

OK EPSCoR State Conference

Thurman J. White Forum Building * University of Oklahoma * Norman, OK
April 21, 2011

CELLULOSIC BIOENERGY RESEARCH POSTER SESSION

- * Hybrid Student Poster Competition (Hallway A)**
 - * Feedstock Development (Hallway A)**
 - * Microbial Conversion (Hallway B)**
 - * Chemical Conversion (Hallway C)**
 - * Other/Misc. (Hallway C)**

Please note: Abstract content appears as submitted by the presenter.

Student Presenters: Hybrid Poster Competition

No.	Hall	Presenter Name	University	Poster Title
1	A	Bhoi, Prakash	Oklahoma State University	Effect of Input Air Preheating on Downdraft Gasification of Switchgrass
2	A	Chapman, Garry Jr.	University of Oklahoma	Sulfite-Driven, Oxorhenium-Catalyzed Deoxydehydration of Glycols
3	A	Couger, Brian	Oklahoma State University	Genome of the Anaerobic Fungi Neocallamastix Strain 4s
4	A	Faria, Jimmy A.	University of Oklahoma	Catalytic Strategies for Bio-oil Refining on Recoverable Catalysts that Stabilize Emulsions in Bi-Phasic Liquid Systems
5	A	Gonzalez B., Miguel	University of Oklahoma	Anisole and Guaiacol Deoxygenation Over Pt-Sn Monoliths
6	A	Pasangulapati, Vamsee	Oklahoma State University	Characterization of Switchgrass Using TGA-FTIR Under Nitrogen and Air Atmospheres
7	A	Sharma, Ashokkumar	Oklahoma State University	Fluidization Characteristics of a Mixture of Chopped Switchgrass, Gasifier Solid Residues and Silica Sand
8	A	Todd, James	Oklahoma State University	Switchgrass Selfing Confirmed by SSR Markers
9	A	Torres, Juan	University of Oklahoma	Syngas to Butanol
10	A	Ukpong, Michael	University of Oklahoma	Physiological Response of Clostridium carboxidivorans during Conversion of Synthesis Gas to Solvents in a Gas-fed Bioreactor
11	A	Vaddepalli, Chandramouli	University of Oklahoma	FTIR Spectroscopic Investigation of the Absorbance of Hydroxyacetone on Catalyst Supports
12	A	Zeng, Xin	Oklahoma State University	Next-Gen Sequencing of Switchgrass Transcriptome

POSTER 1-A

EFFECT OF INPUT AIR PREHEATING ON DOWNDRAFT GASIFICATION OF SWITCHGRASS

PRESENTER: PRAKASH R. BHOI

**Prakash R. Bhoi, Research Engineer; Krushna N. Patil, Assistant Researcher;
Raymond L. Huhnke, Professor**

Biosystems & Agricultural Engineering Department, Oklahoma State University

Air gasification research on low bulk density biomass materials is being conducted utilizing an exploratory downdraft gasifier system. One method to enhance gasifier system performance is the preheating the gasifying air. To date, gasifying switchgrass with an incoming air stream at ambient temperatures (19°C and 31°C) and at 200°C have been evaluated. Preliminary results show that using preheated air drastically increases the temperatures in the twin-shelled pyrolysis section of the reactor, resulting in improved cyclonic combustion of pyrolysis gases. Producer gas tar contents reduced from 16.7 to 12.5 g/Nm³ as the gasifying air temperature increased from 30°C to 200°C. However, marginal differences were observed in the concentration levels for CO (15.6 to 16.2%) and H₂ (10 to 11.2%). Experimental setup and test results obtained so far will be presented.

POSTER 2-A

**SULFITE-DRIVEN, OXORHENIUM-CATALYZED DEOXYDEHYDRATION
OF GLYCOLS**

PRESENTER: GARRY CHAPMAN JR.

Garry Chapman Jr., Irshad Ahmad, and Kenneth M. Nicholas*

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Abstract. Methyltrioxorhenium and perrhenate salts catalyze the deoxydehydration (DODH) of glycols by sulfite, producing olefins regioselectively. Epoxide deoxygenation is achieved also using Na₂SO₃/MeReO₃. MeReO₃ demonstrates more catalytic activity towards sulfite DODH, while perrhenate salts demonstrates enhanced selectivity. The scope and efficiency of these reactions with respect to the polyol substrate, reducing agent, catalyst, solvents and various additives are investigated. Some insights into the reaction mechanism gained from experimental studies will be presented as well.

POSTER 3-A

GENOME OF THE ANAEROBIC FUNGI NEOCALLAMASTIX STRAIN 4S

PRESENTER: BRIAN COUGER

MB Couger, Tyler Werick, Audra S. Liggenstoffer, and Mostafa S. Elshahed

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Anaerobic fungi are highly fibrolytic microorganisms that are found in the rumen of many animals that consume a wide range of plant materials. The lifestyle and competitive environment of an anaerobic rumen in which the main carbon source is plant material would necessitate the organism to produce a wide array of cell-bound and cell-free cellulolytic, hemicellulolytic, glycolytic, and proteolytic enzymes for it to propagate. Therefore, discovery and categorization of the anaerobic fungal repertoire of lignocellulolytic machinery would not only greatly expand our knowledge of their unique physiology, but also increase the catalog of lignocellulolytic enzymes with activity against non-feed plant stocks important in bio-fuel such as Switchgrass. We here present our efforts in annotating the genome of Neocallimastix strain 4S, isolated in a cellobiose and switchgrass-based media in our laboratory. Using 15GB of pair-end Illumina sequence data a working genomic assembly of 110MB was assembled using the velvet algorithm. The genome assembly of strain 4s has an AT content of 79.5% an n50=1kb, with 70MB residing on 1KB contigs. Using a combination of Ab Initio Eukaryotic Gene callers, available transcript data, and protein to genome alignment, 16,300 consensus gene models were generated using Evidence Modeler. A wide range of putative plant degradation genes were found using 1st hit BLAST homology and PFAM domains. Using a criteria for a PFAM domain value of e-4 or less a total of 302 gene models were identified containing CAZy related glycoside hydrolase (GH Family) domains. An extreme concentration genes in families involved with processivity of hemicellulose, the most variable region in plant cell walls were found (74 Gene Models). This suggests that the organism can make use of a wide range of plant substrates due to its endoxylanase plurality. In addition many other genes belonging to additional CAZy related families these included alpha and beta glucosidases, endogluconases, exogluconases, **glycoside transferases**, xylanases, galactosidases, mannases, pectinases, Swollenin related genes, Arabinofuridases, Dextranases, Rhamnogalactases, and carbohydrate esterases Current research involves a more detailed analysis of the lignocellolytic repertoire, comparison of its composition to other organisms including anaerobic bacteria and fungi, and 3-dimensional modeling and functional analysis of bio-fuel relevant enzymes.

POSTER 4-A

SOLID HYBRID-NANOPARTICLES THAT CATALYZE BIOFUEL UPGRADE REACTIONS AT THE WATER/OIL INTERFACE

PRESENTER: JIMMY FARIA

Jimmy Faria, Daniel E. Resasco

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We have developed a new reaction/separation technology based on a family of recoverable and recyclable nanohybrid catalysts that simultaneously stabilize emulsions in biphasic systemsⁱ. These nanostructured solid particles exhibit a unique advantage in streamlining biomass refining, where the immiscibility and thermal instability of crude bio-oil greatly complicates purification procedures. These novel catalyst/emulsifier hybrids can catalyze reactions with high “phase-selectivity” either in the aqueous or organic phases. The amphiphilic-catalysts are obtained by fusing carbon nanotubes to metal-oxide particles, which results in a “Janus” like nanoparticle that is able to stabilize water/oil emulsions by forming a rigid film at liquid-liquid interface of the droplets, increasing the apparent viscosity of the systemⁱⁱ. The inorganic oxide may act both as the hydrophilic side of the emulsifier and as a condensation catalyst. Hence, it is able to catalyze condensation reactions in the aqueous phase, by which small oxygenates soluble in water, with low fuel value, condense via aldol-condensation, ketonization, or etherification. The resulting products are no longer water-soluble molecules and therefore migrate to the organic phase. The oxide used can vary in acid/base characteristics (e.g. MgO, SiO₂, TiO₂ and ZnO).

In the organic phase, transition metals (e.g. Pd, Ni and Cu) can be anchored onto the hydrophobic carbon nanotubes of the nanohybrids to catalyze deoxygenation reactions including hydrogenation, hydrogenolysis, or decarbonylation.

This unique approach could have a great impact in many industrial processes, such as bio-oil upgrading, and production of specialty chemicals and pharmaceuticals, where selective reaction and separation based on water solubility are desirable.

[i] S. Crossley, J. Faria, M. Shen and D. E. Resasco, *Science*, **327**, 68 (2009).

[ii] M. Shen, D. E. Resasco, *Langmuir*, **25**, 10843 (2009).

POSTER 5-A

ANISOLE AND GUAIACOL DEOXYGENATION OVER Pt-Sn MONOLITHS

PRESENTER: MIGUEL A. GONZALEZ B.

Miguel A. Gonzalez B., Phuong T. Do, Daniel E. Resasco

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Catalytic upgrading of pyrolysis oil vapors requires a catalyst bed able to treat high reactant flow rates while generating minimum pressure drop. Monolithic catalysts fulfill both conditions and are therefore good candidates for this purpose. Low surface Inconel monoliths were impregnated with copper metal and then treated with ethylene at high temperature. By these means, surface area was increased by a factor higher than 10, due to formation of a carbon nanofibers coating on the surface of the monolith. Afterwards Pt and Sn were incorporated on the coated monolith, first as single metals and then simultaneously, producing monometallic and bimetallic catalysts, respectively. Synthesized catalysts were tested for the deoxygenation of guaiacol and anisole (products of lignin pyrolysis), two of the most deactivating compounds present in pyrolysis bio-oil. Bimetallic Pt/Sn catalyst showed the highest activity when compared to monometallic Pt and Sn catalysts. Coating with carbon nanofibers provided more surface area for the incorporation of the active phase on the catalyst, therefore increasing the yield of desired products. The main products from deoxygenation of guaiacol and anisole on Pt/Sn coated monoliths (Pt/Sn CNF-Inconel) were phenol and benzene. Pt/Sn CNF-Inconel was proved to be a potential catalyst for deoxygenation of lignin fractions present in bio-oil.

