH 5.5 and agitated for 7 days at 130 RPM. Each 250 mL baffled flask comprised of pretreated switchgrass solids containing 4 g of glucan, 10 mL of yeast peptone medium (YP), 5 mL of 1M citrate buffer and 57 mL of DI water. Commercial cellulase enzyme was used at a loading of 9 FPU/g glucan for hydrolysis of the solid substrate. The released glucose was simultaneously converted into ethanol by *K. marxianus* IMB3. Ethanol and sugar concentrations were determined in order to compare ethanol titer, productivity and yield at the three temperatures used. The highest ethanol concentration measured was 15.2 g/L in flasks maintained at 45°C after 7 days corresponding to 65% of the theoretical yield.

Keywords: switchgrass, thermotolerant yeast, hydrothermolysis, SSF, ethanol

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Reactions of 2-methylpentanal on Bimetallic Pd-Cu Catalysts

Bimetallic Pd-Cu catalysts have been studied for the hydrogenation and deoxygenation of 2-methylpentanal. Compared to Pd catalysts, Pd-Cu shows a decrease in conversion of 2-methylpentanal. At low W/F, 5% Pd-2.5% Cu/SiO_2 exhibits high selectivity for hydrogenation to 2-methylpentanol and substantially reduced decarbonylation activity compared with pure 5% Pd/SiO_2. The bimetallic shows that the ratio of etherification of the alcohol product with the 2-methylpentanal is much greater than for Pd alone, since the parallel decarbonylation is reduced. The product ether has been shown as a valuable fuel component, particularly for diesel blend. Compared at the same 25 % conversion of 2-methylpentanal, the ratio of dimethylpentyl ether/pentane is 4 times higher on the 5% Pd-2.5% Cu/SiO_2 than on the 5% Pd/SiO_2. The addition of Cu decreases the number of ^{2} sites that are needed for both decarbonylation and etherification. However, the bimetallic maintains good ether selectivity due to its ability to readily hydrogenate the aldehyde to the intermediate alcohol and continue to form the required surface alkoxide in proximity to the remaining ^{2} sites.

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Pd Nanoclusters on Pristine and Functionalized Single-wall Carbon Nanotube: DFT and Experimental Studies

The carbon nanotube – inorganic oxide support decorated with Pd nanoclusters hybrid particles have recently shown a promising application in bio-oil catalyzed upgrading. These nanoparticles are capable to create the reactive solid-stabilized emulsion which can handle both aqueous and organic phases presented in bio-oils at the same time. The deposition and dispersion of Pd nanoclusters can be enhanced by creating defect sites on SWNTs which leads to a better metal - support interaction. The ONIOM(DFT:MM) was successfully applied for calculation of Pd nanocluster adsorption on SWNTs. The difference binging energies were investigated between the pristine and oxygen functionalized SWNTs. The calculation suggests that oxygen atoms at the defect sites can modify the electronic structure of SWNTs which results in a stronger bonding with the Pd nanoclusters. The experimental confirmation was conducted by the sputter coating technique. The result agrees well with the calculation, which shows more Pd nanoclusters anchored on the functionalized SWNTs, as compared to the pristine one.

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Catalytic Conversions of Biomass-derived Polyols to Value Added Chemicals and Fuels

We have employed metal carbonyl cations as catalysts and neutral metal carbonyls as a co-catalyst to convert diols to alkenes. As a part of screening a catalytic amount of metal carbonyl cation $[CpFe(CO)_2]^+$ was used to convert 1-phenyldiol under CO (50 psi) to styrene. The major product obtained from this reaction at 150^0C is styrene oxide with no apparent formation of styrene. As the temperature was raised to 200^0C a low percentage (10%) of styrene was observed. By adding a co-catalyst $[CpFe(CO)_2]_2$ the efficiency of this conversion to alkenes is increased by 5-6 fold. Our current effort is to react the Styrene epoxide at 140^0C with metal carbonyls stoichiometrically to isolate the intermediate and also to develop a bench top conversion.

