# Cellulosic Bioenergy Research Poster Session

## Tuesday, April 10, 2012
## Exhibit Hall West * Wes Watkins Conference Center
## Oklahoma State University

**Student Hybrid Poster Competition * Posters 1-12**

**Feedstock Development * Posters 13-23**

**Microbial Conversion * Posters 24-33**

**Chemical Conversion * Posters 34-36**

**Other/Misc. * Posters 37-44**

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

# STUDENT PRESENTERS

## Hybrid Poster Competition

| No. | Student Presenter | University | Scientific Poster Title |
|---|---|---|---|
| 1 | Matthew Brian Couger | Oklahoma State University | Genomic Analysis of the LignoCellulosic Anaerobic Fungus Orpinomyces C1A |
| 2 | Laxman Adhikari | Oklahoma State University | Development of Lowland Inbreds and Upland-Lowland Hybrids in Switchgrass |
| 3 | Fan Lin | University of Oklahoma | Genetics of a Grass Mutant with Decreased Cell Wall Cross-Linking |
| 4 | Gabriela Orquera | Oklahoma State University | Multilocus "DNA Barcodes" for Identification of Switchgrass Rust Populations |
| 5 | David Ponder | University of Oklahoma | Investigating the Cell Wall Changes During Grass Lateral Root Emergence |
| 6 | Xin Zeng | Oklahoma State University | Gene Networks Associated with Tillering Traits in Switchgrass |
| 7 | Kangmei Zhao | University of Oklahoma | Indentification of the Regulation Genes in the Phynelpropanoid Biosynthesis Pathway by Network Analysis of the Model Grass, Rice |
| 8 | Pushpak Bhandari | Oklahoma State University | Biochar and Biochar-derived Activated Carbon as Catalysts for Syngas Tar Removal |
| 9 | Prakash Bhoi | Oklahoma State University | Solubility of Major Producer Gas Tar Compounds in the Water |
| 10 | Taiwo Omotoso | University of Oklahoma | Influence of Ruthenium Titania Catalyst Pretreatment Conditions on the Upgrading of Biomass Fast-Pyrolysis Oil Vapors |
| 11 | Ashokkumar M. Sharma | Oklahoma State University | Effects of Steam Injection Location on Syngas Generated from Fluidized-bed Gasification of Switchgrass |
| 12 | Paula Zapata | University of Oklahoma | Hydrophobic Zeolites for Biofuel Updgrading Reactions at the Liquid-Liquid Interface in Water/Oil Emulsions |

# Cellulosic Biofuels Research

| No. | Primary Presenter | University | Scientific Poster Title |
|---|---|---|---|
| 13 | Anserd J. Foster | Oklahoma State University | Predicting Biomass Yield in Bioenergy Crop Production Systems Using Canopy NDVI |
| 14 | Vijaya Gopal Kakani | Oklahoma State University | Evaluating Energy Beet Potential as Bioenergy Feedstock in Southern Great Plains |
| 15 | Hao Lin | Oklahoma State University | *Stenofolia*, a Potential Biomass Gene, Regulates Lamina Outgrowth by Controlling Cell Proliferation |
| 16 | Linglong Liu | Oklahoma State University | A Useful Molecular Tool to Identify Selfed Progeny in Switchgrass |
| 17 | Lifang Niu | Oklahoma State University | Controlling Flowering Time in Switchgrass and Sorghum for Biomass Yield Improvement |
| 18 | Prasenjit Saha | University of Oklahoma | Understanding Transcriptional Regulation of Ferulic Acid (FA) Incorporation in Grasses Cell Wall |
| 19 | Ramanjulu Sunkar | Oklahoma State University | Identification of Conserved, Novel and Stress-Responsive MicroRNAs in Switchgrass Using High-Throughput Sequencing of Small RNA Libraries |
| 20 | Srinivasa Rao Uppalapati | Samuel Roberts Noble Foundation | Non-host Resistance of *Brachypodium Distachyon* to Switchgrass Rust Pathogen, *Puccinia Emaculata* |
| 21 | Srinivasa Rao Uppalapati | Samuel Roberts Noble Foundation | Characterization of Switchgrass Rust Fungus and Evaluation of Genetic Variability in Rust Resistance of Switchgrass Populations |
| 22 | Srinivasa Rao Uppalapati | Samuel Roberts Noble Foundation | Loss of Abaxial Leaf Epicuticular Wax in *Medicago Truncatula IRG1/Palm1* Mutants Results in Reduced Spore Differentiation of Non-host Rust Pathogens |
| 23 | Srinivasa Rao Uppalapati | Samuel Roberts Noble Foundation | Targeted Lignin Modification Induces Tolerance to Soil-Borne Fungal Pathogens in Alfalfa |

## CHEMICAL CONVERSION

### Cellulosic Biofuels Research

| No. | Primary Presenter | University | Scientific Poster Title |
|---|---|---|---|
| 34 | Garry Chapman, Jr. | University of Oklahoma | Vanadium (V)-Catalyzed Deoxydehydration of Glycols |
| 35 | Zhimin Liu | University of Oklahoma | Suitability of MgO as Catalyst for Aldol Condensation of Bio-Oil Compounds |
| 36 | Michael Mueller | Oklahoma State University | Analysis of Single Enzyme Activities on the Hydrolysis of Grain Sorghum Stover |

## OTHER RESEARCH

| | | | |
|---|---|---|---|
| 37 | Amit Khanchi | Oklahoma State University | Emipirical Model to Predict Infield Thin Layer Drying Rate of Cut Switchgrass |
| 38 | Elizabeth Miller | OKlahoma State University | Sustainable Feedstock Production Supply Systems to Support Cellulosic Biorefinery Industries: Logistics Update |
| 39 | Arjun Pandey | Oklahoma State University | Evaluation of Switchgrass Root Characteristics Influence by Row Spacing and Cultivar Differences |
| 40 | Vince Schielack III | Oklahoma State University | Cost Effectively Measuring the Moisture Content of Grass Bales |
| 41 | Bhavna Sharma | Oklahoma State University | Scenario Optimization Model for Biomass Supply Chain Design and Analysis |
| 42 | Pradeep Wagle | Oklahoma State University | Seasonal Variability in Evapotranspiration, Water Use Efficiency and Energy Partitioning in Switchgrass |
| 43 | Yan Zhu | Oklahoma State University | Harvesting Microalgal Biomass by Flocculation |
| 44 | Dani Bellmer | Oklahoma State University | Utilization of Soft Drink Waste for Production of Ethanol |

# POSTER 1

# GENOMIC ANALYSIS OF THE LIGNOCELLULOTIC ANAEROBIC FUNGUS ORPINOMYCES C1A


## PRESENTER: MATTHEW BRIAN COUGER

**MB Couger, Audra S. Liggenstoffer, and Mostafa S. Elshahed[1]**

**[1] Oklahoma State University, Stillwater, OK**



Direct conversion of complex non-feedstock plants into substrates suitable for ethanol production currently is a major bottleneck preventing the actualization of bio-fuel as a widely used energy source. Here we present extensive genomic analysis of an anaerobic fungal isolate, Orpinomyces C1A, that has the capability to produce ethanol from degradation of complex non-feedstock plants such as Switchgrass. Genomic and Transcriptomic sequencing data was generated using Illumina 100bp paired end which yielded 29GB and 30GB of sequence data respectively. This data was assembled with the short read assembly programs Velvet and Trinity on the NSF XSEDE High Performance Computer Blacklight. Annotation of the whole genome assembly reveals the presence of a wide range of functionally relevant bio-fuel enzymes including, Glycoside Hydrolases, Carboxylesterases, Feruloyl Esterases, Hemicellulase degradation support proteins, and Pectin lyases. The genome contains dramatic expansion of families involved in the degradation of hemicellulose as well as processive exo-celluases when compared to other functionally relevant microbial genomes currently reported on the Carbohydrate Enzyme Database, CAZy. Identification and analysis of dockerin domain containing proteins from the genome found that the cellulosome contains many members of the Glycoside Hydrolase family representing a wide range of activities as well members from the Carboxylesterase families. The analysis also uncovered other classes of enzymes in support roles on the cellulosome to the GH and CE families for plant cell wall degradation which include lipases, proteases, seprins, and phosphorylases. This analysis of the genome of Opinomyeces indicates that the anaerobic fungal genus Neocallmastix contains a large amount of functionally relevant genes that can significantly contribute to the current bio-fuel gene repertoire for degradation of complex plant substrates.

# POSTER 2

# DEVELOPMENT OF LOWLAND INBREDS AND UPLAND-LOWLAND HYBRIDS IN SWITCHGRASS


**PRESENTER: LAXMAN ADHIKARI**

**Laxman Adhikari and Yanqi Wu**

**Department of Plant and Soil Sciences, Oklahoma State University, Stillwater, OK**



Switchgrass (*Panicum virgatum* L.) is naturally an outcrossed species, including two major ecotypes, upland and lowland. Complementary inbred lines and male sterile parents are required to produce heterotic hybrids. The objectives of this study were to generate advanced inbred lines in lowland genotypes and examine male fertility in hybrids obtained from crosses between selected upland and lowland parental plants. Second generation (S2) lowland plants of NL94/85 and SL93/34 will be bagged to produce seeds in a greenhouse. Young plants germinated from the seeds will be examined as to whether they are selfed. The parental origin of seeds will be determined using PCR based SSR markers. Crosses between selected upland and lowland plants will be made to produce upland-lowland hybrids, which will be selfed to produce S1 plants. Male fertility of upland-lowland S1 plants will be examined to seek male sterile genotypes. We have isolated seven pairs of upland and lowland genotypes in two greenhouses for making crosses. If successful, male sterile genotypes will be used to develop cytoplasmic-genic male sterile (CGMS) lines. Development of inbred lines and male sterile systems would be valuable to the production of switchgrass hybrid seed on a farm scale.