POSTER 6-A

CHARACTERIZATION OF SWITCHGRASS USING TGA-FTIR UNDER NITROGEN AND AIR ATMOSPHERES

PRESENTER: VAMSEE PASANGULAPATI

Vamsee Pasangulapati, Ajay Kumar, Carol L. Jones, Raymond L. Huhnke

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Using dedicated crops such as switchgrass is foreseen as an efficient way of generating biofuels. Though there are different ways of converting this biomass into biofuels, thermochemical conversion has always been an attractive process. There is a need to understand the reaction kinetics occurring during biomass decomposition for the process design and optimization. The objectives of the present study are to determine the thermochemical characteristics of switchgrass and analyze evolving gases online using Thermogravimetric analyzer (TGA) coupled with Fourier transform infrared spectrometer (FTIR). The decomposition of switchgrass was found to occur in three stages. The significant weight loss occurred in the temperature range of 220 °C to 400-420 °C in nitrogen atmosphere and 220 to 350-390 °C in air atmosphere depending on heating rate. The weight loss kinetics for switchgrass, cellulose, hemicellulose and lignin were evaluated under inert and non inert conditions. The gases such as CO₂, CO, CH₄ were identified as major end products during switchgrass decomposition.

Key words: TGA,FTIR, switchgrass, weight loss kinetics.

POSTER 7-A

FLUIDIZATION CHARACTERISTICS OF A MIXTURE OF CHOPPED SWITCHGRASS, GASIFIER SOLID RESIDUES AND SILICA SAND

PRESENTER: ASHOKKUMAR SHARMA

Ashokkumar M Sharma, Dr. Ajay Kumar, Dr. Raymond L. Huhnke

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Minimum fluidization velocity is one of the important design parameters for optimizing the reaction conditions of the fluidized-bed gasifier. The minimum fluidization velocity initiates fluidization and depends upon the particle size and composition of the bed material. Fluidization characteristics of a mixture of chopped switchgrass, gasifier solid residues (particle size $\leq 150 \mu\text{m}$) and silica sand in different compositions were studied in a 0.25 m i.d. transparent column. The quantity of silica sand in the mixture was 20 kg by weight. Switchgrass in the mixture ranged from 1 to 5% of the weight of silica sand while gasifier residues ranged from 5 to 35% of the weight of switchgrass. Length of the chopped switchgrass was in the range of 2 to 31 mm and diameter of the silica sand was in the range of 106 to 850 μm . The minimum fluidization velocity and corresponding pressure drop across the gasifier bed were measured. Preliminary results show that the minimum fluidization velocity was influenced by the variation in percentages of gasifier residues and switchgrass. The minimum fluidization velocity increased with increasing percentage of switchgrass. However, the minimum fluidization velocity initially decreased and then remained constant with increasing percentage of the gasifier residues. The pressure drop across the bed at the minimum fluidization velocity was observed to be less sensitive to gasifier residues. The pressure drop increased with an increase in percentage of the switchgrass. Segregation of the switchgrass and silica sand also increased with the increase in the switchgrass percentage. At the highest level of switchgrass (5%), channelization was predominant due to segregation of the switchgrass in the bed.

POSTER 8-A

SWITCHGRASS SELFING CONFIRMED BY SSR MARKERS

PRESENTER: JAMES TODD

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Switchgrass is an allogamous, self-incompatible species currently being bred for biomass as a biofuel feedstock. Genotypic recurrent restricted selection for general combining ability has been used to improve switchgrass yield. This method capitalizes on additive gene effects but neglects non-additive genes. Inbreeding and hybridization have greatly improved the yield of major crops, such as maize (*Zea mays*), sorghum (*Sorghum bicolor*) and rice (*Oryza sativa*). Hybridizing inbreds may have the potential to improve yield in switchgrass. The objective of this study was to investigate seed yield of selfing lowland switchgrass plants by bagging, and confirm selfed progeny by SSR markers. In 2008, 34 S1 Alamo and 34 S1 Kanlow switchgrass plants were bagged using two bag types: paper Lawson bags, fabric microfiber bags. Seeds were collected and counted. A total of 304 Alamo seeds were collected from which 100 progenies were grown and a total of 321 Kanlow seeds from which 91 progenies were grown. In 2009 larger bags were used on the same set of parents, one bag per plant. From the 2009 plants a total of 498 Alamo seeds were harvested from which 123 progenies were grown and a total of 1231 Kanlow seeds from which 267 progenies were grown. From the 2008 plants, bag types had no significant effect on seed yield. Family had a significant effect on seed yield for both 2008 and 2009 plants ($\alpha=0.01$). There was a significant difference between the seed yields of 2008 and 2009 ($\alpha=0.05$). DNA was extracted from the respective parents and the seedlings of the 2008 plants. Six SSR primer pairs were used to confirm selfed progeny identity by determining if all progeny alleles matched its maternal parent. Of the 2008 Kanlow offspring 23 were inbreds (25.3%) and of the Alamo 33 were inbreds (33%) with a combined total of 56 inbreds (29.3%). #

POSTER 9-A

SYNGAS TO BUTANOL

PRESENTER: JUAN R. TORRES

Juan R. Torres and Ralph S. Tanner

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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 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 3-5 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 - 7) experiment for *C. carboxidivorans* strain P7^T and its adapted strain showed that butanol concentrations increased at higher pHs.

POSTER 10-A

PHYSIOLOGICAL RESPONSE OF *CLOSTRIDIUM CARBOXIDIVORANS* DURING CONVERSION OF SYNTHESIS GAS TO SOLVENTS IN A GAS-FED BIOREACTOR

PRESENTER: MICHAEL UKPONG

Michael Ukpog, Bradley Stevenson (Advisor)

Department of Botany and Microbiology
University of Oklahoma

C. carboxidivorans P7 produces acetate, butyrate, ethanol and butanol, from the fermentation of synthesis gas. *C. carboxidivorans* uses a variation of the Wood-Ljungdahl pathway for solventogenesis, some aspects of this fermentation have been described. This study focuses on the response of key enzymes in the pathway and the changes in growth dynamics as P7 is grown in large scale continuously fed batch cultures. Enzyme activity and gene expression were measured in order to examine the role of carbon monoxide dehydrogenase (CODH), two alcohol dehydrogenase (ADH 1 and ADH 2), Fe-Fe and Ni-Fe type hydrogenase genes.

C. carboxidivorans was grown in 7.0 L bioreactors with 3.5 L anaerobic medium. Bioreactors were continuously fed a synthesis gas mix of CO:CO₂:H₂. OD, pH, and products were monitored every 24 hours. Acid and alcohol production was analyzed using a GC, enzyme activities was monitored using a MV reduction whole cell assay and gene expression was measured using qRT PCR. Growth of P7 was typical for acetogenic clostridia, with an early growth and acid production phase followed by solventogenesis accompanying cessation of growth, solventogenesis and increase in pH. Maximum concentrations of acetate (26mM), butyrate (0.7mM) ethanol (32mM) and butanol (7.1mM) were observed. Enzyme activities were highest early during growth phase and not lower during solventogenesis. CODH expression is 100 fold higher during solventogenesis and ADH 1 and ADH 2 expression are highest during butanol formation. Ni-Fe hydrogenases show no expression and Fe-Fe hydrogenases show highest expression during solventogenesis. Increased expression of CODH, ADH and FeFe hydrogenases during butanol formation suggest their roles in solventogenesis.

POSTER 11-A

FTIR SPECTROSCOPIC INVESTIGATION OF THE ADSORPTION OF HYDROXYACETONE ON CATALYST SUPPORTS

PRESENTER: CHANDRAMOULI VADDEPALLI

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Depletion of sweet crude oil resources led to the development of various multistep routes for biomass upgrading to fungible fuels and commodity chemicals. One possibility is to use fast pyrolysis as a first step, which produces a gas mixture, a pyrolysis oil consisting of water and organic compounds, and a carbonaceous residue. The composition of the pyrolysis oil depends on the nature of the lignocellulosic biomass and the process conditions. Typically, a multitude of small oxygenates are obtained, which, unlike petroleum, cannot be separated by distillation. Upgrading of such a mixture by catalytic processing will require the development of new, chemoselective catalysts that convert individual molecules or functional groups while leaving others untouched. Likely, some of these catalysts will be supported metals, and the question arises whether oxygenates will solely interact with the metal if the support is polar. Other questions concern the competition of various molecules in the mixture for sites, or the preferential mode of adsorption of molecules with more than one functional group. Currently, little information is available on the interaction of many pyrolysis constituents with surfaces.

Hydroxyacetone is an excellent candidate for a fundamental investigation because it has two functional groups and is contained in pyrolysis oil in concentrations of several weight percent. We have observed the adsorption and reaction of hydroxyacetone on a variety of oxides that are commonly used as supports or catalysts using in-situ diffuse reflectance IR spectroscopy (DRIFTS) as the primary method. Monofunctional reference compounds, specifically acetone and *n*-propanol, were also adsorbed on these oxides. Hydroxyacetone favored different adsorption modes depending on the nature of the surface. Spectra resulting from temperature-programmed experiments evidence strong interaction of hydroxyacetone with the oxide surfaces and incomplete desorption even after heating to 400°C. In conclusion, hydrophilic supports may be unsuitable for upgrading highly reactive short oxygenates.

POSTER 12-A

NEXT-GEN SEQUENCING OF SWITCHGRASS TRANSCRIPTOME

PRESENTER: XIN ZENG

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Switchgrass (*Panicum virgatum L.*) is a warm season C4 perennial grass widely grown in North America and is a promising bioenergy crop. Concerted efforts by several research groups are underway for developing genetic and genomic resources for switchgrass. Given a genome size of approximately 1600 Mbp, identification of transcribed portions of the genome using expressed sequence tags (ESTs) technology provides a viable alternative for analyzing switchgrass. Next generation sequencing technologies such as pyrosequencing, bypass lengthy steps involved in Sanger sequencing and provide rapid and economical technologies for transcriptomics. We undertook 454 based transcriptome analysis from four different switchgrass tissues – seeds, germinating seedlings, tillers and flowers. About a million EST sequences were generated with an average length of 367 bp. To accommodate the potential for multiple homologs given the polyploidy in switchgrass, the clustering and assembly approach allowed for individual ESTs to exist in more than one cluster. The ESTs were assembled into 413, 161 contigs while 481,189 ESTs remained unassembled. All the 25,000 switchgrass unigenes in the Genbank collection were represented in this EST collection. Assembled contigs were subjected to reciprocal BLAST analysis with Brachypodium, sorghum, maize and rice genomes. Nearly 3% of the assembled contigs showed sequence similarity to retrotransposons. Relative expression levels of 454 ESTs were quantified by a reads per kilobase transcript per million reads (RPKM) analysis. RT-PCR analysis for 25 genes that did not show any similarity to sequences in the GenBank database showed that these novel genes were differentially expressed in the four tested tissues. The 454 ESTs will be used for fabricating high-density oligonucleotide arrays. These arrays will be useful for identifying gene networks associated with tillering, biomass accumulation and stress responses in switchgrass.