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Effect of Increased Headspace in Producer Gas Fermentations using Clostridium Strain P11

A major challenge in producer gas fermentations is the low solubility of the gaseous substrates (CO and H_2) in the culture media. Although bench scale and pilot scale experiments are conducted using CSTR, bubble column or membrane reactors, most research conducted on the laboratory scale uses bottle reactors with gaseous substrates in the headspace. During these experiments, the reaction kinetics is solely dictated by partial pressure of gases. One way of increasing reaction rates is by increasing the amount of available substrates by increasing the headspace. In this study, the headspace of the reactor was increased (from 178 ml to 256 ml) by decreasing the volume of the culture media (from 100 ml to 25 ml) in 250 ml serum bottle reactors. P11 fermentation parameters were compared between the two different headspaces. Producer gas of composition of 20% CO, 15% CO_2, 5% H_2 and 60% N_2 was used and all reactors were pressurized to 20 psig. It was observed that cell mass concentration increased 154% (0.516 g/l with increased headspace as opposed to 0.203 g/l with control), ethanol concentration increased 175% (4.4 g/l with increased headspace when compared to 1.6 g/l with control) and acetic acid concentration increased 25% (4 g/l with increased headspace as opposed to 3.2 g/l with control) when the headspace was increased to 256 ml. Also, ethanol was found to be a growth associated product with the increased headspace. The increased headspace resulted in an increase of ethanol productivities by 865%. This would help in drawing conclusions from any experiment conducted at laboratory scale at a much faster rate.

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Gasification of Eastern Redcedar Using a Small-Scale Updraft Gasifier

Eastern redcedar is considered a nuisance plant in most of Oklahoma. One potential use of redcedar is as a feedstock for biomass gasification systems. A small-scale updraft gasifier was designed for testing the feasibility of gasification of redcedar. The simple fixed bed design consists of a 0.2 m diameter combustion chamber, an agitator that extends into the reactor, and an air-locked hopper and screw conveyor for continuous feedstock delivery. Gasification of redcedar mulch was successful, with gasification temperatures ranging from 600-800^{o}C. In addition, the resulting syngas contained very little tar and had a high heating value of 3400 kJ/kg at an airflow rate of 6.5 SCFM. Results provide evidence that eastern redcedar can be utilized as a biomass feedstock for gasification.

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Development of 1030 Genomic SSR markers in Switchgrass

Switchgrass, *Panicum virgatum* L., is native to the tall grass prairies in North America, and has been grown for soil conservation and herbage production in the USA and recently widely recognized as a promising dedicated cellulosic bioenergy crop. A large amount of codominant molecular markers including simple sequence repeats (SSRs) are required for the construction of linkage maps and implementation of molecular breeding strategies to develop superior switchgrass cultivars. The objectives of this study were (1) to identify SSR-containing clones and to design PCR primer pairs (PPs) in SSR-enriched genomic libraries, and (2) to validate and characterize the designed SSR PPs. Five genomic SSR enriched libraries were constructed using genomic DNA of 'SL93 7x15', a switchgrass genotype selected in an Oklahoma State University (OSU) southern lowland breeding population. A total of 3075 clones from four libraries enriched in (CA/TG)n, (GA/TC)n, (CAG/CTG)n and (AAG/CTT)n SSR repeats were sequenced at the OSU Core Facility. From the sequences, we isolated 1,300 unique SSR-containing clones, from which we designed 1,398 PPs using SSR Locator V.1 software. Among the designed PPs, 1030 (73.7%) amplified reproducible and strong bands with expected fragment size, and 802 detected polymorphic alleles, in SL93 7x15 and 'NL94 16x13', two parents of one mapping population. All of the four libraries contained a high rate of perfect SSR repeat types, ranging from 62.7-76.2%. Polymorphism of the effective SSR markers was also tested in two lowland and two upland switchgrass cultivars, encompassing 'Alamo' and 'Kanlow', and 'Blackwell' and 'Dacotah', respectively. The developed SSR markers should be useful in genetic and breeding research in switchgrass.