## POSTER 3

# GENETICS OF A GRASS MUTANT WITH DECREASED CELL WALL CROSS-LINKING

## PRESENTER: FAN LIN

**Fan Lin, Laura Bartley**

**Department of Botany and Microbiology, University of Oklahoma, Norman, OK**

Grass cell walls are one of the most abundant potential sources for biofuel production. One way to utilize the tremendous amount of cell wall is to degrade them into fermentable sugar. Degradation of grass cell walls is inhibited by cross-linking mediated by phenylpropanoids including ferulic acid (FA) and *p*-coumaric acid(*p*-CA) which are attached to the hemicellulose arabinoxylan in grasses. Recently, our group has characterized two genes, OsAT5 and OsAT10, which are involved in FA and *p*-CA cell wall crosslinking. Both of genes belong to subclade i of a grass diverged and expanded clade of so-called BAHD acyltransferase family. But genes from subclade ii have not been studied in detail. Here I report the study of a T-DNA tagged rice lines of a subclade ii member, OsAT15. I confirmed that the mutant line exhibits increased expression of OsAT15 compared to near isogenic wild type. Furthermore, I observed a decrease in FA content in mutant plant compared to wild type plant. This cell wall change doesn't have effect on plant growth. Further studies with metabolomics and enzymatic biochemistry will help reveal the biochemical mechanism of arabinoxylan modification. These results suggest that genetic manipulation of expression of subclade ii members, in addition to subclade i members, represent a promising strategy for improving grasses for biofuel production.

## POSTER 4

# MULTILOCUS "DNA BARCODES" FOR IDENTIFICATION OF SWITCHGRASS RUST POPULATIONS


**PRESENTER: GABRIELA ORQUERA**

**Gabriela Orquera, Kihyuck Choi, Carla D. Garzon, Stephen M. Marek**

**Department of Entomology and Plant Pathology, Oklahoma State University, Stillwater, OK**



Switchgrass (Panicum virgatum L.), a perennial warm-season grass native to a large portion of North America, is used for forage production, erosion control, and as a renewable biomass energy source. Seed yields, forage quality, and biomass of switchgrass can be negatively impacted by diseases. Leaf rust disease, caused by *Puccinia emaculata* Schwein., *P. graminis* Pers., or *Uromyces graminicola* Burrill, have been reported to affect switchgrass. Currently, little is known about rust of switchgrass and its unclear etiology complicates development of effective management strategies. In order to resolve the molecular identity of the pathogen(s), four "barcode" loci were characterized from switchgrass rust urediniospore collections. These barcode loci consisted of the internal transcribed spacer region of ribosomal DNA (ITS-rDNA), β-tubulin (bTub), translation elongation factor 1-α (TEF1a) and mitochondrial cytochrome b (cytb). While cytb was readily sequenced directly from PCR products, ITS-rDNA, bTub and TEF1a products could not be sequenced directly, because the template DNA included different alleles at one or more corresponding chromosomal loci. Only by subcloning PCR products was sequencing of the individual alleles in collected urediniospores possible. Allelic diversity was highest among ITS-rDNA, followed by TEF1a and bTub. Cytb was monomorphic. BLAST searches with ITS-rDNA sequences matched a previous accession from *P. emaculata* with 99% identity and phylogenetically grouped with *P. asparagi, P. andropogonis* and *P. sorghi.* Additionally, whole genome amplification from a single pustule or a single urediniospore generated suitable PCR templates, which are expected to be homozygous and obviate subcloning. Thus far, only *P. emaculata* has been found among multistate collections.

# POSTER 5

# INVESTIGATING CELL WALL CHANGES DURING GRASS LATERAL ROOT EMERGENCE


## PRESENTER: DAVID PONDER

**David Ponder and Laura Bartley**

**Department of Botany and Microbiology, University of Oklahoma, Norman, OK**


Root architecture, defined as the shape and branching pattern of a root system, governs the efficiency with which plants uptake water and other nutrients. In grasses, lateral, or side, roots form deep within the cortex of the root and must emerge past several layers of cells, with formerly intact cell walls. Understanding the changes during the emergence process and the regulators of this process could then be harnessed to improve the deconstructability of plant biomass. Indeed, light and electron microscopy images show that the normally elongated epidermal cells become more cube-like around the site of emergence which is consistent with cell wall loosening allowing cell shape deformation. One of the goals of this study is to use bulk analysis methods to determine the changes to cell wall composition during lateral root emergence. However, in normal lateral root development only a few laterals form and emerge at any given time. Therefore, to facilitate analysis of cell wall composition changes we have developed a grass inducible synchronous lateral root emergence system which has improved the density and synchronicity of lateral root emergence in rice (*Oryza sativa*). The current treatment improves lateral root density from 4.0 ± 0.6 for untreated control plants to 15 ± 1 (LR/cm) for treated plants. After fine tuning this system, in addition to the cell wall analysis, we plan to use it to enable the study of the changes in gene expression in cells overlying the emerging lateral root that can then be harvested using laser capture micro-dissection. This will allow us to identify regulators of grass lateral root emergence and cell wall remodeling which may provide the means to optimize root architecture for stress tolerance or lead to methods for improving biomass feedstock deconstruction.

# POSTER 6

# GENE NETWORKS ASSOCIATED WITH TILLERING TRAIT IN SWITCHGRASS

**PRESENTER: XIN ZENG**

**Xin, Yixing, Dr. Yanqi Wu and Dr. Ramamurthy Mahalingam**

**Department of Biochemistry & Molecular Biology, Oklahoma State University, Stillwater, OK**

Previous agronomic studies in switchgrass have shown that tillering is an important trait that contributes to increased biomass accumulation. A mapping population derived from a cross between a high tillering and a low tillering switchgrass parental line has been developed to identify biomarkers associated with this trait and also other traits associated with biomass accumulation. In this study we utilized these valuable genetic resources to examine the transcriptomic differences in specific tissues that give rise to tillers. Stems of high tillering and low tillering lines containing single nodes were obtained from field growing plants. Following surface sterilization these stems were allowed to grow in tissue culture media for a period of 3-5 days. RNA from nodes and buds from high tillering and low tillering lines were isolated and used for transcriptome analysis with the switchgrasss Affymetrix gene chips. A four-way comparative analysis between buds and nodes from high and low tillering lines were conducted to identify genes with statistically significant differences in gene expression. In both the buds and nodal tissues, high tillering line showed more number of differentially expressed genes compared to the low tillering line. Interestingly, a comparison between buds and nodes showed 3-4 fold more genes were differentially expressed between these tissue samples in the two switchgrass lines. Significantly enriched gene ontologies among differentially expressed genes was determined using AgriGo (bioinfo.cau.edu.cn/agriGO/index.php). This study lays the foundation for defining the gene networks associated with tillering in switchgrass. Differentially expressed genes associated with GO category of transcriptional regulation will be further analyzed for developing functional biomarkers for marker-assisted selection for high tillering trait in switchgrass.

## POSTER 7

# IDENTIFICATION OF THE REGULATORY GENES IN THE PHENYLPROPANOID BIOSYNTHESIS PATHWAY BY NETWORK ANALYSIS OF THE MODEL GRASS, RICE


**PRESENTER: KANGMEI ZHAO**

**Kangmei Zhao, Prasenjit Saha, Laura E. Bartley**

**Department of Botany and Microbiology, University of Oklahoma, Norman, OK**



Cell wall deconstruction inefficiency is a key problem for biofuel production from cellulosic biomass. In grass cell wall, products from the phenylpropanoid pathway, monolignols, which building the high-molecular weight lignin, and hydroxycinamic acids enhance the crosslink of cell wall and prevent deconstruction. Our lab has identified a family of 20 grass-diverged CoA acyltransferases that act to incorporate ferulic acid into grass cell walls. The goal of this project is to identify new genes that regulates the grass ferulic acid and phenylpropanoid incorporation pathways based on network analysis of three public databases, ROAD (http://www.ricearray.org/index.shtml), PlaNet (http://aranet.mpimp-golm.mpg.de/ricenet) and RiceNet (http://www.functionalnet.org/ricenet/). The three databases apply different criteria to establish relationships between genes. ROAD and PlaNet use raw correlation coefficients and ranking of rice Affimetrix microarray data. RiceNet incorporates diverse genomics data across eukaryotes species. We pulled out all genes with connection to the grass CoA acyltransferases from each network. The overlap of the resulting components was highly significant. The 294 common elements were highly enriched for phenylpropanoid biosynthesis genes and cell wall carbohydrate metabolic enzymes, including transcription factors that are prime candidates for regulating grass ferulic acid incorporation. We are now following up on these leads, exploring the use of generalized linear model and other methods to combine the networks quantitatively and building a larger network seeded with known CoA acyltransferase, phenylpropanoid biosynthesis genes and secondary cell wall transcription factors. Our goal is to use this analysis to learn about cell wall biology and improve grass for biofuel production.