Cellulosic Bioenergy: Feedstock Development

No.	Hall	Presenter Name	University	Poster Title
13	A	Bartley, Laura	University of Oklahoma	Coexpression Network Analysis to Identify Genes That Function in Incorporation of Ferulic Acid Into Grass Cell Walls
14	A	Dunford, Nurham (2)	Oklahoma State University	Evaluation of Microalgae Strains Native to Oklahoma for Waste Water Remediation and Oil and Biomass Production
15	A	Fahej, Mohamed	Oklahoma State University	Screening Switchgrass for Water Stress Tolerance & Utilizing <i>In Vitro</i> Culture Technique to Induce Variation
16	A	Gao, Linfang	University of Oklahoma	Characterization of a Novel Fungi <i>Penicillium sp.</i> YT02 with High Lignocellulosic-Saccharification Activity Against Various Substrates
17	A	Lin, Hao (1)	Oklahoma State University	Regulation of Leaf Lamina Outgrowth and Vascular Patterning by a Homeobox Gene <i>Stenofolia</i> in <i>Medicago truncatula</i>
18	A	Lin, Hao (2)	Oklahoma State University	Molecular Dissection of Key Plant Development Programs Crucial for Improving Total Biomass Production in Sorghum and Switchgrass
19	A	Wu, Yanqi	Oklahoma State University	An SSR-based Genetic Linkage Map of the Model Biofuel Crop Switchgrass
20	A	Pandey, Arjun	Oklahoma State University	Switchgrass Root Distribution and Yield as Modified by Row Spacing
21	A	Sripathi, Raghuveer	Oklahoma State University	Genotype by Environment Interactions Among New Switchgrass Populations in Oklahoma

Cellulosic Bioenergy: Feedstock Development

No.	Hall	Presenter Name	University	Poster Title
22	A	Srivastava, Avinash	Samuel Roberts Noble Foundation	Identifying Novel Transcripts Involved in Secondary Cell Wall from Vascular Tissues of Switchgrass (<i>Panicum virgatum L. cv Alamo</i>) Using Genomics Tools
23	A	Uppalapati, Rao	Samuel Roberts Noble Foundation	irg1 is a Novel Leaf Epicuticular Wax Mutant of Medicago Truncatula That Confers Enhanced Resistance Against Switchgrass Rust
24	A	Wagle, Pradeep	Oklahoma State University	Net Ecosystem Exchanges of Bioenergy Cropping Ecosystems
25	A	Wang, Zeng-Yu	Samuel Roberts Noble Foundation	Overexpression of PvmiRNA156b Enhances Biomass Yield of Transgenic Switchgrass
26	A	Wilson, Tracy	Oklahoma State University	Carbon Cycling in Perennial Biofuel Management Systems
27	A	Zhang, Jiye	Samuel Roberts Noble Foundation	Development of Resources for Switchgrass Functional Genomics

POSTER 13-A

**COEXPRESSION NETWORK ANALYSIS TO IDENTIFY GENES
THAT FUNCTION IN INCORPORATION OF FERULIC ACID INTO
GRASS CELL WALLS**

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Grass cell wall properties affect the quality of this abundant family as a food, feed, and biofuel feedstock. Unlike dicots, grasses incorporate the phenyl propanoid, ferulic acid (FA), into the cell wall matrix polysaccharide arabinoxylan, which can form covalent cross-links between neighboring phenylpropanoid residues of arabinoxylan and lignin. The pathway for cell wall FA incorporation has only begun to be described. Through a reverse genetics examination of a grass-expanded group of putative CoA acyltransferases, we have identified four rice (*Oryza sativa*) genes, for which modification of gene expression modifies FA composition and cell wall digestability. To develop hypotheses regarding other gene products in the pathway and the biological functions of wall FA, we are using Pearson correlation coefficients to examine public microarray data for genes coexpressed with the putative acyltransferases. Initial network analysis demonstrates that phylogenically related acyltransferase genes have similar coexpression partners and that all are within the same coexpression module. In contrast, a closely related gene that codes for a protein with a non-canonical acyltransferase domain belongs to a disparate network module. Among the proteins the transcripts of which are coexpressed with the large number of acyltransferases are a 4-coumaroyl ligase, which is likely synthesizing the substrate of the acyltransferases, and a germin-like protein, involved in rice resistance to pathogens. We are now following up on these leads toward developing grasses with cell walls that are easier to digest but still maintain a robust plant.

POSTER 14-A

EVALUATION OF MICROALGAE STRAINS NATIVE TO OKLAHOMA FOR WASTE WATER REMEDIATION AND OIL AND BIOMASS PRODUCTION

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Various types of algae are among the most efficient plants to convert solar energy to chemical energy. Microalgae are capable of taking a waste form of carbon (CO₂) and converting it into oil. Microalgae can produce up to 55,000 L oil /acre depending on the oil content (20% -70%, dry weight basis) and biomass productivity of microalgae strain while soybean produces only about 180 L oil/acre.

Oklahoma is the eighth largest swine production state in the US with over 2.4 million pigs and about 2700 Hog & Pig operations. Currently, the management of lagoon nutrients, odor and maintenance of environmental quality pose a great challenge for swine producers. An integrated system optimized for utilization of swine lagoon liquid for growing microalgae with high oil content will improve the economic feasibility of the biomass production and help to clean waste water.

Our long term goal is to develop a semi-continuous microalgae system that maximizes production of algal biomass with high oil content, captures carbon dioxide from flue gas produced by power plants and ethanol production facilities and reduces the adverse impact of agricultural waste water on environment in Oklahoma. The main objective of the current project is to select high performance microalgae strains that are suitable for waste water remediation and high oil accumulation.

Six commercial microalgae strains have been used for the screening studies; *Botryococcus braunii*, *Nannochloropsis oculata* and *Dunaliella tertiolecta*, *Picochlorum Oklahomensis* (PO) and *Dunaliella* species SP19 and SP20. Microalgae strains PO, SP19 and SP20 are native to Oklahoma and isolated from Salt Plains National Wildlife Refuge, OK.

PO was the fastest growing microalgae among the strains examined in this study. Algal biomass had high oil content, 20-40% (dry biomass basis). We have also shown that swine lagoon waste water supports algal biomass production without additional nutrients. Significant differences were observed between fatty acid composition of algal oil and vegetable oils. Adjustment of the culture medium pH to 11 was effective in flocculation of algal biomass for easy harvest. Impact of these finding on microalgae based biofuel production needs to be further studied.

POSTER 15-A

SCREENING SWITCHGRASS FOR WATER STRESS TOLERANCE & UTILIZING *IN VITRO* CULTURE TECHNIQUE TO INDUCE VARIATION

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Screening the performance of switchgrass genotypes under water stress will enable to identify traits needed for improving tolerance to water stress. Improved tolerance can be reached by using *in vitro* culture to enhance somaclonal variation and screen for variation can be beneficial. The objectives of this study are, first to evaluate growth and physiological parameters and identify switchgrass traits that can contribute to increased water use efficiency and second is screening for somaclonal variation to identify cell lines with more water stress tolerance. Under the first objective, thirteen genotypes were screened using physiological parameters including photosynthesis, fluorescence, electron transport rate and stomatal conductance. 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. Under the second objective, Alamo and Forestburg were used in vitro culture procedure to produce callus and induce variation under water stress MS media. Poly Ethylene Glycol (PEG) was used to induce stress (- 0.6 MPa, -1.6 MPa) in addition to control. Callus development was observed in all treatments and cell division was found better performed in all treatments of Forestburg cultivar than Alamo.

POSTER 16-A

CHARACTERIZATION OF A NOVEL FUNGI *PENICILLIUM* SP. YT02 WITH HIGH LIGNOCELLULOSIC-SACCHARIFICATION ACTIVITY AGAINST VARIOUS SUBSTRATES

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Penicillium spp. have been identified as potential cellulase producers for bioconversion of lignocellulosic material but their cellulase systems have not been investigated in detail. In this study, the enzyme activity and saccharides production profiles of a newly isolated *Penicillium* strain (*Penicillium* sp. str. YT02) was characterized. The optimal culture conditions for the strain based on the highest cellulase activity and saccharification ratio, were 30 °C, pH 5.5, and an agitation speed of 130 rpm. Compared with the most commonly used fungal strain *Trichoderma reesei* (ATCC 24449), high cellulolytic activity and saccharification capacity were observed in YT02 with Avicel and various insoluble lignocellulosic substrates. Similar total filter paper and CMCase activity (0.5-0.6 IU/mg) but much higher β -glucosidase (0.78 IU/mg) and xylanase (9.0 IU/mg) activity was detected in YT02, which is consistent with the results of the enzyme activity staining for xylanase, glucanases and β -glucosidase. The volumetric activities in relation to the amount of protein of *P. sp.* YT02 cellulases were determined with a model substrate, Avicel, a microcrystalline cellulose. In addition, the theoretical saccharification ratio of YT02 reached about 70% with pretreated cellulosic materials. The protein content in the supernatant of the YT02 culture was less, but more stable over time than that of *T. reesei*, which provides an advantage for cellulose degradation. These results suggest that *P. sp.* YT02 may be a potential strain for industrial bioprocessings of cellulosic plant biomass.

POSTER 17-A

REGULATION OF LEAF LAMINA OUTGROWTH AND VASCULAR PATTERNING BY A HOMEBOX GENE *STENOFOLIA* IN *MEDICAGO TRUNCATULA*

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Photosynthetic CO₂ assimilation almost exclusively occurs in leaves, and leaves are said to be the best solar panels ever built. Wider and flatter surface areas improve photosynthetic efficiency by promoting capture of solar energy and gaseous exchange. However, redesigning leaf size and plant architecture for increasing cellulosic biomass feedstock production is a challenge at present because the molecular mechanism of lamina outgrowth is not well understood. We seek to understand the mechanism of this fundamental process by dissecting the molecular events leading to lamina expansion using *Tnt1*-tagged *Medicago truncatula* mutants as genetic and genomic tools. We have identified a key gene called *STENOFOLIA* (*STF*), as a central regulator of lamina outgrowth and leaf vascular development in *M. truncatula*. *STF* is a *WUSCHEL*-like homeobox transcriptional regulator required for cellular differentiation and expansion in lateral organ primordia. In the classical *bladeless lam1* mutant of *Nicotiana sylvestris* leaf blades are reduced to vestigial strips lacking mesophyll differentiation. We discovered that the *lam1* lesion of *N. sylvestris* is caused by deletion of the *STF* homologue *NsSTF1*. In both *stf* and *lam1* mutants, blade outgrowth, venation and dorsoventral polarity are initiated but arrested while growth in the proximodistal axis is apparently unaffected. We show that *STF* and *LAM1* are interchangeable functional homologs and encode for a homeodomain protein of the WOX class. *STF* expression is confined to the adaxial-abaxial boundary layer in leaf primordia coincident with the position of the vascular strands. By using a combination of transcript and metabolite profiling and plant transformation technology, we are uncovering that the *STF/LAM1* function is required for auxin homeostasis and multiple hormonal crosstalk at the leaf margins.