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Scenario Optimization Approach for Supply Chain and Logistics Management of Switchgrass to Biorefinery

The US dependence on foreign oil has been growing steadily. To achieve oil independence, biofuel is recognized as the future alternative energy source. The expanding biofuel industry is facing challenges due to uncertainty in supply and cost (transportation cost, handling cost etc) of the biomass feedstock. The operations involved in delivery of the biomass feedstock to the biorefinery include harvesting, pre-processing, storage, transportation, and handling. The collection and the transportation accounts for more than 50 % of the overall biomass supply cost (Fiedler et al, 2007). There are different biomass feedstocks that can be used by the biorefinery like wood, agricultural residues and herbaceous crops. All the biomass feedstocks differ in their physical properties, production cost, storage and transportation methods, harvesting and handling techniques. Efficient logistics and supply chain management of a particular biomass feedstock is crucial. Therefore the objective of this study is to develop optimization model for ensure cost effective and in-time delivery of switchgrass to the biorefinery. The field operations like harvesting, handling, baling of switchgrass are weather dependent, which is random and uncertain. Scenario optimization technique can capture this uncertainty. A deterministic model with different weather scenarios will be developed.

Fiedler. P., M. Lange, and M. Schultze. 2007. Supply logistics for industrialized use of biomass-principles and planning approach. International symposium on logistics and industrial informatics. 13 September-15 September, 2007. Wildau, Germany

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Effects of Equivalence Ratio on Pollutant Emissions and Radiation from Flames of Biofuel, Petroleum Fuel and their Blends

An alternative energy source that has become popular for use in engines is biofuel. Biofuels are renewable, can be made from various feed stocks grown in the U.S., have energy content similar to those of gasoline and diesel fuels, and can run in standard engines and combustors with minor modifications. Knowledge of the combustion and pollutant emission characteristics of biofuels and their blends is important in the application of both existing and newly developed fuels. Canola Methyl Ester (CME) is a biofuel that is produced by the transesterification of canola oil. In order to understand the effects of fuel chemistry on the combustion properties, partially-premixed laminar flames of prevaporized fuels of CME and Diesel in pure form and their blends are studied. A laminar flame arrangement is used, which provides a convenient venue to study the effects of *only* fuel composition and chemistry on the combustion properties. The radiative heat fraction and global pollutant emissions of CO and NO_x are measured at equivalence ratios of 1.2, 2, 3 and 7 to simulate the partial premixed to non-premixed flame combustion that exist in diesel engines.

The liquid fuel is injected into a heated carrier gas stream (temperature of 425^oC) with the help of a syringe pump through a high temperature silica-based septum and sent to a 9.5 mm inner diameter tube burner. The vaporized fuel is ignited using a pilot flame and is removed after ignition. CME, Diesel and their three blends containing 25, 50 and 75% of CME by volume are studied. The initial equivalence ratio is altered by changing the flow rate of air, while maintaining a constant flow rate of fuel. Radiative heat flux measurements are made with a high-sensitivity pyrheliometer and the radiant fraction of heat release is calculated, which serves as an indication of sooting tendency of the fuels. Concentrations of NO_x, CO, and CO_2 in the exhaust gases are measured by collecting samples and passing the samples through gas analyzers. The results indicate that for each equivalence ratio, the pure CME flames produce the highest emission index of NO_x and the diesel flames produce the lowest. In contrast, the diesel flames produce the highest emission index of CO and radiant heat fraction; both the CO emission index and the radiant heat fraction decrease as the volume percentage of CME is increased in the blend.