# POSTER 8

# BIOCHAR AND BIOCHAR-DERIVED ACTIVATED CARBON AS CATALYSTS FOR SYNGAS TAR REMOVAL

## PRESENTER: PUSHPAK BHANDARI

**Pushpak Bhandari, Dr. Ajay Kumar*, Dr. Danielle Bellmer, and Dr. Raymond L. Huhnke**

**Biosystems and Agricultural Engineering, Oklahoma State University, Stillwater, OK**

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In the quest for low-cost and efficient technologies to convert biomass into fuels, chemicals, and power, gasification offers great potential. However, to effectively utilize syngas, the main product of biomass gasification, it must be cleaned of its tars. Syngas tars are comprised of organic compounds having molecular weight higher than benzene. These tars cause many problems downstream of the gasifier, such as clogging process lines and deactivating catalysts. The primary objective of this work was to investigate the use of carbon-based catalysts, biochar and activated carbon derived from biochar, for tar removal. Biochar is a low surface area waste product generated during biomass gasification. Biochar has shown good potential for tar removal in previous studies. To improve upon the low surface area of biochar, activated carbon was synthesized using the biochar derived from switchgrass gasification. Activated carbon was also modified using dilute acid to give an acidic surface to help improve toluene conversion. The three catalysts (biochar, activated carbon, and modified activated carbon) were evaluated at temperature of 700 °C and 800 °C and a gas residence time of 0.001 kg/($m^3$/hr) in a fixed-bed catalytic reactor fed with syngas, steam, and toluene. Several key findings are following. First, both the activated carbons and biochar were effective in tar removal (up to 92%). Second, as compared to biochar, activated carbon catalysts with its higher surface area (~900 $m^2$/g compared to <10 $m^2$/g for biochar), larger pore diameter (19 A° compared to 15.5 A° for biochar) and larger pore volume (0.44 cc/g compared to 0.085 cc/g) resulted in higher toluene conversion.

# POSTER 9

# SOLUBILITY OF MAJOR PRODUCER GAS TAR COMPOUNDS IN WATER

## PRESENTER: PRAKASH R. BHOI

**Prakash R. Bhoi, Research Engineer; Krushna N. Patil, Assistant Researcher;**

**Ajay Kumar, Assistant Professor; Raymond L. Huhnke, Professor**

**Biosystems & Agricultural Engineering Department, Oklahoma State University, Stillwater, OK**

Wet scrubbing process removes producer gas impurities such as tars and particulates. Water is the most common scrubbing solvent in biomass producer gas applications. Major tar compounds in biomass-based producer gas include benzene, toluene, ethyl benzene, styrene, m-xylene, and naphthalene. Solubility, which is defined as the fraction of a selected solute in a selected solvent, is one of the crucial parameters for designing the wet scrubbing columns. The primary goal of this study is to develop a solubility model to generate theoretical data in Aspen Plus. Using this software package, water solubility data for these selected producer gas tar compounds under varying operating temperatures is determined. It was determined the solubility (g/100ml) of benzene, toluene, ethyl benzene, styrene, m-xylene, and naphthalene varies in the range of 0.16-0.26, 0.032-0.062, 0.015-0.023, 0.016-0.043, 0.0075-0.019, and 0.004-0.0067, respectively as the temperature increases from 5°C to 70°C. Results show that all tar compounds have poor water solubility and are significantly affected by operating temperature. These data are in close agreement with those values published in NIST-IUPAC and DECHEMA databases. Using this knowledge, solvent-water mixtures will be selected and explored to develop a low-cost producer gas wet scrubbing system.

## POSTER 10

# INFLUENCE OF RUTHENIUM TITANIA CATALYST PRETREATMENT CONDITIONS ON THE UPGRADING OF BIOMASS FAST- PYROLYSIS OIL VAPORS


**PRESENTER: TAIWO OMOTOSO**

**Taiwo Omotoso, Richard Mallinson, Daniel Resasco, and Steven Crossley**

**School of Chemical, Biological and Materials Engineering, University of Oklahoma, Norman, OK**



Fast pyrolysis of biomass for liquid bio-oil production is an appealing strategy for the production of liquid fuels due to the relatively low cost of the process and wide range of biomass sources that may be incorporated. A major drawback is that the liquid bio-oil produced has characteristics that deem it unsuitable for transportation fuels such as thermal instability, corrosivity, and low heating value. This study investigates the use of a dual-function $Ru/TiO_2$ catalyst for the vapor-phase upgrading of guaiacol, a lignin derived model compound present in bio-oil, in a reducing environment. Catalyst stability and activity at 400°C towards deoxygenation and transalkylation reactions are compared for various derivatives of this catalyst. Catalyst pretreatment conditions, including calcination temperature, support phase, and surface area yield significant shifts in catalytic performance. The results showed that carrying out the calcination procedure at a lower temperature produces a catalyst that is more active and selective to aromatics production than its counterpart calcined at a higher temperature` over the time on stream studied. Both types of catalysts were stable in terms of guaiacol conversion over the same time of stream. Also, the effect of lower surface area of the P25 titania support compared to the anatase titania support was reflected in the results obtained as the anatase supported catalyst was more selective for aromatics production at the same conversion than the P25 supported catalyst. Explanations of the observed trends will be discussed.

# POSTER 11

# EFFECTS OF STEAM INJECTION LOCATION ON SYNGAS GENERATED FROM FLUIDIZED-BED GASIFICATION OF SWITCHGRASS

**PRESENTER: ASHOKKUMAR M. SHARMA**

**Ashokkumar M Sharma, Research Engineer, Ajay Kumar, Assistant Professor,**

**Raymond L. Huhnke, Professor**

**Biosystems & Agricultural Engineering Department, Oklahoma State University, Stillwater, OK**

Researchers have shown that steam injection into gasifier increases $H_2$ content of syngas making it more suitable feedstock to convert into liquid fuels and chemicals. We hypothesized that the location of steam injection has significant influence on syngas quality. Syngas $H_2$ and CO contents can be maximized if steam injection location is optimal. The goal of present study was to investigate effect of steam injection location on gas composition and yield, gas tar and particulates contents, as well as gasifier efficiencies for syngas generation in fluidized-bed air-steam gasification of switchgrass. Experimental design included a total of 18 experiments (3 steam injection locations × 3 steam-to-biomass ratios × 2 replications). Steam injection locations were at the heights of 51, 152, and 254 mm above the distributor plate. Steam-to-biomass ratios were 0.1, 0.2, and 0.3. Preliminary results (first replication of 9 experiments), showed significant ($p < 0.05$) influence of steam injection location on syngas CO content, as well as cold and hot gas efficiencies. However, syngas $H_2$ content, carbon conversion efficiency, and tar and particulates contents were not significantly depend on steam injection location. Maximum $H_2$ of 9.8%, CO of 17.9%, cold gas efficiency of 75%, and hot gas efficiency of 80% were observed at a steam injection location of 254 mm and at a steam-to-biomass ratio of 0.1. A maximum carbon conversion efficiency of 98% was observed at the steam injection location of 254 mm and steam-to-biomass ratio of 0.3. Remaining experiments are currently underway.

## POSTER 12

# HYDROPHOBIC ZEOLITES FOR BIOFUEL UPGRADING REACTIONS AT THE LIQUID-LIQUID INTERFACE IN WATER/OIL EMULSIONS

### PRESENTER: PAULA ZAPATA

**Paula A. Zapata, Jimmy Faria, M. Pilar Ruiz, Rolf E. Jentoft, Daniel E. Resasco**

**School of Chemical, Biological and Material Engineering**

**Center for Interfacial Reaction Engineering (CIRE)**

**University of Oklahoma, Norman, OK**

HY zeolites hydrophobized by functionalization with organosilanes are much more stable in hot liquid water than the corresponding untreated zeolites. Silylation of the external surface of the zeolite crystals increases hydrophobicity without reducing the density of acid sites. This hydrophobization with organosilanes makes the zeolites able to stabilize water/oil emulsions and catalyze reactions of importance in biofuel upgrading, i.e., alcohol dehydration and alkylation of m-cresol and 2-propanol in the liquid phase, at high temperatures. Moreover, the hydrophobization reduces the high susceptibility of HY zeolite to hot liquid water. While at 200°C the crystalline structure of an untreated HY zeolite collapses in a few hours in contact with a liquid medium, the functionalized hydrophobic zeolites keep their structure practically unaltered. Detailed XRD, SEM, HRTEM and BET analysis indicate that even after reaction under severe conditions, the hydrophobic zeolites retain their crystallinity, surface area, microporosity, and acid density. It is proposed that by anchoring hydrophobic functionalities on the external surface, the diffusion of water into the zeolite is hindered, thus preventing the collapse of the framework during the reaction in liquid hot water.