POSTER 18-A

MOLECULAR DISSECTION OF KEY PLANT DEVELOPMENTAL PROGRAMS CRUCIAL FOR IMPROVING TOTAL BIOMASS PRODUCTION IN SORGHUM AND SWITCHGRASS

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Sustainable lignocellulosic biofuel production from biomass feedstocks at the moment poses significant challenges in total biomass production as well as in bioconversion. Current biomass production needs to be increased by a significant margin to relieve pressure off the cultivated land. This project aims to contribute to the molecular understanding of biomass production by dissecting the genetic pathways that lead to higher total biomass accumulation in C4 grasses. We focus on C4 grasses using sorghum as a model and switchgrass as the main target species, but molecular genetic information is being integrated from other well studied species including Arabidopsis, Medicago, rice, and maize. We aim to uncover major genetic control points and identify key transcription factors that invoke a systems level response in C4 grass developmental programs. We are targeting four major but interconnected plant developmental programs; leaf development, flowering time, plant height, and tiller number, which are all critically important for understanding total biomass accumulation. Using a reverse genetics approach, we have identified *FT*, *SOC1*, *AP1/FUL*, *ID1*, *SD1*, *SLR1* and *MOC1* homologues from sorghum and switchgrass and we are characterizing and evaluating their contribution to total biomass. We are also developing a functional genomics tool in sorghum using fast neutron mediated deletion which will help us to discover key biomass regulatory genes and genetic networks in sorghum, switchgrass and other C4 grasses. We have established an optimized fast neutron treatment dose for 50% seedling survival in R1 progeny. Using this dose, we have so far mutagenized approximately 34,000 R1 seeds with an estimated ~12,000 fertile independent R2 families. Our plan is to generate ~50,000 R2 families which will provide a reasonable coverage of the sorghum genome. When completed, this population will be publicly available and will help to advance functional genomics studies in annual and perennial C4 grasses.

POSTER 19-A

AN SSR-BASED GENETIC LINKAGE MAP OF THE MODEL BIOFUEL CROP SWITCHGRASS

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Switchgrass (*Panicum virgatum*), indigenous to North America, has been developed as a model cellulosic biofuel crop. Genetic improvement is one of the major areas focused to develop the biofuel crop. Construction of a genetic linkage map is fundamental for switchgrass breeding through marker assisted selection and elucidation of genetic mechanisms for economically important traits. Here, a mapping population consisting of 132 individuals was created by randomly selecting selfed progeny of a northern lowland genotype, NL 94 LYE 16X13, from an OSU switchgrass breeding population. Total 1943 SSR-based markers, including 1049 switchgrass genomic simple sequence repeat markers (gSSRs), 666 switchgrass EST-SSRs (eSSRs), 39 selected sorghum SSRs, and 189 foxtail millet gSSRs were screened for polymorphism using the parent and 7 selfed progeny for the linkage map construction. Of 423 polymorphic, easily scoring SSR loci, 254 (60%) had goodness-of-fit of 1:2:1 segregation ratio with χ^2 testing, directly indicating the inheritance mode of the lowland switchgrass was the same as that of a diploid species. The data suggested that switchgrass parent genotype had an allotetraploid genome structure. However, remaining 169 (40%) markers demonstrated distorted segregation, i.e. deviated from the Mendelian ratio. Using JoinMap 4.0, 23 linkage groups of total 269 markers harboring 2 to 76 markers in each group have been constructed. The map total length was 1293.9 cM with the largest linkage group being 205.9 cM long. Because our population was derived from selfing a highly heterozygous genotype, only one linkage map was constructed, instead of the two maps usually constructed for a cross-fertilized population of two highly heterozygous parents. This research should provide an excellent opportunity to facilitate QTL mapping and marker assisted selection.

POSTER 20-A

**SWITCHGRASS ROOT DISTRIBUTION AND YIELD AS MODIFIED
BY ROW SPACING**

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Plant root systems are very crucial for above ground biomass, fluxes of energy and nutrients cycling, anchoring the plant in soil, and to absorb water and nutrients. Root characteristics such as length, average diameter, and surface area are used to determine quantity and functional size, and to predict responses to the environmental changes. Above ground biomass positively correlates with root size. The objective of the study was to determine the root length density (RLD) of switchgrass (*Panicum virgatum* L.) at different row spacings (0.19m, 0.38m, and 0.76m) at different soil depth (0-0.10m, 0.10-0.20m, 0.20-0.40m, 0.40-0.80m, and 0.80-0.110m). Root parameters were measured using an image analysis system (winRHIZO) with grey level image type in resolution of 100 dpi. Both RLD and average root diameter were calculated for different soil depths. RLD value decreased with increase in soil depth. Average diameter of roots at the specific soil depth of 20-80cm is found to be greater rather than at other depths. Smaller RLD values were observed with increasing row spacing probably due to lower plant density in wider spacing. The lowest aboveground biomass was observed at 0.19 m whereas similar aboveground biomass was achieved at 0.38 and 0.76 m. Lower yield at closer spacing may be due to the dense population of plants that limit nutrients and sunlight. The root parameters developed can be used to improve current switchgrass models.

POSTER 21-A

GENOTYPE BY ENVIRONMENT INTERACTIONS AMONG NEW SWITCHGRASS POPULATIONS IN OKLAHOMA

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Department of Plant and Soil Sciences, Oklahoma State University

Evaluation of potential breeding methods in transferring economically viable traits into new genotypes is important to develop high biomass Switchgrass (*Panicum virginatum* L.) cultivars. The objective of this study was to evaluate genotype x environment (G x E) interaction for agronomic and biofuel traits among 19 new switchgrass populations and two check cultivars at five strategically selected locations (Chickasha, Lane, Stillwater, Tipton and Woodward) in Oklahoma. Plant height and stand density were measured during vegetative growth stage, and tiller number and biomass were observed after killing frost. Switchgrass populations were different across the locations in terms of biomass, plant height, tiller number, and stand density. The G x E interaction was evident in biomass and plant height. Dry matter yield and tiller density of Southern Lowland (SL) populations were greater than Northern Upland (NU) and Northern Southern Lowland (NSL). Across the locations, genotype SL93 C2-3 produced more biomass than the high yielding check cultivar 'Alamo'. The highest tiller density was noticed in genotype SL93 C2-4. Plant height was high in NU populations compared to SL and NSL populations. These preliminary results were based on one year study. Multi-year evaluations could provide better understanding of G x E interaction and will be useful to rank switchgrass populations.

POSTER 22-A

IDENTIFYING NOVEL TRANSCRIPTS INVOLVED IN SECONDARY CELL WALL FROM VASCULAR TISSUES OF SWITCHGRASS (*PANICUM VIRGATUM* L. CV ALAMO) USING GENOMICS TOOLS

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Lignin is an integral component of secondary plant cell walls that strongly interferes in the hydrolytic process during conversion of cellulose to fermentable sugars. Thus, various efforts are in progress worldwide to reduce lignin in plants for efficient production of cellulosic ethanol. To understand the complex genetic network that governs secondary cell wall formation, and to uncover novel genes involved in lignification, we used a targeted approach to isolate transcripts of genes from vascular tissues using laser-capture microdissection (LCM). This enabled us to identify vascular tissue (VT) specific candidate genes by comparing transcription profiles between the VT with that of the whole stem (WS). By using PAVE software and combined assembly, we determined that 846 consensus sequences were putatively VT-specific with at least 5 EST reads from the VT and none from the WS. Tissue-specific expression of these genes is being confirmed using real time qRT-PCR and *in situ* hybridization. Genes that show preferential expression in lignified versus non-lignified tissues are being considered as targets to reduce recalcitrance.

POSTER 23-A

***IRG1* IS A NOVEL LEAF EPICUTICULAR WAX MUTANT OF *MEDICAGO TRUNCATULA* THAT CONFERS ENHANCED RESISTANCE AGAINST SWITCHGRASS RUST**

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To identify genes that confer nonhost resistance to biotrophic fungal pathogens, we have conducted a forward-genetic screen using *Medicago truncatula Tnt1* insertion lines and identified an *irg1* (inhibitor of rust germ-tube differentiation₁) mutant that inhibited pre-infection structure differentiation of switchgrass rust pathogen, *Puccinia emaculata* and Asian soybean rust pathogen, *Phakopsora pachyrhizi* and the host pathogen *Colletotrichum trifolii* on the abaxial leaf surfaces. Inhibition of rust pre-infection structure formation in *irg1* mutants is associated with the complete loss of the abaxial epicuticular wax crystals and surface hydrophobicity. *IRG1* encoded a Cys(2)His(2) zinc finger transcription factor (*PALM1*) that also controls dissected leaf morphology in *M. truncatula*. Transcriptome analysis further revealed down-regulation of genes involved in the acyl reduction pathway and upregulation of several lipid transfer proteins in *irg1* mutants, suggesting a regulatory role for *IRG1/PALM1* in wax biosynthesis and secretion. This study provides a novel approach to improve fungal resistance by engineering abaxial leaf surface features without altering the protective adaxial leaf surface and also provides avenue to decipher the mechanism of epicuticular wax loading (asymmetric) on leaf surfaces.

POSTER 24-A

NET ECOSYSTEM EXCHANGES OF BIOENERGY CROPPING ECOSYSTEMS

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Growing concern on energy security, increasing impact of greenhouse gas emissions on climate, and escalating oil prices have led to the use of renewable energy sources in place of conventional fossil fuels. Biomass is the America's largest domestic source of renewable energy and the only current renewable source of liquid transportation fuel. Expansion of bioenergy crop production in a larger scale may have considerable impact on the environment influencing CO₂, H₂O, and energy balances. Net ecosystem exchanges (NEE) of CO₂, H₂O, and heat are being monitored in 30 acre plot of each of the feedstock species [switchgrass (*Panicum virgatum* L.), sorghum (*Sorghum bicolor* (L.) Moench), and mixed-species grasses] at South Central Research Station, Chickasha, OK, using a micrometeorological technique - eddy covariance (EC) method. The NEE and carbon balance of a whole ecosystem across a spectrum of time scales from hours to years will be determined using fluxes measured with EC technique. Seasonal and inter-annual variability in NEE with respect to the major environmental variables and dynamic seasonal plant phenology will be documented. The study will compare ecosystem-level water use efficiency in three different ecosystems. Leaf level flux measurements will be scaled up to the landscape-level and compared with the concurrent measurement of surface-atmosphere CO₂ exchanges using EC technique. Dependency of energy balance closure on the flux footprints, friction velocity, stability/instability conditions, and time of day will be tested. Preliminary result of comparatively much higher amplitude of the net ecosystem CO₂ uptake to the amplitude of CO₂ released indicates the high potential of the bioenergy crops in sequestering atmospheric carbon during active growing season. The study will enhance our understanding of biophysical controls on land surface-atmosphere energy, H₂O, and gas exchanges and will allow us to predict the response of terrestrial ecosystems to global environmental change in the future.