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Selective Deoxygenation of Furfuraldehyde on Bimetallic Catalysts

Furfuraldehyde is commonly found in bio-oil, and this remarkably reduces fuel properties and stability during storage. Deoxygenation is typically needed to produce the bio-oil more fungible with standard gasoline and diesel. This can be achieved by hydrotreating using various noble metal catalysts. The reaction mainly involves decarbonylation and hydrogenation of the carbonyl group. In the present work, the silica supported Pd-Cu and Fe-Ni bimetallics were used for hydrodeoxygenation of fufuraldehyde. The reactions were performed at the temperature between 210-230^{o}C under atmospheric pressure of hydrogen. In the case of Pd/SiO_2 catalysts, TPR suggests that the addition of Cu to supported Pd catalyst results in formation of Pd-Cu bimetallic alloy. The activity and selectivity of the catalysts were largely influenced by the Cu content. The yield of furan from decarboylation was significantly reduced when Cu was incorporated. However, increasing the hydrogenation activity to produce furfuryl alcohol can be observed. For Ni/SiO_2 catalyst, the main products were furan and butane which is derived from ring opening reaction. 2-Methyl furan was the minor product on Ni catalyst. However, the addition of Fe to Ni catalyst dramatically increased the yield of 2-methyl furan while reducing the yield of furan. It is clearly seen that the addition of Cu into Pd catalyst and Fe into Ni catalyst can suppress the decarbonylation activity while enhancing the hydrogenation activity.

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Pyrolysis of Switchgrass in a 1 kg/h Pilot Plant

The search for a renewable energy supply will likely be supported by a new generation of integrated pyrolysis processes for biomass, among other options, to produce high quality products that must be compatible with standard fuels products. Many years of global effort in the research and development of different alternatives for the economic production of fuels from biomass have confirmed the needs for a new approach to valorize biomass. The production of compatible high quality fuels required a customized molecular engineering transformation of lignin, cellulose and hemicellulose type polymers to produce particular fuel structures and properties, and have a positive CO_2 and energy balance. Properties such as viscosity, octane and/or cetane number, acid number, biodegradability, water solubility, density, heat power and fuel stability are essential to blend these small streams produced from biomass with other conventional fuels. The upgrading requires new types of pretreatment, pyrolysis and post-treatment.

The conventional pyrolysis of switchgrass produces excessive amount of coke (char), light acid and phenolic type compounds, and CO_2. The liquid product is instable, contains water, ash, microcoke and reactive oxygen, and is very viscous.

A 1 kg/h pilot plant has been built and is currently in operation. This plant is composed of a solid feeder, a fluidized bed reactor, and several gas-solid separations and cooling stages using water. The research objectives for this pyrolysis plant are: 1) *control of thermal reactions* of lignin, cellulose and hemicelluloses biomass components during the pyrolysis to maximize the liquid yields (minimum gas and char) boiling in the gasoline-diesel ranges. 2) *Separate the unwanted compounds* by boiling point and by solubility in water. 3) Prepare sample for further catalytic upgrading. CNMR, GCxGC-MS, acid titration, water and elemental analysis were performed on the liquid products, and char was analyzed by TPO.

The plant produces several pyrolysis oil fractions diluted in the aqueous phase. Water is injected to control the separation and the stability the liquid products. Light acids, levoglucosan and alcohols are concentrated in the aqueous phase. Organic compounds insoluble in the water phase are mainly composed of phenolic derivatives. The properties of the pilot plant products are discussed.

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Response to Temperature of Upland and Lowland Genotypes of Switchgrass

The objectives of this study were (a) to quantify the effect of temperature on growth, development, partitioning, photosynthesis and feedstock quality of switchgrass genotypes, (b) to determine if there is ecotype and/or genotype difference in upland and lowland switchgrass in response to growth temperature and (c) to screen switchgrass genotypes for traits for improved biomass production in present and future climates. Two lowland (Alamo and Kanlow) and two upland (Caddo and Cave-in-Rock) genotypes of switchgrass were grown in pots filled with pure, fine sand. Growth chambers at the Controlled Environment Research Laboratory (CERL) facility at Oklahoma State University were used for this study and four different day/night temperature treatments of 23/15, 28/20, 33/25, and 38/30^{o}C with 14 hours daylight and 10 hours night condition was imposed at four leaf stages. High temperature significantly decreased the biomass yield across all cultivars but effect varied for other traits among genotypes. Stem elongation rate (SER) and leaf elongation rate (LER) decreased at the highest temperature treatment but lowland genotypes had significantly higher SER and LER across the temperature treatments. Upland genotypes produced higher number of tillers across the temperature treatment. Both shoot/root and leaf/stem ratios increased at the highest temperature condition across all genotypes but upland genotypes partitioned more biomass to the leaf and root in higher temperature. Net photosynthesis rate (P_n), photochemical efficiency of leaves (F_v'/F_m'), and rate of electron transport (ETR) increased in the highest temperature in Alamo and Cave-in-Rock genotypes. Among lowland genotypes, Alamo genotype showed wider adaptation to temperature gradient compared to Kanlow genotype. Among upland types, Cave-in-Rock genotype was more tolerant to higher temperature than Caddo genotype. Caddo was the most tolerant genotype and Kanlow was the most sensitive genotype to cold temperature.