# POSTER 13

# PREDICTING BIOMASS YIELD IN BIOENERGY CROP PRODUCTION SYSTEMS USING CANOPY NDVI

**A.J. Foster[1], V.G. Kakani[1], J.Mosali[2], and J.Ge [3]**

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**[2]The Samuel Roberts Noble Foundation, Inc., 2510 Sam Noble Parkway, Ardmore, OK**

**[3]Department of Geography, Oklahoma State University, Stillwater, OK**

Remote sensing technology has been successful in monitoring crop N status and estimating crop yield in numerous traditional cropping systems. However, there is limited information on the use of remote sensing technology in bioenergy crop production systems. Therefore, objective of the study was to determine the relationships between plant height and canopy NDVI with biomass yield under different cropping systems. Variable crop N was generated by supplying N at different rates (winter legume (hairy vetch), 0, 75,150 and 225 $kgNha^{-1}$) in two field studies at Stillwater and Woodward, Oklahoma. Fields were planted with switchgrass "Alamo" (*Panicum, virgatum* L.) and mix grass (Indian grass (*Sorghastrum nutans),* big bluestem (*Andropogon gerardii)* and Switchgrass) in a split plot design. Canopy heights, canopy NDVI, LAI and biomass during the season were measured on an individual plot basis. Regression analysis was done across locations, cropping systems and N treatments. Strong linear relationships were observed between NDVI and LAI ($r^2$ =0.76) and NDVI and canopy height ($r^2$ =0.64). However, canopy height alone ($r^2$ =0.68) was a good predictor of plot biomass yield. The index of NDVI x canopy height ($r^2$=0.60) did not improve the prediction for biomass yield achieved using height alone, but did for NDVI alone ($r^2$=0.41). These results suggest that there is great potential for the use of remote sensing in predicting biomass yield in bioenergy crop production systems.

## POSTER 14

# EVALUATING ENERGY BEET POTENTIAL AS BIOENERGY FEEDSTOCK IN SOUTHERN GREAT PLAINS

**Vijaya Gopal Kakani, Pradeep Wagle, Anserd J. Foster, Chad B. Godsey, Aparna Kakani, Raghuveer Sripathi, Arjun Pandey**

**Department of Plant and Soil Sciences, Oklahoma State University, Stillwater, OK**

Sugar feedstocks are easy to convert and are most efficient for biofuel production. Energy beets (*Beta vulgaris* L.) in Great Plains of USA have the potential to replicate ethanol yields of sugarcane in Brazil. Energy beet can be readily incorporated into crop rotations and serve as a cash-crop to producers. The objective of this study was to evaluate energy beet sugar and biomass yield and finally ethanol production. Five energy beet lines in collaboration with BetaSeed Inc. were evaluated during 2010 winter and 2011 summer at Agronomy Research Farm, Stillwater, OK. Crop was established on 28 Sept 2010 and 14April/18 May 2011. Crop growth, leaf area, root weight, diameter and length, juice expression, bagasse dry weight, and brix were evaluated at monthly interval. Juice was extracted using a commercial fruit juice extractor. The winter 2010 planted crop survived the winter and started to regrow after March 2011, while the summer 2011 crop survived the extreme drought and high temperatures of 2011 with a supplemental irrigation of 25 cm. The final root weight for the winter 2010 crop ranged from 42 to 65 wet Mg $ha^{-1}$, while the summer 2011 crop yield ranged from 43 to 100 wet Mg $ha^{-1}$. The brix was 15-18% for the winter 2010 crop harvested in June 2011, while it was 18-20% for the summer 2011 crop harvested in October. The crop moisture content ranged from 80-85%. Based on biomass yield and brix, the theoretical ethanol yield potential ranged from 4500 to 10,000 L $ha^{-1}$ or 1200 to 2700 gal $ha^{-1}$.

## POSTER 15

# *STENOFOLIA*, A POTENTIAL BIOMASS GENE, REGULATES LAMINA OUTGROWTH BY CONTROLLING CELL PROLIFERATION

**Lin, Hao[1]; Niu, Lifang[1]; Mysore, S. Kirankumar[2] and Tadege, Million[1]**

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The *WUSCHEL*-related homeobox (*WOX*) gene *STENOFOLIA* (*STF*) performs a conserved function during lamina outgrowth and leaf vascular development in plants. *STF* is expressed at the adaxial–abaxial boundary layer of leaf primordia and is required for cell proliferation and expansion in lateral organ primordia. The classical bladeless *lam1* mutant of *Nicotiana sylvestris* is caused by deletion of the *STF* homologue *NsSTF1*, and full rescue of vegetative and floral *lam1* mutant phenotypes by *STF* indicates the conservation of *STF* function in regulating lamina outgrowth in diverse species. When driven by the *STF* promoter, full rescue of vegetative *lam1* mutant phenotypes is provided by *WUSCHEL* (*WUS*), a founding member of WOX which functions in meristem maintenance, suggesting conserved mechanisms in stem cell maintenance and cell proliferation in lateral organs governed by the WOX gene family. Using transcript profiling analysis, we uncovered that specific types of GRAS and TCP transcription factors as well as D-type cyclins are downregulated in the *stf* mutant. EMSA results show that STF protein can directly bind to the promoter regions of GRAS transcription factors and D-type cyclins indicating that *STF* may regulate cell proliferation by directly activating GRAS transcription factors and D-type cyclins. Loss-of-function of *STF* in *Medicago* leads to a three-fold decrease in total above ground biomass. We found that introducing *STF* gene into the model grass *Brachypodium distachyon* by genetic transformation increases leaf blade outgrowth (leaf width) by more than 65% of the corresponding wild-type. This suggests that *STF* could be used to significantly improve photosynthetic efficiency and biomass yield in priority biofuel crops such as switchgrass and sorghum.

# POSTER 16

# A USEFUL MOLECULAR TOOL TO IDENTIFY SELFED PROGENY IN SWITHGRASS

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Switchgrass is a herbaceous model plant for the cellulosic biofuel feedstock development in the USA and Europe. Accurate identification of selfed progeny is important to produce switchgrass inbreds, which can be used in the production of heterotic hybrids. Development of a technically reliable and easily used marker system is required to quantify and characterize breeding origin of progeny plants of targeted switchgrass parents. Here a genome-wide screening of 915 mapped microsatellite markers was conducted, and 842 (92%) produced clear and scorable bands on a pooled DNA samples including eight major switchgrass varieties. A total of 166 primer pairs were selected on the basis of their evenly distribution in switchgrass genome and PCR amplification quality on 16 tetraploid genotypes. Mean polymorphic information content value for these 166 selected loci was 0.810 ranging from 0.116 to 0.959. Among them, a core set of 48 loci, which evenly distributed on 17 linkage groups and had mean null allele frequency of 0.059 with a range from -0.196 to 0.281, suggesting low mutation rates, was further refined and optimized to develop 24 sets of duplex markers. Most of (up to 87.5%) non-allelic bands within each duplex were separated by more than 10-bp. Assuming one known parent, 10 randomly selected markers provided combined non-exclusion probability of less than 0.0001 (i.e., accuracy being > 99.99%) in the identification of selfed progeny. Using the established duplex PCR protocol, selfing ratio was 0 for an open-pollinated ‘Kanlow’ genotype, 11.3% for ten selected and bagged parents, and 77.3% for a breeding line grown in a growth chamber. In conclusion, the duplex PCR of 48 loci provides ample choices for unlinked loci on switchgrass whole genome, and represents a powerful and reliable method for the identification of selfed progeny in switchgrass.

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## POSTER 17

# CONTROL OF FLOWERING TIME IN SWITCHGRASS AND SORGHUM FOR BIOMASS YIELD IMPROVEMENT

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Plant biomass can be a significant source of renewable energy, and biomass yield is an important component of feedstock improvement to ensure long term sustainability and profitability of the bioenergy industry. The timing of the transition from vegetative to reproductive growth is a very important biomass yield parameter, as delaying of flowering can increase vegetative biomass yield by allowing the continuous generation of photosynthetic tissue for a longer time period until growth ceases. Thus, delaying floral transition is one important approach to increase biomass yield in biofuel crops. In this project, our objective is to increase the biomass yield of switchgrass and sorghum by delaying the transition to flowering. Using a reverse genetics approach, we identified three strong flowering promoters *FT*, *SOC1*, and *ID1* homologues from switchgrass and sorghum. Phylogenetic analysis showed that the switchgrass flowering time genes are similar to their dicot and monocot counter parts but closer to homologues from C4 plants than from rice or other C3 grasses. First, we tested their flowering promoter function by overexpressing each of them in the model grass *Brachypodium distachyon*. Our results show that the *PvFT* gene is functional in *Brachypodium* and leads to very early flowering. We are now knocking down the expression of *PvFT* in switchgrass by a transgenic approach in collaboration with colleagues from the noble Foundation to delay flowering in switchgrass and evaluate repercussions on biomass yield. 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 M1 progeny. We have so far mutagenized approximately 34,000 M1 seeds with estimated 12,000-14,000 fertile independent M2 families. When completed, this population will be publicly available and will help to advance functional genomics studies in annual and perennial C4 grasses.

## POSTER 18

# UNDERSTANDING TRANSCRIPTIONAL REGULATION OF FERULIC ACID (FA) INCORPORATION IN GRASSES CELL WALL

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The major limitation for biological conversion of biomass to biofuel is the low saccharification and deconstruction efficiency due to cell wall crosslinking by ferulic acid (FA) and lignin, which are the products of the phenylpropanoid pathway. Transcription factors (TFs) have been proven to be key regulators of phenylpropanoid metabolism in plants. Using gene network analysis we have identified two putative TFs that are consistently associated in three rice network databases with CoA acyltransferase that incorporate FA into cell wall, phenylpropanoid biosysthesis genes and secondary cell wall TFs in rice. We are now conducting functional analysis of these genes. Based on sequence homology, one of the transcription factors OsMYBL1 is annotated as a MYB-like, about which little is known in any species. The other, OsMYB61a belongs to the R2R3-MYB family, which is similar to a protein in *Arabisopsis* involved in controlling stomatal aperture. OsMYB61a has one other close homolog in rice, OsMYB61b, that we are examining in case OsMYB61a and OsMYB61b might function redundantly. Phylogenetic analysis reveals that all three of these genes are present in other grass genomes and contained distinct conserved functional motifs. We determined the expression of these genes using quantative reverse transcription-PCR (qRT-PCR) in rice. We have cloned the genes and made both overexpression and silencing constructs. We are now using *Agrobacterium*-mediated transformation to alter the expression of these genes in rice for functional characterization. Further, we have identified T-DNA insertional mutant lines of these MYBs from public mutant repositories for reverse genetic study. Our aim is to develop a mechanistic understanding of gene regulatory networks controlling cell wall synthesis in rice, a model grass. Moreover, this study is likely to lead to the development of superior lignocellulosic feedstock quality with reduced cell wall recalcitrance for enhanced bio-fuel production from rice and other grasses.