POSTER 25-A

OVEREXPRESSION OF PVMIRNA156B ENHANCES BIOMASS YIELD OF TRANSGENIC SWITCHGRASS

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Switchgrass (*Panicum virgatum* L.) is a productive, perennial warm-season species with a broad cultivation range and minimal requirement of agricultural inputs. It has been predicted that a significant portion of switchgrass will be grown in Oklahoma as a biofuel crop. The development of switchgrass lines with enhanced biomass yield is one of the goals of the EPSCoR biofuel program. Plant miRNA156 is a family of non-coding, small, endogenous RNAs with high expression levels in the juvenile phase of plants. We have constructed a PvmiRNA156b overexpression vector and introduced it into switchgrass. More than 50 transgenic switchgrass plants were produced by *Agrobacterium*-mediated transformation. The transgenics in the greenhouse showed increased tiller numbers. Quantitative RT-PCR analysis of selected transgenics indicates that the transgene was overexpressed in the materials. The level of miRNA156b in the transgenic plants was 2 to 66-fold higher than the wild-type plants. The transgenics that exhibit more than a 14-fold difference in the level of miRNA156 failed to flower. Five transgenics were harvested to evaluate biomass yield and forage quality after 6-month growth in the greenhouse. The dry matter biomass of four transgenics was 1.58-1.73-fold higher than the wild-type plants. The transgenic line with the highest miRNA156b expression level showed less dry matter biomass and stunted plant height. All the lines analyzed showed significant increase in forage digestibility and soluble protein content. Evaluation of other traits, such as lignin content and sugar release efficiency, is in progress.

POSTER 26-A

CARBON CYCLING IN PERENNIAL BIOFUEL MANAGEMENT SYSTEMS

Tracy M. Wilson and Jason G. Warren

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Demand for alternatives to fossil fuels has driven interest in cellulosic biofuel and the desire to mitigate CO₂ emissions has steered researchers towards various methods of sequestering carbon. Perennial grasses hold promise for being both biofuel feedstock and capable of sequestering carbon. In this study, Switchgrass (*Panicum virgatum*), Miscanthus (*Miscanthus* spp.) and Eastern Gammagrass (*Tripsacum dactyloides*) were evaluated with harvest frequency to determine the best management practices to achieve these dual goals of energy production and sustainability in the Southern Great Plains. The harvest frequency treatments include a single harvest at the end of the growing season (Oct-Nov.) and a split harvest treatment harvested midseason (Aug.) and again at the end of the growing season. Residue samples were collected from a 0.35 m² area within each harvested plot and residue samples were evaluated for moisture and total carbon. Soil samples were taken in May 2009 and March 2010 to a depth of 80 cm. Soils were analyzed for organic carbon, bulk density and moisture. This data showed no significant treatment effects on soil carbon concentration or soil carbon stock. A measurable change in soil carbon was not detected during the 1 year between sampling. In 2009, no significant yield differences were found between species, however the single harvest yields were significantly higher than the split harvest yields by 30 %. The 2010 yield data showed no effect of harvest frequency yet the Miscanthus yields were 25% higher than the remaining species. The carbon mass removed in the biomass followed the same trends as biomass yield. The data suggest that carbon cycling and sequestration are influenced by species and harvest frequency but that these effects interact with growing season conditions. Continued monitoring will determine what role species and harvest frequency selection play in the race for sustainable energy production.

POTER 27-A

DEVELOPMENT OF RESOURCES FOR SWITCHGRASS FUNCTIONAL GENOMICS

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Switchgrass (*P. virgatum* L.) is a perennial C4 grass native to North America. It has been used as forage or for soil conservation and has the potential to become a major source of biomass for biofuel production. To realize this potential, breeding and genetic engineering efforts are underway to improve existing germplasm. As the first step toward developing a set of functional genomics resources that are essential for gene discovery, basic biology research, and molecular breeding efforts, large numbers of expressed sequence tags (ESTs) have been generated for AP13 and VS16, two tetraploid switchgrass genotypes selected from lowland cultivar "Alamo" and upland cultivar "Summer" respectively. In addition to over 11.5 million high quality ESTs generated using 454/Roche pyrosequencing technology, three full-length enriched Sanger cDNA libraries were constructed with RNAs from multiple AP13 tissues grown under optimal and stress conditions. About 93,000 clones were sequenced from both ends with the Sanger method and over 169,000 high quality longer reads were produced. To optimize sequence assembly strategies, different programs including the classical CAP3 and CAP3-based assemblers were tested, and a two-stage approach was finally selected to assemble AP13 ESTs into uni-transcripts. First, 454 ESTs were assembled into 102,000 isotig/contigs using the Newbler program with stringent parameters (overlap 100 bp and identity at 99%). PAVE was then used to assemble Sanger reads and the processed 454 isotig/contigs into ~80,000 unique transcript sequences. Separately, the VS16 454 ESTs were assembled into ~34,000 isotig/contigs using Newbler with the same parameters. To create a switchgrass gene index with better representative, a total of 545,000 Sanger ESTs of other genotypes in the public domain were downloaded, grouped, and assembled using the PAVE program. A final 139,200 unigene set (PviUT1.2) was generated from all assembled ESTs with priority order of AP13, Alamo, Kanlow, VS16, and other sequences including about 1502 virtual transcripts predicted from AP13 BAC sequences. An Affymetrix cDNA microarray chip (Pvi_cDNAa520831) based on PviUT1.2 was designed. This chip has an 11µm feature size, with 11 probes for each transcript without mismatch probes. It contains ~122,400 probe sets that represent 104,871 switchgrass unitranscript sequences. The chip is available to the public through Affymetrix Inc. A switchgrass gene expression atlas (PviGEA) is being generated with this platform. The sequence resources will be used for gene annotation, prediction of transcription factor and other gene families of interest, and SNP identification. All switchgrass ESTs generated by this project and the assembled unigene set PviUT1.2 have been deposited to the Switchgrass Genomics database hosted by the Noble Foundation and accessible through this web link: <http://switchgrassgenomics.noble.org> .

Cellulosic Bioenergy: Microbial Conversion

No.	Hall	Presenter Name	University	Poster Title
28	B	Adetola, Tihamiyu	Oklahoma State University	Effect of Nitrogen, Iron and Temperature on Three Microalgae Strains
29	B	Dunford, Nurhan (2)	Oklahoma State University	Ethanol Production from Winter Hulled Barley
30	B	Gao, Jie	Oklahoma State University	Development of Low Cost Medium for Ethanol Production by Clostridium Strain P11
31	B	Liu, Kan	Oklahoma State University	Ethanol Fermentation From CO and H ₂ Using Novel Acetogens "Alkalibaculum Bacchi" Strain CP11, CP13 and CP15
32	B	Phillips, J.R.	Oklahoma State University	Acetogenic Fermentation of Synthesis Gas: A Simple Thermodynamic System
33	B	Prabhakaran, Madhu S.	Oklahoma State University	Degradation of Lignin by Bacterial Enrichment Cultures Metagenomic and Proteomic Identification of Lignocellulose Degrading Genes
34	B	Ramachandriya, K.D.	Oklahoma State University	Production of isopropanol from Acetone by "Clostridium ragsdalei"
35	B	Ray, Anamika	Oklahoma State University	<i>Phanerochaete Chrysosporium</i> Produces a Wide Array of Enzymes on Sorghum Stover
36	B	Remondet, Nicole	Oklahoma State University	Medium Study for Synthesis Gas Fermentation with Clostridium Strain P11
37	B	Terrill, Jennine	Oklahoma State University	Response Surface Optimization of Seven Media Components for Simultaneous Saccharification and Fermentation with K. Marxianus IMB3
38	B	Wilkins, Mark	Oklahoma State University	Operational Strategy for Improved Syngas Fermentation Efficiency
39	B	Zhu, Xiaoguang	Oklahoma State University	Fermentation of Syngas by "Clostridium Ragsdalei" in the Presence of Small Hydrocarbons

POSTER 28-B

EFFECT OF NITROGEN, IRON AND TEMPERATURE ON LIPID AND BIOMASS YIELD OF THREE MICROALGAE STRAINS

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Microalgae have been identified as a potential source of biodiesel production and the economical viability is largely dependent on its biomass, lipid production and storage capability. The class biomolecules of interest are neutral lipids or triacylglycerol (TAGs) which can be processed into biodiesel by transesterification, while the biomass can be used as feedstock in other farm processes.

A laboratory study was conducted to evaluate the sustainability of high lipid and biomass production of microalgae strains *Nannochloropsis sp.*, *Chlorella sp.*, and *Pleurochysis carterae* under conditions that leads to nitrogen (N), iron (Fe) and temperature (T) stress to these organisms. The three strains were cultured in three different media- F2 media, K media and Hogland's media, while inducing low and high extreme environmental and nutrient conditions. The response to the treatments reflects in their biomass and lipid productivity which were compared to the control.

Considering response to nitrogen concentration at 21days cultivation, *Nannochloropsis sp.* and *Chlorella sp.* had highest growth rate when cultured in low N concentration (37.5g/L) in the F2 media, while *Pleurochysis carterae* did not respond well to Nitrogen stress. *Nannochloropsis sp.*, *Chlorella sp.*, *Pleurochysis carterae* maximum growth rate were high at 14 days in Iron concentration treatment (7.2g/L) in K, H and F2 media respectively. *Nannochloropsis sp.*, and *Pleurochysis carterae* response to temperature had its highest growth rate at high temp. 32 °C in K and F2 media respectively while *Chlorella sp* at 25 °C (control).

POSTER 29-B

ETHANOL PRODUCTION FROM WINTER HULLESS BARLEY

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This study examined the potential of two winter hulless barley varieties, Eve and VA125, as feedstock for ethanol production. Chemical composition of the barley samples were analyzed by using official analytical techniques. Both whole grain and flour samples were hydrolyzed by enzymes and heat treatment preceding fermentation experiments. A Simultaneous Saccharification and Fermentation method was used to convert starch to ethanol.

Eve variety had higher starch content than that of VA125. β -Glucan content of VA125 was higher than Eve. The highest ethanol conversion efficiency, 88.6%, was attained with the mash prepared from whole grain Eve. This study demonstrated that winter hulless barley can be a viable feedstock for bio-ethanol production with similar starch content to corn. Dry milling can be used to obtain high starch content flour fractions from barley grain. It is possible to produce ethanol from winter barley varieties with acceptable conversion yields. Optimization of dry milling and ethanol conversion process parameters could improve the economic feasibility of barley to ethanol conversion operations.