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Genetic Diversity in Tetraploid Switchgrass Revealed by AFLP Polymorphisms

Switchgrass (*Panicum virgatum* L.) is a perennial grass native to North America, which has been identified as a dedicated biofuel crop. Traditional breeding techniques are currently being utilized to increase its biomass yield. Long term improvement in breeding requires a high level of diversity in the germplasm. The objectives of this study were to quantify genetic diversity in tetraploid switchgrass germplasm collected at Oklahoma State University, and to characterize genetic relatedness among the collections from distinct regions. Fifty six tetraploid accessions including upland and lowland ecotypes from throughout the US were used in the investigation. Genomic DNA samples were isolated for each clonal accession using Zymo Research Plant/Seed Kit^{TM}. Fluorescence-labeled amplified fragment length polymorphism (AFLP) procedure was utilized to generate DNA profiling patterns. Amplified fragments were visualized using a Li-Cor 4300 DNA Analyzer and scored visually. Sixteen selective AFLP primer combinations were used to amplify polymorphic bands for the switchgrass collections. The accessions' genetic similarity coefficients, UPGMA (unweighted pair-group method with arithmetic averaging) cluster analysis, and principle coordinate analysis were performed using NTSYS-pc (ver. 2.02i) (Numerical Taxonomy System) software. The upland and lowland accessions clustered according to type, with one exception (TN104). The similarity coefficients ranged from to 0.73 to 0.95. An AMOVA performed using GenAlEx (ver. 6.3) showed that there was a significant difference (alpha=0.5) between the upland and lowland genotypes. The *trn*L marker confirmed that TN104 was Lowland, but the *trn*L marker identification of upland and lowland genotypes was not consistent with the AFLP in two germplasm (Miami and AR4).

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Microbial Production of Butanol via Indirect Fermentation

Butanol has several advantages over ethanol as a biofuel. Synthesis gas (syngas), which consists primarily of carbon monoxide, carbon dioxide and hydrogen, can be converted to a number of desired products, including butanol; however, most butanol producing species do not tolerate elevated concentrations of butanol. One way to improve butanol tolerance and, potentially enhance production, is through strain and process development by means of physiological adaptation. Solvent production could also be enhanced by evaluating fed-batch cultures and varying initial pHs. In this study, several microbial catalysts were evaluated for their potential to produce butanol from syngas. It was possible to increase butanol tolerance in all five organisms 3 to 5 fold through serial transfers in batch and fed-batch cultures; however, there was no increase in butanol production compared to the wild types. Fed-batch cultures proved to be best for production of solvents when compared to batch. A pH range (4.0 - 7.0) experiment for *C. carboxidivorans* strain P7^T and its adapted strain showed that butanol concentrations increased at higher pHs.

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Potential of Mean Force between Aqueous Single Walled Carbon Nanotubes in Surfactant Solutions

Molecular dynamic simulations were conducted to calculate the effective potential of mean force (PMF) between two (6,6) SWNTs in the presence of aqueous surfactants at room conditions. In an effort to relate the surfactant structure to effective CNT-CNT interactions in aqueous solutions, the surfactants considered include sodium dodecyl sulfate (SDS) and flavin mononucleotide (FMN) surfactants.