## POSTER 19

# IDENTIFICATION OF CONSERVED, NOVEL AND STRESS-RESPONSIVE MICRORNAS IN SWITCHGRASS USING HIGH-THROUGHPUT SEQUENCING OF SMALL RNA LIBRARIES

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Future production of renewable transportation fuels will require a consistent supply of biomass produced specifically for biofuel production. Switchgrass has emerged as one of the major sources of bioenergy crops in the United States, including southern plains. Switchgrass can be grown on marginal lands, and it is also tolerant to frequent drought or heat episodes. However, little is known about the basic biology of the traits that contribute for switchgrass's biomass accumulation and stress tolerance. Recently discovered genome-encoded 21-nt long microRNAs (miRNAs) have emerged as critical regulators of gene expression important for growth and development including biomass production, and adaptation to stress conditions. To gain an insight into miRNA networks that control these traits in switchgrass, several small RNA libraries were generated from flowers and emerging tillers as well as from untreated seedlings (control) and seedlings exposed to drought and heat stress conditions. Small RNA libraries were also generated from uninfected leaves or leaves infected with rust. These small RNA libraries were sequenced using Illumina GAII analyzer. Together, more than 100 million small RNA reads were generated for switchgrass. Sequence analyses revealed the identification of ~30 conserved miRNA families. More importantly, our analysis has identified ~20 novel miRNAs in switchgrass. Most conserved miRNA families are significantly altered in response to drought or heat stress. Similarly, our preliminary analysis revealed that several miRNA families are differentially regulated (up-regulated or down-regulated) by rust infection.

# POSTER 20

# NONHOST RESISTANCE OF *BRACHYPODIUM DISTACHYON* TO SWITCHGRASS RUST PATHOGEN, *PUCCINIA EMACULATA*

**Srinivasa Rao Uppalapati and Kirankumar S Mysore**

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Rust disease of switchgrass, caused by *Puccinia emaculata*, is a concern in Oklahoma and other parts of the United States and could become an important factor once switchgrass is grown in monoculture over a long period of time. Nonhost resistance is defined as a form of resistance exhibited by an entire plant species to a particular microbial pathogen and is an attractive and durable alternative to host resistance breeding. To identify a suitable monocot nonhost model system for *P. emaculata* we have screened several monocot systems from BEP (Bambusoideae, Ehrhartoideae, Pooideae) and PACCMAD (Panicoideae, Arundinoideae, Chloridoideae, Centothecoideae, Micrairoideae, Aristidoideae, and Danthonioideae) clades. All the tested monocots were nonhost to *P. emaculata*. *P. emaculata* germtubes failed to recognize the host surfaces and show oriented growth and form appressoria on the abaxial or adaxial leaf surfaces of corn, sorghum, foxtail millet belonging to the PACCMAD clade. Unlike, on rice or barley, on *Brachypodium distachyon* belonging to the BEP clade, *P. emaculata* germ tubes recognized the topographic and/chemical signals on the host surface and showed oriented growth perpendicular to the long axis of the epidermal cells. Furthermore, ca. 40% of the germ tubes encountered stomata and formed appressoria within 72 hours post inoculation on *B. distachyon* (Bd21). To further identify natural variation within *B. distachyon* populations, we screened six different core accessions and found significant variation in differentiation of preinfection structure formation and percentage of appressorium formation between accessions collected from Spain and Iraq. In summary, we identified *B. distachyon* as an ideal nonhost grass model for switchgrass rust.

## POSTER 21

# CHARACTERIZATION OF SWITCHGRASS RUST FUNGUS AND EVALUATION OF GENETIC VARIABILITY IN RUST RESISTANCE OF SWITCHGRASS POPULATIONS

**Srinivasa Rao Uppalapati[1], Serba, Desalegn[2], Yasuhiro Ishiga[1], Les J. Szabo[3], Kirankumar S. Mysore[1] and Malay C. Saha[2]**

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Several fungal pathogens have been identified on ornamental and native strands of switchgrasss (*Panicum virgatum* L.). Diseases of switchgrass have been largely neglected and pathogens could become the major limiting factors to biomass quality, and yield of switchgrass; especially when planted in monocultures. Leaf and stem rust is a major emerging disease in switchgrass research fields of Oklahoma. Based on teliospore morphology and ITS-based diagnostic primers we have identified the rust pathogen as *Puccinia emaculata*. The current morphological methods for identification of *P. emaculata* and *U. graminicola* depend on the teliospore morphology. It is common to make collections of rust infected switchgrass that contain only urediniospores and not teliospores, therefore making positive identification of the causative rust pathogen is difficult. We therefore developed ITS-based diagnostic primers for early detection and diagnosis of *P. emaculata*. The molecular phylogeny based on ITS sequences suggested that *P. emacualata* is closely related to *P. andropogonis*. Furthermore, to identify genetically diverse source(s) of rust resistance, we evaluated half-sibling families from upland (Summer and Cave-in-Rock) and lowland (Alamo and Kanlow) switchgrass populations in Ardmore, OK in 2008 and 2009 and in growth chamber assays. Field and growth chamber evaluations revealed a high degree of genetic variation within and among switchgrass populations. Alamo in general showed moderate resistance to *P. emaculata*, while Summer was highly susceptible. These results suggested a potential for improvement of rust resistance via the selection of the resistant individuals within the populations.

## POSTER 22

# LOSS OF ABAXIAL LEAF EPICUTICULAR WAX IN *MEDICAGO TRUNCATULA IRG1/PALM1* MUTANTS RESULTS IN REDUCED SPORE DIFFERENTIATION OF NONHOST RUST PATHOGENS

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To identify genes that confer nonhost resistance to rust pathogens, we performed a forward-genetics screen using *Medicago truncatula Tnt1* retrotransposon insertion lines and identified an inhibitor of rust germ tube differentation1 (*irg1*) mutant. *Irg1* mutants were identified from the screen because they failed to promote preinfection structure differentiation of switchgrass rust (*Puccinia emaculata*) on the abaxial leaf surfaces. Interestingly, *irg1* mutants also failed to promote preinfection structure differentiation of Asian Soybean Rust (*Phakopsora pachyrhizi*) and anthracnose pathogen, *Colletotrichum trifolii*. Cytological and chemical analyses revealed that the inhibition of rust preinfection structures in *irg1* mutants is due to complete loss of the abaxial epicuticular wax crystals and reduced surface hydrophobicity. The composition of waxes on abaxial leaf surface of *irg1* mutants had >90% reduction of C30 primary alcohols and a preferential increase of C29 and C31alkanes compared with the wild-type. *IRG1* encodes a Cys(2)His(2) zinc finger transcription factor, PALM1, which also controls dissected leaf morphology in *M. truncatula*. Transcriptome analysis of *irg1/palm1* mutants revealed downregulation of eceriferum4, an enzyme implicated in primary alcohol biosynthesis, and MYB96, a major transcription factor that regulates wax biosynthesis. Our results demonstrate that PALM1 plays a role in regulating epicuticular wax metabolism and transport and that epicuticular wax influences spore differentiation of host and nonhost fungal pathogens.

## POSTER 23

# TARGETED LIGNIN MODIFICATION INDUCES TOLERANCE TO SOIL-BORNE FUNGAL PATHOGENS IN ALFALFA

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Lignin modification benefits the biofuel, pulp, and forage industries through improving the accessibility of cell wall polysaccharides to chemical, microbial or enzymatic digestion. However, lignin reduction is suggested to negatively impact plant defense against pathogens, and down-regulation of cinnamate 4-hydroxylase (*C4H*), an early enzyme in the lignin pathway, resulted in increased susceptibility to fungal pathogens in alfalfa. Surprisingly, however, down-regulation of hydroxycinnamoyl CoA: shikimate hydroxycinnamoyl transferase (*HCT*), caffeoyl CoA 3-*O*-methyltransferase (*CCoAOMT*) and caffeic acid methyltransferase (*COMT*) resulted in increased tolerance with restricted growth of *Fusarium oxysporum* f. sp. *medicaginis* and *Phymatotrichopsis omnivora*. *HCT* down-regulated lines showed higher amount of tolerance than *CCoAOMT* and *COMT* down-regulated lines tested. Metabolite and gene expression profiling revealed that the induced tolerance in these lignin modified plants may result from increased accumulation and/or spillover of flux towards the (iso)flavonoid pathway. A continuous increase in accumulation of liquiritigenin, coumestrol, 7,4 dihydroxyflavone and medicarpin from 3 to 12 dpi with *F. oxysporum* were observed in lignin modified alfalfa roots when compared to the wild-type. Although none of these compounds completely inhibited the growth of *F. oxysporum*, *in vitro*, both 7,4 dihydroxyflavone and medicarpin caused 50-60% reduction in growth at 0.5 mM and 0.1 mM, respectively. These results demonstrate an additional commercial value of targeted lignin modification.