POSTER 30-B

DEVELOPMENT OF LOW COST MEDIUM FOR ETHANOL PRODUCTION BY *CLOSTRIDIUM* STRAIN P11

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Oklahoma State University

A low cost medium is designed to produce ethanol and acetic acid through syngas fermentation by *Clostridium* strain P11. Morpholinoethane sulfonic acid (MES), an expensive buffer, was used to control pH in standard medium for strain P11. Initially, the growth and ethanol production by P11 with MES were investigated. Next, MES was eliminated and sodium bicarbonate was added to maintain the fermentation pH above 4.5. The cell concentration was determined by optical density measurement using a spectrophotometer, and gas chromatography was used to measure the composition of H₂, CO, CO₂ and N₂ in the head space and acetic acid and ethanol in the fermentation broth. Growth, pH, gas composition and fermentation products acetate and ethanol were recorded. The results for growth of P11 were similar with or without MES. Subsequent experiments will focus on other expensive nutrients in the standard medium for P11, such as yeast extract, cysteine-sulfide, and ammonium chloride. These will be successively eliminated or reduced in the media. To identify the necessity of individual components, different concentrations of the individual nutrient component will be used to define the required concentration for growth and ethanol production. The cost of the standard medium for P11 is currently \$5.62 per liter. Medium must cost less than \$0.10 per liter to be economical for industrial ethanol production.

POSTER 31-B

**ETHANOL FERMENTATION FROM CO AND H₂ USING NOVEL ACETOGENS
“*ALKALIBACULUM BACCHI*” STRAIN CP11, CP13 AND CP15**

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Ethanol fermentation from CO and H₂ using new strains of “*Alkalibaculum bacchi*” CP11, CP13 and CP15 was studied in 250-mL bottles containing 100 mL of yeast extract medium at 37°C and pH 8.0. Two commercial syngas mixtures (Syngas I: 20% CO, 15% CO₂, 5% H₂, 60% N₂) and (Syngas II: 40% CO, 30% CO₂, 30% H₂) were used. Strains CP11, CP13 and CP15 were fed syngas every 24 h for 360 h. Liquid samples were collected to measure pH, optical density (OD) and product concentrations. Gas samples were collected to measure the concentration of the gases in the head space and to calculate the product yields and conversion efficiencies of syngas components to ethanol. The results showed that all three strains were able to convert CO and H₂ components of the syngas to ethanol and acetic acid. Maximum ethanol concentration (1.7 g/L) and yield (61%) were obtained in fermentations with CP15 and Syngas II for 360 h. CP15 produced over two fold more ethanol in Syngas I, compared to strains CP11 and CP13. However, CP15 produced 18% and 71% more ethanol than strains CP11 and CP13, respectively, with Syngas II. Strain CP13 produced over two fold more acetic acid than strains CP11 and CP15 in both gas mixtures after 360 h of fermentation. These results showed the potential of the new strains of “*Alkalibaculum bacchi*” CP11, CP13 and CP15 to produce ethanol from CO and H₂.

Keywords: Alkaliphilic bacterium, Fermentation, Ethanol, Yeast extract, Syngas

POSTER 32-B

ACETOGENIC FERMENTATION OF SYNTHESIS GAS: A SIMPLE THERMODYNAMIC SYSTEM

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Acetogenic fermentation of synthesis gas to acetic acid and ethanol follows a simple system of elementary reactions that comprise the Wood-Ljungdahl pathway. The pathway reactions occur in sequence (series and parallel series) and are associated with and mediated by the cell membrane. These elementary reactions are primarily electrochemical oxidation/reduction reactions that reflect the electrochemical potential within the cell. The electrochemical potential inside the cell is set by (or sets) the equilibrium position of the half cell reactions involved. Oxidation/reduction half cells involved include $\text{NAD}^+:\text{NADH}$, ferredoxin $\text{Fd}_{\text{ox}}:\text{Fd}_{\text{red}}$, $\text{CO}_2:\text{CO}$ and $\text{H}^+:\text{H}_2$. Electrons derived from the reduced substrates, CO and H_2 , supply energy for growth and production. Product formation yields no ATP from substrate level phosphorylation, and growth is driven by a membrane potential via an ATP synthase. The free energy change for each progressing elementary reaction is negative, and the thermodynamics of the active fermentation depart significantly from the free energy change and electrochemical potential at standard conditions, ΔG° and E_m° . The thermodynamics can be estimated by analysis of substrate mass transfer and the electrochemical potential defined by the concentration of dissolved H_2 within the cell.

Seven electrochemical half cell reactions prominent in the production pathway are incorporated in a simple spreadsheet model of fermentation. The model input of intracellular electrochemical potential predicts thermodynamic equilibrium positions of these half cell reactions as an approximate prediction of the fermentation inputs and outputs. Quantities calculated include the dissolved H_2 pressure, the ratio of CO to CO_2 as dissolved pressure inside the cell, and the ratio of ethanol to acetic acid that can be achieved at a given pH.

POSTER 33-B

DEGRADATION OF LIGNIN BY BACTERIAL ENRICHMENT CULTURES: METAGENOMIC AND AND PROTEOMIC IDENTIFICATION OF LIGNOCELLULOSE DEGRADING GENES

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Biomass represents one of the world's most abundant renewable energy sources. However, its utilization for biofuels and other commodity chemicals requires the use of expensive and harsh pretreatments methods to breakdown lignin, a recalcitrant plant cell wall polymer that hinders the digestion of plant polysaccharides to simple sugars. The goal of this project was to develop bacterial consortia that degrade lignin and identify genes that encode enzymes needed for lignin degradation/depolymerization. We have enriched several lignin-utilizing bacterial cultures from decaying wood, rumen fluid, and termite gut. Microcosms were setup to test their ability to degrade lignin in plant residues such as alfalfa and switchgrass. The cultures developed from decaying wood obtained from a thermal pond at Yellowstone National Park and cow creek in Stillwater degraded 80-90 % of the lignin in alfalfa in 80 days. However, it took > 6 months to degrade 10-15 % lignin in switchgrass. To characterize the phylogenetic and functional profile of the enrichments, metagenomic sequencing was done for one of the enrichment cultures. Initial analysis of the metagenome revealed that Proteobacteria and Firmicutes were the dominant members of the community and majority of these organisms have been shown to degrade a variety of aromatic compounds similar to lignin structures. Using bioinformatic tools, we identified several enzymes pertinent to lignin degradation in the metagenome. Also, we also isolated several pure cultures from the enrichments that have the ability to grow on lignin and lignin model compounds as the sole carbon source. Our data clearly establishes that bacteria have the ability to degrade lignin and thus represents a novel strategy for the bioconversion of agricultural residues.

POSTER 34-B

**PRODUCTION OF ISOPROPANOL FROM ACETONE BY
“*CLOSTRIDIUM RAGSDALEI*”**

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Acetone scrubbers are often used to remove condensable hydrocarbons from syngas. Acetone can be volatilized in the scrubbers and contaminate syngas. If the syngas is fermented by bacteria to produce biofuels, the acetone present may affect the product profile of the fermentation. This study investigated the effect of acetone on the product profiles and growth of two bacteria that are known to consume syngas and produce ethanol, *Clostridium carboxidivorans* and “*Clostridium ragsdalei*.” Acetone was added to media for these bacteria at a concentration of 2 g/L. In some flasks acetone was added at the beginning of the experiment, in some bottles acetone was added while the bacteria were producing ethanol, and in some bottles no acetone was added to serve as a control. *C. carboxidivorans* does not utilize acetone and from an analysis of its genome, it does not have enzymes needed for acetone production or utilization. In contrast “*C.ragsdalei*” consumes acetone and produces isopropanol, which is the result of a reduction of acetone by NADH. No isopropanol is produced by “*C.ragsdalei*” when acetone is not present in the media.

POSTER 35-B

***PHANEROCHAETE CHRYSOSPORIUM* PRODUCES A WIDE ARRAY OF ENZYMES ON SORGHUM STOVER**

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Phanerochaete chrysosporium, a white rot fungus is a model organism which has been shown to be efficient in the degradation of lignocelluloses. The goal of this project is to study the growth of this fungus on sorghum under solid-state cultivation, and to identify the enzymes necessary to degrade sorghum for efficient conversion of sugars to bioethanol. We monitored the growth of the fungus for 1D, 2D, 3D, 5D, 7D and 14D on sorghum by estimation of chitin, a key component in the fungal cell wall. The chitin content increased steadily with days of growth till 5th day followed by decrease in growth on the 7th day and then showing a plateauing effect till 14th day of growth. After establishing growth of the fungus on sorghum, the next logical step was to identify the enzymes required for successful utilization of lignocellulosic biomass. For this, we decided to study the secretome of this fungus by using the powerful technique of liquid chromatography-tandem mass spectrometry (LC-MS/MS). 68, 127 and 110 proteins were identified in extracellular extracts from 1st, 7th and 14th day of fungal growth on sorghum. The most abundant proteins identified belonged to the cellulase and hemicellulases families along with enzymes belonging to peroxidases family. The repertoire of proteins produced by this fungus and identified by LC-MS/MS should help in the efficient conversion of cellulose and hemicelluloses to fermentable sugars.

POSTER 36-B

**MEDIUM STUDY FOR SYNTHESIS GAS FERMENTATION WITH
CLOSTRIDIUM STRAIN P11**

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A fermentation medium was designed for the production of ethanol and acetic acid from syngas using *Clostridium* strain P11. The designed medium was developed by successive reduction or complete removal of several components from the standard medium used for isolation and growth of strain P11. Cost and function of individual components in the designed medium guided the revision of the medium recipe. This process resulted in the elimination of morpholinoethanesulfonic acid (MES), a buffer used to maintain the pH near 6.0. Instead, a buffer was formed from the acetic acid produced during the fermentation by addition of bicarbonate, keeping the pH around 4.75 to enhance ethanol production. The performance of fermentation without MES and with pH control using acetate buffer was similar to that with standard medium. Yeast extract, an undefined growth promoter, was also eliminated. Fermentation without yeast extract did not affect the initial substrate uptake and production rates. However, elimination of yeast extract resulted in lower growth compared to standard medium. The cost of the designed fermentation medium was reduced by 95% compared to standard medium, which will increase the cost effectiveness of syngas fermentation technology.

POSTER 37-B

**RESPONSE SURFACE OPTIMIZATION OF SEVEN MEDIA COMPONENTS FOR
SIMULTANEOUS SACCHARIFICATION AND FERMENTATION WITH *K.*
MARXIANUS IMB3**

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Thermotolerant yeast, such as *K. marxianus* IMB3, have the ability to overcome some of the failings of conventional yeast strains in simultaneous saccharification and fermentation (SSF) reactions due to their ability to ferment sugars to ethanol at temperatures closer to those optimal for enzymatic hydrolysis. Improving the feasibility of this process makes it necessary to optimize the constituents in the supplemented fermentation media. Defining and optimizing these constituents for ethanol production can increase the rate of fermentation as well as the ethanol yield by reducing undesired by-products of fermentation. Important components of the media used in previous experiments with IMB3 were determined through initial screening experiments using an industrial characterization of yeast extract as the basis for the defined media composition. Eight parameters; nicotinic acid, calcium pantothenate, thiamine, ammonia sulfate, calcium chloride, magnesium sulfate, zinc sulfate and potassium phosphate, were all determined to be important to the media through initial screening experiments utilizing glucose. Zinc sulfate was determined to be necessary, but at a very low level, so only the other seven components were optimized. Optimization was done using SAS version 9.2 design of experiments interface. The optimized media for a glucose media was then employed in an SSF (with and without pH adjustment) and compared to the original fermentation media.