In the absence of surfactants our results show, as expected, a strongly attractive SWNT-SWNT PMF at short nanotube-nanotube separations. The presence of surfactants modulates the PMF profile, as detailed by the results presented herein. In the case of SDS we employed two surface densities adsorbed on the (6,6) SWNTs to calculate the PMF as a function of nanotube-nanotube distance. Restricted to the conditions considered, we found that the potential of mean force does not depend significantly on the SDS surface density. In both cases, the PMF shows a long-ranged weak repulsion between the SWNTs in the presence of the surfactants, coupled to strong attractive and repulsive regions when the SWNTs are close to each other. Unfortunately, the repulsive peak is not strong enough to prevent the aggregation of carbon nanotubes, probably because of the large mobility of SDS surfactants adsorbed on (6,6) SWNTs. Because FMN surfactants, the phosphorilated form of vitamin B_2, contain an aromatic isoalloxazine moiety and a chiral phosphate group, they couple more tightly with the SWNTs and yield a much more pronounced repulsive force between approaching SWNTs than that observed in the presence of SDS. Our results will help us identify the surfactant properties that allow us to manipulate nanotube-nanotube effective interactions. This is the key for designing nanotube-specific dispersing agents.

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Changes in Microbial Population During Fermentation of Sweet Sorghum Juice

Sweet sorghum represents a promising alternative feedstock for ethanol production in temperate climates due to its ability to generate high levels of directly fermentable sugar with relatively low inputs. One disadvantage of the sweet sorghum feedstock is that freshly pressed juice is unstable and degradation of the sugars begins soon after pressing. The objectives of this study were to determine the types and numbers of microorganisms in the sweet sorghum juice immediately after pressing and during fermentation under conditions of delayed inoculation and with two different levels of yeast inoculation. Targeted microorganisms included lactobacilli, coliforms, E coli and yeast. Sweet sorghum was pressed with a small scale roller press, and the resulting juice was fermented at room temperature in 0.5 L vessels. Yeast was inoculated at levels of 0.13 and 0.26 g/l, and inoculation time was varied from 4 to 48 hours after pressing. Samples were plated at intervals of 12, 24 and 48 hours after inoculation onto petrifilms containing selective media for the organism of interest. Results show that delayed inoculation time resulted in increased bacterial loads, especially Lactobacilli. Coliforms generally decreased during the fermentation process, regardless of inoculation factors, and E. Coli were present in only very small numbers. Level of yeast inoculation did not significantly impact microbial counts at the two levels tested here.

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Xylitol Production by Kluyveromyces Marxianus IMB Strains

Production of products in addition to fuels from biomass can improve the economics of bioenergy processes. One such product is xylitol, which is a sweetener safe for use by diabetics. Five novel *Kluyveromyces marxianus* yeast strains, called the IMB strains, have shown the ability to grow on xylose and produce xylitol. In comparison to current chemical catalysis xylitol production processes, the biotechnological production method can be more cost effective, since no expensive metal catalysts and high temperatures are needed. All five yeast strains of K. marxianus IMB were tested at five different temperatures ranging from 25°C to 45°C with xylose as sole carbon source. IMB4 showed highest growth rate of 0.20 h^{-1} at 40°C. IMB2 produced the highest xylitol concentration at 40°C with a value of 7.84 g/l ($Y_{X/X}$ = 0.42). IMB1 produced the highest ethanol concentration of 1.64 g/l ($Y_{E/X}$ = 0.08) at 40°C. Furthermore, a test to determine the effect of inoculum carbon source on subsequent xylitol production was conducted. Xylitol production was higher in fermentations using cells grown on xylose than fermentations with cells grown on glucose.