## POSTER 24

# NUTRIENT REQUIREMENTS FOR *CLOSTRIDIUM RAGSDALEI* IN PRODUCTION OF ETHANOL FROM SYNGAS

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Cellulosic ethanol can be used as an alternative to fossil fuels if produced economically. Cellulosic ethanol can be produced by fermentation of sugars or syngas. Biomass is gasified to syngas (a mixture of CO, $CO_2$ and $H_2$), then fermented to ethanol by a novel *Clostridium ragsdalei* strain P11. The objective of this study is to determine the nutrients' requirement for P11 and develop a cost effective medium for ethanol production. Experiments with removal of morpholinoethane sulfonic acid (MES) buffer, reducing nutrients such as yeast extract (YE) and replacing YE with inositol were performed. The results indicated that MES can be removed from the medium to reduce the cost by over 90%. YE was necessary for P11cell growth. However, more ethanol was produced with the medium containing lower concentrations of YE when the gas mixture was 20 CO: 15 $CO_2$: 5 $H_2$. Replacing YE with inositol did not improve ethanol production. Future research will be undertaken to evaluate the effects of other nutrients on ethanol production. Providing only necessary nutrient to the medium for P11 to produce ethanol will make syngas fermentation more competitive on a cost basis.

## POSTER 25

# GENOME-WIDE ANALYSIS OF A NOVEL FUNGUS *PENICILLIUM EXPANSUM* YT02 FOR EFFICIENT SACCHARIFICATION OF LIGNOCELLULOSIC BIOMASS

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The conversion of plant materials into biofuels using biocatalysts (e.g., enzymes, microorganisms) remains challenging in terms of efficiency, yield, and economy. A novel fungal strain, Penicillium expansium YT02 was isolated and characterized in terms of its physiology, efficiency and capability of biomass depolymerization, and potential for biofuel production. The isolated strain could efficiently produce sugars from plant materials with the highest theoretical sugar yields reported, and produce 30% more sugars than the control fungus, Trichoderma reesei. Also, the produced sugars could be directly used for ethanol production with a yeast strain, and two times more ethanol was produced with sugars generated by YT02 than by T. reesei, indicating that YT02 may serve as an effective microbial catalyst for cellulosic ethanol production and a good source of new enzymes for various industrial applications. To gain more insight into the potential of YT02 for biofuel production, the YT02 genome was sequenced using 454 Roche GS-FLX and Illumina technologies, and many novel genes involved in lignocellulosic biomass were identified. Illumina RNA-Seq technology was used to determine the gene expression profiling of YT02 with different lignocellulosic substrates, such as alfalfa and switchgrass. To further explore possible applications of novel enzymes in the biofuels industry, several functional genes encoding xylanase, xylosidase, endoglucanase, exo-cellobiohydrolase and beta-glucosidase have been cloned and expressed. All results indicate P. expansum YT02 has a great potential for efficient conversion of lignocellulosic substrates and saccharide production. Further studies will focus on gene regulation, process optimization and scaling up, especially with woody materials.

## POSTER 26

# CLOSTRIDIUM THERMOCELLUM STRAINS AND THEIR COMPETITION ON CELLULOSE

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*Clostridium thermocellum*, an anaerobic, cellulolytic, and thermophilic bacterium, efficiently degrades plant cell wall polysaccharides using an extracellular enzyme complex called the cellulosome. Based on the genome sequences, three *C. thermocellum* strains including the type strain ATCC 27405, JW20 and LQR1 showed high level of similarity in every aspect of phenotypic and genetic traits; however, their kinetic behaviors on the degradation of cellulose in the presence of each other are largely unknown. In addition to the genome comparisons of these three strains, here we present their physiological characteristics of cellulose degradation in the same batch. The genome size and the numbers of CDS, cellulosome-related genes and tRNA among three *C. thermocellum* strains were quite similar with 4-10% variations. Strain LQR1 showed the highest ratio of cellulosome-related genes against CDS (2.7%), followed by ATCC 27405 (2.6%) and JW20 (2.5%). To study their physiological and functional differentials of the cellulosomes in the same culture condition, we established a tri-culture consisting of three *C. thermocellum* strains, developed real-time PCR assays for each strain, and quantitatively determined the ratio of three strains in the culture. Strain JW20 was predominant in the co-cultures with and without a saccharolytic strain, *Thermoanaerobacter* sp. X514, indicating its rapid adherence to cellulose and high affinity to the products of cellulose hydrolysis. Our study in a limited single carbon system showed the real behavior of the strains beyond the predictions complied only based on the information of genome sequences. The co-culture studies also showed evidence of selection of a strain better able to utilize cellulose in a given condition. Thus examining ecophysiological traits of a cellulolytic strain built on its genomic information provides better insight into the composition of the intact cellulosome, its behavior, and the way natural selection drives relevant functional gene evolution.

## POSTER 27

# EXPLORING THE GENES FOR BIOMASS DEGRADATION IN THE FILAMENTOUS FUNGUS *PENICILLIUM EXPANSUM* THROUGH WHOLE GENOME ANALYSIS

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*Penicillium expansum* strains are most widely known as pathogens of stored pome fruits. We previously isolated and characterized a novel *P. expansum* strain TY02 able to efficiently degrade a variety of biomass substrates and produce sugars, producing 40% and 65% more reducing sugars than *Trichoderma reesei* ATCC 24449 from switchgrass and corn stover, respectively. In this study, to further understand its mechanisms and to explore its potential for production of high activity biomass-degrading enzymes for bioenergy industries, we have sequenced this genome using Illumina and 454 sequencing technologies, and assembled 52 scaffolds with a total of 31 Mbp nearly contiguous *P. expansum* genome sequence and 10,064 predicted genes. The genome of *P. expansum* encodes more cellulases and hemicellulases than *T. reesei*, *Penicillium chrysogenum*, or *Aspergillus niger* for hydrolyzing plant cell wall polysaccharides. Also, this genome encodes more pectin-degrading genes than *T. reesei* or *P. chrysogenum*. In addition, there are five genes encoding feruloyl esterase and two genes encoding cellobiose dehydrogenase in the genome of *P. expansum*, which could facilitate the hydrolysis of polysaccharide from lignocellulosic matrix. This study provides genetic background for our understanding of mechanisms for efficient biomass degradation and the potential for production of biomass-degrading enzymes in bioenergy related industries.

## POSTER 28

# FIBROLYTIC CAPABILITIES OF *ORPINOMYCES* SP. STRAIN C1A ON UNTREATED AND PRETREATED SWITCHGRASS IN BATCH CULTURE

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The fibrolytic capabilities of an *Orpinomyces* species of anaerobic gut fungi were tested when grown on untreated and pretreated switchgrass in batch cultures over a six day period. This potential was demonstrated by measuring the resulting loss in dry weight (DW), measuring the compositional change and loss in structural carbohydrates within the switchgrass, and through measuring the fermentative end products produced by the *Orpinomyces* sp. strain C1A. Anaerobic gut fungi have a mixed-acid fermentation metabolism, and therefore in addition to ethanol, formate, acetate and lactate were also monitored. On switchgrass pretreated with dilute sulfuric acid, a common pretreatment used to remove hemicelluloses, the *Orpinomyces* strain degraded 20% of the total DW, including 32% of the cellulose and 26% of the remaining hemicellulose present in the sample. Using untreated switchgrass did not diminish the fibrolytic capabilities of *Orpinomyces* strain C1A, with 19% of the total DW and 33% of the cellulose and 25% of the hemicellulose removed within six days. On switchgrass pretreated with sodium hydroxide, which reduced the total lignin in the sample and made the cellulose and hemicellulose components more accessible, the percentage DW loss was 41%, with the cellulose loss at 48% and the hemicellulose loss reaching 57% after 6 days of incubation with the fungal strain. Fermentation end product profiles on each of the three switchgrass substrates indicated faster production on hemicellulose containing samples with the greatest amounts achieved on the sodium hydroxide pretreated switchgrass. Overall, *Orpinomyces* sp. strain C1A did not require pretreatment of the switchgrass in order to effect substantial removal of the cellulose and hemicellulose components. Additionally, the ability of this fungus to access and utilize both pentose and hexose structural components within the switchgrass indicates that common pretreatment methods such as dilute sulfuric acid may be removing a substantial resource for biofuel production.

## POSTER 29

# EXAMINING LOW COST MEDIUM FOR SYNGAS FERMENTATION BY *ALKALIBACULUM BACCHI* STRAIN CP15

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The use of low cost medium for any fermentation process is critical to the feasibility of the overall process. This study reports on designing a low cost medium for syngas fermentation for production of ethanol from *Alkalibaculum bacchi* CP15. Costly TAPS buffer was removed from standard yeast extract (YE) medium. YE, minerals and vitamins were replaced with low cost corn steep liquor (CSL) in 250-mL bottle fermentations with 100-mL working volume. Commercial syngas mixture 20% CO, 15% $CO_2$, 5% $H_2$, 60% $N_2$ was used and strain CP15 was fed syngas every 24 h for 15 days. Four treatments were tested, including 10 ml/L or 30 ml/L minerals in YE medium without TAPS, 20 g/L or 50 g/L CSL medium without TAPS. The results showed that CSL medium produced over twofold more ethanol, compared to YE medium. The medium with 50 g/L CSL produced the highest amount of ethanol (2.7 g/L) and acetic acid (6.4 g/L) among all treatments. At least 94% of the medium cost was reduced when TAPS and YE were removed from the medium and replaced by CSL. These results showed TAPS buffer could be removed from CP15 medium as well as YE could be replaced by CSL.