POSTER 38-B

**OPERATIONAL STRATEGY FOR IMPROVED SYNGAS
FERMENTATION EFFICIENCY**

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The effect of three limiting nutrients, calcium pantothenate, vitamin B₁₂ and cobalt chloride (CoCl₂), on syngas fermentation using "*Clostridium ragsdalei*" was determined using serum bottle fermentation and fermenter studies. Significant results from the bottle studies were translated into single- and two-stage continuous fermentor designs. Studies indicated that three-way interactions between the three limiting nutrients, and two-way interactions between vitamin B₁₂ and CoCl₂ had a significant positive effect on ethanol and acetic acid formation. In general, ethanol and acetic acid production ceased at the end of 9 d corresponding to the production of 2.01 and 1.95 gL⁻¹ for the above interactions. Reactor studies indicated the three-way nutrient limitation in a two-stage fermentor showed improved acetic acid (17.51 gg⁻¹ cells) and ethanol (14.74 gg⁻¹ cells) yield compared to treatments in single-stage fermentors. These results further support the hypothesis that it is possible to modulate the product formation by limiting key nutrients during "*C. ragsdalei*" syngas fermentation.

POSTER 39-B

**FERMENTATION OF SYNGAS BY “CLOSTRIDIUM RAGSDALEI” IN THE
PRESENCE OF SMALL HYDROCARBONS**

**Xiaoguang Zhu, Mark R. Wilkins, Karthikeyan D. Ramachandriya, Hasan K.
Atiyeh, Raymond L. Huhnke**

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Clostridium ragsdalei is a bacterium that converts CO and H₂ into ethanol. Syngas produced from biomass gasification provides CO and H₂ for P11 fermentation. The main components of syngas are CO, CO₂, N₂, and H₂; however, few studies focus on effects of hydrocarbons in the syngas. The purpose of this study was to estimate the effect of small hydrocarbons in syngas on P11 fermentation. Methane, ethane, ethylene and acetylene were studied. Spectrophotometer was used for measuring optical density at 660 nm. An Agilent 6890 N GC was used for gas analysis. Methane and ethane had no effect on cell growth or product formation. Acetylene inhibited ethanol, acetic acid and cell production. Ethylene had a positive effect on ethanol production at a concentration of 0.5% in the syngas, but ethanol production was the same for syngas with 1% ethylene and syngas with no ethylene.

Cellulosic Bioenergy: Chemical Conversion

No.	Hall	Presenter Name	University	Poster Title
40	C	Boonyasuwat, Sunya	University of Oklahoma	The Role of Acidic Catalyst Support on the Catalytic Upgrading of Bio-oils
41	C	Elam, Kyle	University of Oklahoma	Deoxygenation of Lauric Oils to Produce Fungible Transportation Fuels on Supported Platinum Catalysts
42	C	Grisanti, Maria	University of Oklahoma	Measurement of Fundamental Properties of Biofuels and Their Blends with Petroleum-based Fuels
43	C	Marin, Luz	Oklahoma State University	Use of Char for Reduction of Tar in Synthesis Gas
44	C	Rownaghi, Ali	Oklahoma State University	Selective Dehydration of Methanol-to-Hydrocarbons
45	C	Sitthisa, Surapas	University of Oklahoma	Conversion of Furfural Over Ni-Fe Catalyst
46	C	To, Anh T.	University of Oklahoma	Pulse Reaction Technique for Studying the Transformation of Tetralin, Anisole and Their Mixture Over HY and HZSM-5 Under Catalytic Cracking Condition

POSTER 40-C

THE ROLE OF ACIDIC CATALYST SUPPORT ON THE CATALYTIC UPGRADING OF BIO-OILS

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Center for Biomass and Refining
School of Chemical, Biological and Materials Engineering
University of Oklahoma

Conversions of anisole and guaiacol, phenolic model compounds commonly existing in bio-oils, were studied over an acidic silica-alumina catalyst at varying space times (W/F) and time-on-streams. The reactions were carried out in gaseous phase fixed bed reactor under hydrogen at atmospheric pressure and 300 °C. It is found that transalkylation is the predominating pathway, while none of deoxygenation activity was detected under the investigated reaction conditions. Deactivation of the catalysts is rapid and more severe in the case of guaiacol feed. As a result, conversion of anisole is generally higher as compared to that of guaiacol. It is suggested that the strong adsorption of the phenolic compounds is responsible for the catalyst deactivation. In this contribution, Density Functional Theory (DFT) calculation was performed to simulate the adsorption of the phenolic compounds on acid sites. Based on this present calculation, it exhibited the adsorption energy of guaiacol is significantly higher than that of anisole.

POSTER 41-C

DEOXYGENATION OF LAURIC OILS TO PRODUCE FUNGIBLE TRANSPORTATION FUELS ON SUPPORTED PLATINUM CATALYSTS

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University of Oklahoma

The removal of oxygen from vegetable oils via catalytic deoxygenation exhibits high potential for the production of compounds present in modern transportation fuels. This work focuses on the conversion of model oxygenate compounds and natural lauric oils over supported Pt catalysts with and without the addition of hydrogen. Supported Pt catalysts were used in a 300 mL Parr reactor at 200-300 psi and 593 K, in both batch and semi-batch modes. Catalytic studies have shown variations in triglyceride conversion rate, selectivity to hydrocarbons, and selectivity to undesired condensation products, depending on the presence of metal, catalyst amount, and reaction atmosphere. The conversion of different triglycerides initially displays similar kinetics and overall product distributions. Primary products include: hydrocarbons, fatty acids, and diglycerides with ketones and esters identified as intermediate products. Mixtures of triglycerides reveal that transesterification reactions also occur under the given conditions. Gas phase analysis reveals the production of hydrogen and shows both CO and CO₂ production. The final products in the absence of added hydrogen are primarily C-11 alkanes and alkenes.

POSTER 42-C

MEASUREMENT OF FUNDAMENTAL PROPERTIES OF BIOFUELS AND THEIR BLENDS WITH PETROLEUM-BASED FUELS

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Biofuels are relatively recent fuels, produced from biological materials such as vegetable matter or animal fats. Biofuels are produced from local resources; they are renewable and environmentally-friendly and have energy content similar to that of petroleum-based fuels. It is a common practice to mix petroleum-based fuels and biofuels and use the blends. But there is a lack of information regarding the fundamental properties of biofuel blends. These values need to be known to understand the combustion process of the flame. The objective of this study was to measure and compare the fundamental properties of a fuel such as the density, fire point, flash point, viscosity and refractive index of biofuels (Soy-Methyl Ester SME, and Canola Methyl Ester CME) and their blends with petroleum based fuels (Jet A and Diesel No. 2). The blends were prepared with different concentrations, and were designated as B25, B50 and B75 where the number represents the volumetric percent of biofuel in the blend. The biofuels SME and CME were denser and more viscous than diesel and jet A. Biofuels are also safer to handle than petroleum based fuel, because their flash and fire point temperatures were considerably higher than those of diesel and jet A. The properties such as density, fire point, flash point, refractive index and viscosity of the fuel blends (B25, B50 and B75) were between the corresponding values for the pure fuels. The values for the density, fire point, flash point, fire point and viscosity increase with a non-linear trend as the volumetric concentration of biofuel in the blend increases.

POSTER 43-C

USE OF CHAR FOR REDUCTION OF TAR IN SYNTHESIS GAS

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The destruction of tars is a crucial technological barrier for the development and further commercialization of biomass gasification. It is known that the decomposition of tar compounds using catalysts is a suitable solution to this problem. Biochar, which is generated during gasification, is also a potential catalyst for tar destruction. Biochar along with three commercial steam reforming catalysts were evaluated for tar removal using toluene as a model tar compound. Two of the commercial catalysts were nickel based (Reformax 250 and Hifuel R-110) and one was platinum based (Nextech-A). The biochar was generated during switchgrass gasification in a down draft gasifier at the OSU thermochemical conversion facility. Tar destruction experiments were performed in a bench scale fixed bed reactor between 600 and 800°C under atmospheric pressure using a synthetic gas mixture with similar composition to the syngas generated from switchgrass gasification. Toluene conversion results showed that the biochar performance was comparable to the commercial catalysts and Reformax was the most active of the tested catalysts. The activation energy of toluene steam reforming over these catalytic materials was found to be 50.26 KJ/mol for Reformax 250; 51.18 for Hifuel R-110 KJ/mol; 59.44 KJ/mol for Nextech-A and 61.59 KJ/mol for biochar. Using the same experimental set up, a system of two catalysts in series was tested, using char along with a commercial catalyst. Results showed that at 700°C the mixture of char and Nextech-A performed better than the individual compounds. Catalyst characterization by SEM, XPS, TGA, and FTIR was also conducted on the used catalysts and according to the XPS spectra graphitic carbon was found on all catalysts.

POSTER 44-C

SELECTIVE DEHYDRATION OF METHANOL-TO-HYDROCARBONS

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The conversion of methanol on ordered mesoporous single nanocrystals and conventional crystallite ZSM-5 at 370 °C and atmospheric pressure has been studied. Here I studied, for the first time, the synthesis of ordered mesoporous ZSM-5 single nanocrystals without use of the secondary various carbon templates or other treatments. Compared with conventional ZSM-5, the mesoporous ZSM-5 single nanocrystals exhibited significantly higher external surface area and larger mesopore volume. Methanol conversion process was carried out over the catalysts to evaluate the catalytic resistance to coke poisoning. Improved catalyst stability was observed on mesoporous ZSM-5 single nanocrystals due to faster removal of products with the shorter diffusion path length, reducing the formation of coke precursors. Good correlation was observed between catalyst lifetime and mesoporosity. The new mesoporous ZSM-5 containing large pores enhanced selectivity towards alkyl aromatics. It is concluded that the use of new mesoporous ZSM-5 single nanocrystals improves the production of alkyl aromatics from methanol at mild conditions that may prove useful for upgrading of biomass thermochemical intermediates to hydrocarbons. This research was initiated at Sweden and I intend to continue this area of studies at Oklahoma State University.