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Expressed Sequence Tag Analysis of Switchgrass Seeds

Switchgrass (*Panicum virgatum L*,) is a warm season C4 perennial grass widely grown in North America. It has been recognized as a promising feedstock for bioenergy industry by the U.S government. Seed dormancy is one of the major problems that potentially hinder the large-scale production of switchgrass. Genomic resources for analyzing the molecular basis of dormancy in switchgrass are lacking. To facilitate this process we are conducting Expressed Sequence Tag (EST) analysis of dormant and sprouting switchgrass seeds. In this study we isolated messenger RNA from dormant seeds and sprouting seeds of switchgrass. The mRNAs were converted into double stranded cDNAs and following size selection, were cloned into the pSPORT1 vector. We have collected approximately 4000 clones from these two EST libraries. We are in the process of sequencing these seed ESTs. Preliminary analysis of the seed EST sequence data and their eventual use in constructing switchgrass cDNA-based microarrays will be discussed.

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Effect of Contaminants in Producer Gas on *Clostridium* Strain P11 Fermentation

Clostridium strain P11 is one of several bacteria that are able to convert CO and H_2 to ethanol, a renewable transportation fuel. Gas produced from biomass gasification will be required for a commercially feasible process; however, few studies have used this gas from gasifiers for fermentation. The purpose of this study was to estimate the effect of impurities in gasifier gas on cell growth, acetic acid and ethanol production. Switchgrass was gasified by fluidized bed gasification to produce switchgrass producer gas (SGPG). The composition of SGPG was 12.4% $H_{2,}$ 51.4% $N_{2,}$ 14.7% CO, 17.7% CO_2, 3.1% CH_4, 0.2% C_2H_6, 0.7% C_2H_4, and 0.2% C_2H_2. The control gas contained 12.3 % H_2, 54.2% N_2, 15.2% CO, and 18.4% CO_2. The fermentation was conducted for 11days. Cells grew faster and produced more ethanol and acetic acid using the control gas than using SGPG. Oxygen was detected in the SGPG fermentations after 6 days, which caused cell death. Acetic acid and ethanol production in fermentations using control gas also ceased after 6 days, but no oxygen was detected in these fermentations. It is hypothesized that a combination of 5g/L acetic acid and a pH of 4.4 combined to inhibit these cell growth. Isopropanol accumulated in fermentations using SGPG due to acetone reduction. The presence of acetone was the result of SGPG being scrubbed with acetone to remove tars. Acetone reduction consumed electrons that could have been used for ethanol production. Acetone was not found in the control fermentations.

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Understanding the Nonhost Resistance Mechanisms of *Medicago truncatula* to Switchgrass Rust

Switchgrass rust caused by *Puccinia emaculata* is a growing concern for bioenergy crop production. We found that *Medicago truncatula*, a model legume, displays nonhost resistance to *P. emaculata*. Initial characterization of the *M. truncatula*-*P. emaculata* incompatible interaction has shown that the spores germinate and forms long germ-tubes on the leaf surface and fails to penetrate *M. truncatula* epidermal cells. Interestingly, the *M. truncatula* nonhost response to *P. emaculata* was not associated with major transcriptional changes in phenylpropanoid pathway or pathogenesis related genes. To identify *M. truncatula* genes conferring nonhost resistance to *P. emaculata*, we have established a forward-genetic screen of *M. truncatula Tnt1* insertion lines. Screening of 1,200 *Tnt1* lines identified several mutants that showed alteration in spore germination or infection. Detail characterization of some of these mutants will be presented.

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An Integrated Approach to Rust Resistance in Switchgrass (*Panicum virgatum* L.)