**Keywords:** *Alkalibaculum bacchi*, Syngas fermentation, Ethanol, Yeast extract, CSL

# POSTER 30

# INTERACTIONS OF TWO SPECIES IN A HIGH HYDROGEN YIELD BACTERIAL CONSORTIUM

**Wenbin Liu[1], Xinxing Liu[2], Xueduan Liu[2], Guanzhou Qiu[2], Zhili He[1*], Jizhong Zhou[12*]**

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Hydrogen gas production via dark fermentation of cellulose has been investigated as a potential source of renewable energy. Some microorganisms, such as *Clostridium* species, are capable of both cellulose hydrolysis and $H_2$ production. However, both $H_2$ yield and cellulose degradation efficiency remain very low with *Clostridium* species. In this study, a co-culture of *Clostridium cellulolyticum* H10 and *Desulfovibrio vulgaris* Hildenborough was established for an efficient cellulose degradation and high-yield $H_2$ production using a consolidated bio-processing (CBP) strategy. The results showed that the co-culture performed better in terms of cellulose degradation and $H_2$ production (3.3 mol $H_2$ $mol^{-1}$glucose) than the mono-culture (1.8 mol $H_2$ $mol^{-1}$ glucose). Interactions of the two species in this high hydrogen yield bacterial consortium was further analyzed by gene expression and physiological characterization. The results showed that *D. vulgaris* Hildenborough not only used lactate to produce $H_2$ in the system, but also was able to help *C. cellulolyticum* colonize cellulose to speed up cellulose degradation. The microarray data showed that *C. cellulolyticum* genes involved in cellulose degradation were up-regulated under co-culture conditions. However, genes related to the pyruvate /acetate –CoA metabolic pathway were down-regulated, suggesting that *D. vulgaris* Hildenborough in this co-culture system may use lactate produced by *C. cellulolyticum* and relieve a possible inhibition of *C. cellulolyticum* in the regulation of excessive accumulation of pyruvate and lower NADH/NAD+ ratios. This enabled the growth resumption of *C. cellulolyticum* originally inhibited by a self-intoxication of excessive pyruvate resulting from an inefficiently regulated carbon flow. The results suggested that in the co-culture system, the rate limiting step was no longer the pyruvate /acetate –CoA metabolic node as in the mono-culture, but cellulose degradation. Future work will focus on further understanding the mechanisms of cellulose degradation and $H_2$ production and the cell-cell interactions in the co-culture.

## POSTER 31

# IMPROVED XYLITOL PRODUCTION IN THE CHLOROPLAST OF *CHLAMYDOMONAS REINHARDTII* THROUGH CODON OPTIMIZATION AND FUSION OF 16S PROMOTER TO THE 5' ATPA UTR

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In recent years, there has been increased interest in the use of microalgae for biotechnological applications and as a model plant system. Microalgae can potentially be employed for the production of valuable proteins and other biomolecules with applications in the food, nutritional, cosmetic, pharmaceutical, and precursors for biofuel generation. For achieving the full potential of microalgae, genetic improvements are necessary in order to enhance the capabilities of native strains to make algae-based processes more economically attractive. Microalgae are well known for their ability to grow photoautotrophically; however, higher biomass yields and oil content have been reported when algae is grown heterotrophically. Currently significant research efforts are being devoted to breaking down lignocellulosic biomass into fermentable sugars. The fermentable sugars derived from cellulosic feedstocks vary significantly depending on the feedstock and the conversion process. The pentose sugar xylose typically constitutes a substantial portion of the total sugar content from these different feedstocks. In this work, we genetically engineered the microalgae strain *Chlamydomonas reinhardtii* by introducing a fungal D-xylose utilization pathway and for the first time we demonstrate the production of a key intermediate xylitol. For increasing xylitol production, we developed the 'CODON OPTIMIZER' program to optimize the codon usage of the xylose reductase gene (*XR*) for expression in the chloroplast of *C. reinhardtii*. In this program all the codons of *XR* that encode the same amino acid were substituted by the most frequently used synonymous codon. In addition to codon optimization the 16S rRNA was fused to the 5' atpA UTR for increasing the expression level.

## POSTER 32

# CONVERSION OF EASTERN RED CEDAR TO ETHANOL: EFFECT OF PRETREATMENT TIME ON OVERALL WOOD GLUCAN TO GLUCOSE YIELDS

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Eastern red cedar is the most widely distributed indigenous conifer in Oklahoma. The invasiveness of red cedars has brought many ecological concerns to farmers, ranchers and wildlife species. The removal of red cedar and conversion of the polysaccharides into ethanol is a viable option that can be achieved using a pretreatment-enzymatic hydrolysis-fermentation process. Acid bisulfite pretreatment has attained tremendous success with softwoods in recent years. Preliminary studies on this process indicated complete digestion of biomass, but with high (45%) glucan losses. The objective of the current study was to prevent glucan loss during pretreatments by reducing the reaction time during pretreatments. Reaction times of 5 and 10 min were compared to a control of 20 min. Pretreatments were conducted using sulfuric acid loading of 3.75% (w/w) and sodium bisulfite loading of 20% (w/w) at 200˚C with a dry biomass-to-liquid ratio of 1:5. The efficacy of pretreatment was tested using enzymatic hydrolysis with Accelerase 1500 at 0.5 g/g glucan enzyme loading. Wood glucan-to-glucose yield was the response variable used for comparison. Highly digestible biomass (90% digestibility) and low glucan loss (6%) was achieved when a pretreatment time of 10 min was used. This condition resulted in the highest wood glucan-to-glucose yield of 85%. Such a high biomass digestibility was achieved due to removal of lignin and hemicellulose from the biomass. Future experiments will focus on the statistical optimization of the pretreatment process with focus on reducing chemical loading to reduce the cost of the process.

Keywords: Pretreatments, acid bisulfite pretreatment, enzymatic hydrolysis.

## POSTER 33

# THE INHIBITION OF ACETYLENE ON THE FERMENTATION OF PRODUCER GAS BY "*CLOSTRIDIUM RAGSDALEI*"

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Acetylene is a two-carbon unsaturated hydrocarbon that may affect fermentation. *C. ragsdalei*" can utilize CO and $H_2$ in producer gas to produce ethanol and acetic acid. 0.2% Acetylene can exist in the producer gas because of the presence of air during the gasification process. Thus, the objective of this paper is to estimate the effect of acetylene on producer gas fermentation by *C. ragsdalei*. All experiments were done in 250 mL serum bottles with 50 mL working volume. All serum bottles were pressurized to 20 psig and incubated on an orbital shaker (150 rpm) at 37°C. Three concentrations of acetylene (0%, 0.2%, and 0.4%) were fed to *C. ragsdalei* in triplicate. Gas analysis was conducted with GC and FID and TCD detectors. Products were analyzed with GC and a FID detector. Cell growth was inhibited in acetylene treatments. Cell growth ceased at 48h in acetylene treatments with maximum cell mass concentration 0.125 g/L, while maximum cell mass concentration was 0.245 g/L in the control treatment. Maximum ethanol concentration was only 0.2 g/L in acetylene treatements, while maximum ethanol concentration was 0.43 g/L ethanol in the control treatment. $H_2$ and CO consumptions were less in acetylene treatment than the control treatment.

# POSTER 34

# VANADIUM (V)-CATALYZED DEOXYDEHYDRATION OF GLYCOLS

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Reactions which convert biomass-derived polyol substrates into partially/completely deoxygenated products are of great potential value for the sustainable production of chemicals and fuels. We have discovered that metavanadate salts, vanadium(V) oxide and oxo-vanadium(V) complexes catalyze the deoxydehydration (DODH) of glycols and the deoxygenation of epoxides by sulfite or alcohols, producing olefins. The scope, efficiency and selectivity of these reactions with respect to the polyol substrate, the reducing agent, the catalyst, and the reaction conditions will be presented.

## POSTER 35

# SUITABILITY OF MgO as CATALYST FOR ALDOL CONDENSATION OF BIO-OIL COMPOUNDS

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MgO-containing catalysts are known to be active in aqueous-phase aldol condensations of ketones and furanic aldehydes, which are reactions with a high potential in a future bio-oil upgrading process scheme. The model reaction of acetone and furfural has been studied in several papers, but the effects of acid, alcohol and other organics, which are abundant in bio-oil, on the catalysis and the catalyst are not clear. In order to evaluate the potential of MgO-based catalysts for bio-oil upgrading, the performance of these catalysts for aqueous-phase aldol condensation of acetone and furfural was investigated. Acetic acid, methanol, and tetrahydrofuran (THF) were introduced into the reaction mixture to simulate a fraction of bio-oil, and the effect of these components on the aldol condensation of furfural and acetone was analyzed. The reusability of these catalysts was also studied. All of the MgO-based catalysts in this study, including MgO, Mg–Al-oxide, and MgO-$ZrO_2$, showed high activity and selectivity for this aldol condensation in aqueous solution. As shown by infrared spectroscopy, the MgO-based catalysts are instable in acidic solution, and their activities decrease with the concentration of acid. The presence of methanol or THF in aqueous solution also inhibits aldol condensation on MgO-based catalysts to various degrees and makes the reusability of these catalysts worse than it is after use in water. Therefore, instability in acid media, strong solvent effects and poor reusability indicate that MgO and perhaps other strongly basic oxides are unsuitable for bio-oil upgrading by liquid-phase aldol condensation.