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POSTER 45-C

CONVERSION OF FURFURAL OVER NI-FE BIMETALLIC CATALYSTS

Surapas Sitthisa, Wei An and Daniel E. Resasco

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Furfural is commonly found in bio-oil, and this remarkably reduces fuel properties and stability during storage. Hydrodeoxygenation 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, hydrogenation and hydrogenolysis of the carbonyl group. In the present work, the silica supported Ni-Fe bimetallics were used for hydrodeoxygenation of fufural. The reactions were performed at the temperature between 210-230°C under atmospheric pressure of hydrogen. In the case of Ni/SiO₂ catalyst, the main products were furan and butane which is derived from decarbonylation and ring opening reaction, respectively. 2-Methyl furan was the minor product on Ni catalyst. However, the addition of Fe to Ni catalyst forming Ni-Fe alloy catalysts dramatically increased the yield of 2-methyl furan while reducing the yield of furan. It is clearly seen that the addition of Fe into Ni catalyst can suppress the decarbonylation activity while enhancing the hydrogenolysis activity.

POSTER 46-C

PULSE REACTION TECHNIQUE FOR STUDYING THE TRANSFORMATION OF TETRALIN, ANISOLE AND THEIR MIXTURE OVER HY AND HZSM-5 UNDER CATALYTIC CRACKING CONDITION

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Pulse reaction technique was used to study the cracking reaction of tetralin over commercial HY and HZSM-5 zeolites at 450°C. Strong effect of shape selectivity to the cracking reaction was observed as tetralin conversion over HZSM-5 was much lower than that over HY, even though the HZSM-5 has more acid density than the HY. Cracking reaction of pure anisole at the same condition over these two zeolites confirmed the stronger activity of HZSM-5 than HY, as complete anisole conversion and more severe coking and adsorption of the products (etc. phenol, cresols and xylenols) were always observed in all pulses over HZSM-5, but not HY. Anisole was also co-fed with tetralin at the same condition to study the effect of co-processing phenolic compound derived from bio-oil with the Fluid Catalytic Cracking (FCC) feedstock. Deactivation of tetralin cracking was observed in experiment with HZSM-5 when anisole was co-fed because of severe coking and adsorption of anisole cracking products. However, in HY zeolite, strong hydrogen donor as tetralin promotes desorption of phenol, which is a deactivating compound. Hence tetralin cracking over HY did not show strong deactivation as in HZSM-5. Experiments with subsequent pulses of anisole and tetralin were also carried out to elucidate the transalkylation reaction pathway between anisole and tetralin. Over HZSM-5, dissociative pathway occurs dominantly, while over HY, both dissociative and non-dissociative bimolecular pathways occur.

Other Research

No.	Hall	Presenter Name	University	Poster Title
47	C	Devarapalli, Mamatha	Oklahoma State University	Analysis of Trickle Bed Reactor Mass Transfer Characteristics
48	C	Fan, Heng	University of Oklahoma	Nanoparticles Simulated at the Water-Decane Interface
49	C	Hickey, David	University of Oklahoma	New Biofuel Cell Anode Materials Based on Linear Poly(ethylenimine) and Ferrocene
50	C	Love, Kayla	Langston University	The Use of Special Peanut Hair, Emergence, as Explant for Shoot Organogenesis
51	C	Sharma, Bhavna	Oklahoma State University	Scenario Optimization Approach for Supply Chain and Logistics Management of Switchgrass to Biorefinery
52	C	Singh, Vinay	University of Oklahoma	Effects of Equivalence Ratio on Temperature and Soot Volume Fraction in Laminar Partially Premixed Flames of CME/Diesel and SME/Diesel Blends

POSTER 47-C

ANALYSIS OF TRICKLE BED REACTOR MASS TRANSFER CHARACTERISTICS

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and Raymond L. Huhnke¹**

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Syngas fermentation involves complex biochemical reactions of gaseous substrates to produce liquid products such as ethanol and acetic acid using special group of acetogenic microorganisms. The solubility of the gaseous substrates (CO, H₂ and CO₂), which are provided as the main source for growth of acetogens, is very low in the fermentation medium. The productivity of syngas fermentation is often severely limited due to low mass transfer of CO and H₂ into the liquid medium. The aim of the current study is to design a lab-scale trickle bed reactor (TBR) and analyze its mass transfer capabilities. The effect of temperature, size of packing materials, liquid and gas flow rates on mass transfer characteristics of the TBR will be examined. A counter-current flow of liquid and gas configuration will be used. Understanding of mass transfer characteristics of the TBR and optimizing the conditions to maximize mass transfer is expected to help improve the conversion efficiency of CO and H₂ and increase ethanol productivity of syngas fermentation.

POSTER 48-C

NANOPARTICLES SIMULATED AT THE WATER-DECANE INTERFACE

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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 pyrolysis 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.

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 interfacial tension, contact angle, desorption energy, density profile and dynamic properties including rotational relaxation. The results are quantified based on the chemical features of the nanoparticle surface. Methodology and some preliminary results of coarse grain simulation are also presented.

POSTER 49-C

NEW BIOFUEL CELL ANODE MATERIALS BASED ON LINEAR POLY(ETHYLENIMINE) AND FERROCENE

**David Hickey(1), Matthew Meredith (2), Daniel T. Glatzhofer (3),
David Schmidtke (4)**

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University of Oklahoma Bioengineering Center, and School of Chemical, Biological, and
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Linear poly(ethylenimine) ($-\text{[CH}_2\text{CH}_2\text{NH]}_n-$, LPEI) was modified by attachment of 3-(dimethylferrocenoyl)propyl groups to ca. 17% of its nitrogen atoms (FcMe₂-C₃-LPEI) to form a new redox polymer for use as an anodic mediator in glucose/O₂ biofuel cells. Electrochemical properties of this polymer were compared to those of 3-ferrocenylpropyl-modified LPEI (Fc-C₃-LPEI). When Fc-C₃-LPEI or FcMe₂-C₃-LPEI was mixed with glucose oxidase (GOx) and cross-linked with ethylene glycol diglycidyl ether (EGDGE) to form hydrogels on planar, glassy carbon electrodes, limiting catalytic bioanodic current densities of up to $\sim 2 \text{ mA/cm}^2$ at 37° C were produced. The use of dimethylferrocene moieties in place of ferrocene moieties lowered the $E_{1/2}$ of the films by 0.09 V and significantly increased electrochemical and operational stabilities. FcMe₂-C₃-LPEI was shown to be the more effective polymer for use in biofuel cells and, when coupled with a stationary O₂ cathode comprised of laccase and cross-linked poly[(vinylpyridine)Os(bipyridyl)₂Cl^{2+/3+}] (PVP-Os) as a mediator, produced power densities of up to $56 \text{ } \mu\text{W/cm}^2$ at 37° C. Power density increased to $146 \text{ } \mu\text{W/cm}^2$ when a rotating biocathode was used. The stability of biofuel cells constructed with FcMe₂-C₃-LPEI was higher than that of cells using Fc-C₃-LPEI. The results of further modification to the bioanode materials will also be discussed.

POSTER 50-C

**THE USE OF SPECIAL PEANUT HAIR, EMERGENCE, AS EXPLANT FOR
SHOOT ORGANOGENESIS**

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The main purpose of this experiment was to determine whether special peanut hair, emergence, could be used as an “*explant*” and cultured *in vitro* autonomously for inducing efficient shoot organogenesis comparable to standard plant organs such as leaf, stem, root, or cotyledon. The results showed unequivocally that indeed special peanut hair is potentially a valuable explant that could be used as effectively as, if not better, than any other standard plant organs for shoot organogenesis. The studies showed that efficient shoot formation was observed in single units as well as in clusters of two to four hair units. In all cases new shoots also induced repetitive multiple shoot, similar to those observed standard plant organs. These observations could be beneficial for improving peanut protocols for genetic engineering.

POSTER 51-C

SCENARIO OPTIMIZATION APPROACH FOR SUPPLY CHAIN AND LOGISTICS MANAGEMENT OF SWITCHGRASS TO BIOREFINERY

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Increasing demand and dependence of US on foreign oils has focused the attention of researchers in exploring alternative energy sources. Biofuel is recognized as the future renewable energy source. The supply chain of biomass is combination of several distinct processes such as harvesting, baling, pre-processing, storage, transportation, and transshipment. Efficient logistics and supply chain management of a particular biomass feedstock is crucial. A scenario optimization model is developed to ensure cost effective and in-time delivery of switchgrass to the biorefinery. The field operations such as harvesting, baling etc are weather dependent. Weather is the major factor for randomness and uncertainty in field operations. Scenario optimization technique deals with uncertainty along with providing a single reasonable solution which performs well under all scenarios. A deterministic model with 12 year different weather scenarios is developed. The case study for Abengoa Bioenergy Biomass of Kansas (ABBK) is developed.

POSTER 52-C

EFFECTS OF EQUIVALENCE RATIO ON TEMPERATURE AND SOOT VOLUME FRACTION IN LAMINAR PARTIALLY PREMIXED FLAMES OF CME/DIESEL AND SME/DIESEL BLENDS

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In order to provide energy independence and control pollutant emissions during combustion, alternative fuels are being developed. Biofuels, such as canola methyl ester (CME) & soy methyl ester (SME), have considerable potential for use as fuels in internal combustion engines. In the near-term, blends of biofuels with petroleum fuels can be used with no modifications to current engines. Therefore, knowledge of the combustion and pollutant emission characteristics is important in the application of biofuels and their blends. In order to understand the effects of equivalence ratio on the combustion properties of petroleum-biofuel blends, partially-premixed laminar flames of prevaporized blends were investigated. A laminar flame environment was chosen to simplify the fluid mechanics. The primary objective of this study was to compare the combustion characteristics of blends of CME/diesel and SME/diesel at equivalence ratios (Φ) of 1.2, 2, 3 and 7. The equivalence ratios were chosen to simulate the partial premixed to non-premixed flame combustion zones that exist in the far-injector regions in diesel engines. The documented combustion characteristics included the flame temperature and soot volume fraction.

The liquid fuel was injected using a syringe pump into a heated air stream (temperature of 425°C to pre-vaporize the fuel before burning) and sent to a 9.5 mm inner diameter tube burner. The volumetric flow rate of the air was measured using a calibrated rotameter. The feed line temperature was monitored using K-Type thermocouples. The resulting flame was laminar whose characteristics were dependent on the chemistry of the fuel alone. Three blends of CME with petroleum-based diesel & three blends of SME with petroleum-based diesel were used with 25, 50 and 75% volume concentration of the biofuels respectively. The radial flame temperature profiles were documented with a platinum – platinum 13% rhodium (R-type) thermocouple at three distances above the burner exit. The soot volume fraction was measured by beam attenuation through the flame with a Helium-Neon laser ($\lambda = 632.8$ nm). Peak temperatures occurred in the near-burner region (0.25 flame height) and reached a maximum at $\Phi = 1.2$ and became lower as the equivalence ratios were increased. The maximum soot volume fraction was also measured in the near-burner region, and the peak soot volume fraction increased with equivalence ratio for all fuels.

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