Switchgrass (*Panicum virgatum* L.) is chosen as one of the main herbaceous bioenergy species. Diseases of switchgrass have been largely neglected and pathogens could become the major limiting factors to seedling establishment, biomass quality, and yield of switchgrass; especially when planted in monocultures. We have limited knowledge of the range of (rust) pathogens on wild and pasture grasses that can cause diseases in switchgrass. Leaf and stem rust caused by *Puccinia emaculata* is a major emerging disease of switchgrass. Therefore, there is an urgent need for integration of disease resistance traits into switchgrass. The current morphological methods for identification of *P. emaculata* and *U. graminicola* depend on the teliospore morphology. It is common to make collections of rust infected switchgrass that contain only urediniospores and not teliospores, therefore making positive identification of the causative rust pathogen difficult. We therefore developed ITS-based diagnostic primers for early detection and diagnosis of *P. emaculata*. The molecular phylogeny based on ITS sequences suggested that *P. emacualata* is closely related to *P. andropogonis*. Furthermore, to identify genetically diverse source(s) of rust resistance for a successful breeding program, we have developed growth chamber assays to evaluate the switchgrass germplasm. Large-scale phenotyping revealed a high degree of genetic variation within and among switchgrass populations. Alamo in general showed resistance to an Oklahoma isolate of *P. emaculata* (PE-OKC1), while Summer was highly susceptible to PE-OKC1. These results suggest a potential for genetic improvement of leaf and stem resistance in switchgrass through integration of host resistance traits.

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Effect of Energetic Gas Composition on Hydrogenase Activity and Ethanol Production in Syngas Fermentation by *Clostridia* P11

Thermochemical conversion of biomass to energetic gases and then biological conversion of these gases to ethanol is one of the promising technologies for biofuel technology. With the advancement of gasification technology, more specifically the ability of gases to be shifted to specific compositions through chemical reactions prior to fermentation, it becomes important to evaluate how the composition of these gases affects the production of ethanol and ability of these microorganisms to utilize the gas mix provided. Increasing overall efficiency of this biofuel platform involves the optimization of the organism's use of all energetic gases available, such as carbon monoxide (CO) and hydrogen (H_2). H_2 utilization can be evaluated by measuring the activity of hydrogenase enzymes present in the microorganism. This study focuses on the bacteria *Clostridia* P11 and the effect of three concentrations of CO (20%, 30%, 40%) and three concentrations of H_2 (10%, 20%, 30%) in the gaseous substrate on ethanol production and hydrogenase activity. Hydrogenase activity appeared to be least inhibited by the lowest concentrations of H_2 and CO. The highest levels of hydrogenase activity appeared early in the growth phase of the microorganism prior to solventogenesis. At lower concentrations of CO and H_2, the activity of the enzyme appeared to recover slightly through the solventogenesis stage. At the lowest level of CO (20%) the maximum ethanol concentration (0.8 g/L for 30% H_2) was reached 24 hours later in the fermentation than for higher CO concentrations (30% and 40%) reached maximum ethanol concentration (0.9 g/L for 30% H_2). Both depletion of acetic acid and increase in ethanol concentration were seen in only the trial at the lowest concentration of H_2 and CO. The highest concentration of ethanol (1.4 g/L) was seen after 10 days in the bottle with 10%H_2 and 20%CO.

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Testing-configuration and Lubricant Effects on Cartilage Lubrication

The friction coefficient between wet cartilage surfaces was measured using a pin-on-disc tribometer adopting different testing-configurations: cartilage-on-pin vs. alumina-on-disc (CA); cartilage-on-pin vs. cartilage-on-disc (CC); and alumina-on-pin vs. cartilage-on-disc (AC). Several substances were dissolved in the phosphate buffered saline (PBS) solution to act as lubricants: 10,000 molecular weight (MW) polyethylene glycol (PEG), 100,000 MW PEG and chondroitin sulfate (CS), all at 100 mg/ml concentration. Scanning electron microscopy photographs of the cartilage specimens revealed limited wear due to the experiment. The measured friction coefficient strongly depends on the testing configuration. Our results show that the friction coefficient measured under CC and AC testing configurations remains very low as the sliding distance increases, probably because during the pin displacement the pores present in the cartilage replenish with PBS solution. Under such conditions the fluid phase supports a large load fraction for long times. Both 100mg/ml CS and 100,000 MW PEG in PBS reduce the friction coefficient of articular cartilage by ~40%. Relating the measured friction coefficient with the Hersey number, our results are consistent with a Stribeck curve, indicating that the friction coefficient of cartilage under AC testing-configuration depends on a combination of hydrodynamic and boundary lubrication mechanisms.

Keywords: articular cartilage; friction coefficient; testing configuration; pin-and-disc tribometer