# POSTER 36

# ANALYSIS OF SINGLE ENZYME ACTIVITIES ON THE HYDROLYSIS OF GRAIN SORGHUM STOVER

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Grain sorghum stover (GSS) is a valuable lignocellulosic side product of grain sorghum cultivation. The demand for sustainable feedstocks for bioproduct or biofuel production increases where GSS, similar to corn stover, can be utilized. The enzymatic hydrolysis of GSS needs to be optimized to achieve maximum sugar production. Cellulose degradation is well understood. However, hemicellulose has a unique composition for each substrate and requires a mixture of different enzymatic activities for complete breakdown. Enzyme mixtures have shown synergistic effects and can improve lignocellulose degradation. A factorial design was conducted on liquid hot water pretreated and non-pretreated GSS using xylanase (XynB), mannanase (Man) and ferulic acid esterase (FAE) expressed in *Aspergillus nidulans*, as well as Novozyme Cellic CTec2. In each test, the protein concentration was kept at 7.5 mg/g glucan and divided into equal amounts when more than one enzyme was present. The results on pretreated GSS showed the best glucose conversion using CTec2 alone with a value of 90.0%. The maximum glucose conversion on non-pretreated GSS was only 21.2% with CTec2, XynB and FAE. Future tests will concentrate on the addition of stabilizers to improve enzyme stability over time, milder pretreatment conditions, and the effect of higher enzyme concentrations.

## POSTER 37

# EMPIRICAL MODEL TO PREDICT INFIELD THIN LAYER DRYING RATE OF CUT SWITCHGRASS

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A series of 62 thin layer drying experiments were conducted to evaluate the effect of environmental conditions on drying rate of switchgrass. An environmental chamber was fabricated that can simulate field drying conditions of solar radiation, vapor pressure deficit and wind speed. The range of environmental variables tested during the study was based on the environmental conditions encountered during the field drying of switchgrass. An empirical model based on maturity stage of switchgrass was also developed during the study. Separate drying rate equations were developed for seed development stage of maturity, and seed shattering and seed shattered stage of maturity. During both maturity stages, solar radiation intensity was positively and strongly correlated with drying rate. Vapor pressure deficit was also positively correlated with drying rate but the effect was not significant for later stages of maturity. The effect of wind was to decrease the drying rate and was significant during both maturity stages. A variable effect of wind was also observed during low radiation intensity. An increase in wind speed increased the drying potential of switchgrass under low radiation intensity. Initial moisture content was weakly correlated with drying rate having both negative and positive affect.

## POSTER 38

# SUSTAINABLE FEEDSTOCK PRODUCTION SUPPLY SYSTEMS TO SUPPORT CELLULOSIC BIOREFINERY INDUSTRIES: LOGISTICS UPDATE

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Oklahoma State University is currently leading a large multi-disciplinary USDA Biomass Research and Development Initiative project. A major component of this project is the feedstock supply system evaluation. Collaborators include: AGCO Industries, Stinger Inc., The Samuel Roberts Noble Foundation, and Idaho National Laboratories. The project deploys and assesses an integrated system of supplying energy crops (switchgrass, mixed grasses, and forage sorghum) from the Panhandle and the Central/South-Central regions of the state. The feedstocks were harvested at three time periods: (1) plant physiological maturity; (2) soon after the first killing frost; and (3) early winter. A fourth cutting of switchgrass took place in late winter/early spring. These four periods roughly corresponded to harvests in August, October, December, and February, respectively. For forage sorghum, an additional 2-cut harvest system was employed with the 1st harvest in August and 2nd in December. The harvesting, baling and transport to storage area were completed using commercially available equipment. Data was collected throughout the process for equipment evaluations. Large square bales were stacked and stored at three different conditions: (1) tarped stacks on gravel pads; (2) uncovered stacks on soil; and (3) wrapped stacks. At two-, four-, and six-month intervals, bales were retrieved from the stacks for quality evaluations. Samples are being collected to assess bale moisture, density, dry matter loss, and quality changes. This is the third year of the four-year project. This poster will provide an overview and current status of this large scale multi-region Oklahoma bioenergy feedstock logistics study.

## POSTER 39

# EVALUATION OF SWITCHGRASS ROOT CHARACTERISTICS INFLUENCE BY ROW SPACING AND CULTIVAR DIFFERENCES

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Biofuels that replace fossil fuels have the potential to reduce greenhouse gases in the atmosphere. Switch grass (*Panicum virgatum* L.), a dedicated biofuel crop has high potential for carbon sequestration from atmosphere as well as for improving soil quality via its root system. However, information on switchgrass root growth and its distribution is extremely limited. The objective of this study was to analyze root characteristics and distribution pattern influence by row spacing and cultivar differences. The influence of row spacing (0.19m, 0.38m and 0.76m), differences in root characteristics of 3 lowland and 7 upland switchgrass cultivar were determined. Measurement of root parameters was carried out with an image analysis system (winRHIZO) with grey level image type with 100 dpi resolution. The cultivar Blackwell had 58% of root biomass in the surface 0-10 cm layer, while Southlow had 62% of root biomass below the surface from 10-110cm. Similarly, root length density (RLD) and root weight density (RWD) of lowland cultivar was found to be increased at the end of growing season. The root length across cultivars during the July harvest was greater (27%) in the top 20 cm, while at second harvest in December the root length was greater by 30% at 20-110 cm depth. No significant interactions were found between row spacing and harvest time, but it was significant with soil depth for root biomass and root characteristics. By the end of season, RLD decreased at upper depth of 0-10 cm while average diameter and RWD increased all over soil profile. At the end of the season the fine roots (0-0.05 mm) decreased by more than 24 %, which reduced RLD at top depths. Differences between the cultivars suggest that cultivar selection owing to different cultivation practices will be an important determinant of C sequestration by its root system.

# POSTER 40

# COST EFFECTIVELY MEASURING THE MOISTURE CONTENT OF GRASS BALES

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The moisture content of feedstock bales used for conversion to biofuels varies significantly within each bale. The moisture content of bales is often measured before going into storage, but the variance within each bale, and the lack of a standard measuring point or points leave the measurement of moisture content open for unnecessary error. While one way to reduce the error and account for varied moisture within a bale is to increase the number of sample points, increasing the number of samples also increases the cost and time associated with taking moisture measurements. The goal of this presentation is to find a way to increase the accuracy of the estimated moisture profile of a bale while minimizing the number of core samples necessary to generate the estimate. In January 2012, between 18 and 60 core samples were taken from switchgrass and mixed-grass bales that had been in storage. These core samples were taken from pre-selected points within each bale, and measured for moisture content to create a moisture content profile of each bale as well as each storage stack. Analysis of the data included reducing the sampling points down to one point per bale and comparing an estimated moisture profile with the profile generated by the whole dataset. This analysis lays the groundwork for creating a standard procedure to measure moisture content within both single bales, and stacks of stored bales.

# POSTER 41

# SCENARIO OPTIMIZATION MODEL FOR BIOMASS SUPPLY CHAIN DESIGN AND ANALYSIS

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The commercialization of biofuel industry is highly dependent on the development of efficient biomass supply chain system. The effect of uncertain parameters on biomass supply chain needs to be investigated for realistic and robust economic assessment of the system. The present study focuses on the development of scenario optimization model for maximizing profit of biomass supply to biorefinery under weather uncertainty. The model determines material flow, number of harvest units, in-field transportation units, roadway transportation units, storage treatment, and allocation of units to sites. The applicability of proposed model is demonstrated by developing a case study for Abengoa ethanol biorefinery at Hugoton, Kansas. The model optimizes the design and operation of biomass supply chain network and minimizes the financial risk on investment by the biorefinery in terms of harvest, inventory, and transport logistics.

# POSTER 42

# SEASONAL VARIABILITY IN EVAPOTRANSPIRATION, WATER USE EFFICIENCY, AND ENERGY PARTITIONING IN SWITCHGRASS

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Evapotranspiration (ET), water use efficiency (WUE), and energy partitioning in switchgrass (*Panicum virgatum* L.) ecosystem is crucial to understand its water and energy balances. We measured $CO_2$, $H_2O$, and energy fluxes over switchgrass field in Chickasha, OK, USA using eddy covariance method. The major objective of this study was to quantify and examine seasonal variations in ET, WUE, and seasonal distributions of energy partitioning in response to environmental controls. Seasonal (May to mid November) cumulative ET (450 mm) exceeded cumulative rainfall (432 mm) for the period. More than 192% of rainfall water was lost to the atmosphere via ET during June to September. It indicated that the crop experienced severe drought during the growing season. Evapotranspiration showed clear seasonality with 3 – 4 mm $day^{-1}$ during late May and June to low rates of about 0.5 mm $day^{-1}$ during late growing season in November. The ET rate decreased during dry periods. On seasonal scale, more energy was partitioned to sensible heat flux (H) than latent heat (LE) due to drought. However, LE was the dominant flux under wet conditions during active growing season. Regression of daily day time net $CO_2$ ecosystem exchange (NEE) or gross ecosystem photosynthesis (GEP) to daily daytime ET on a seasonal scale yielded WUE of 5.72 and 7.55 g $CO_2$ $m^{-2}$ $mm^{-1}$ ET, respectively. Seasonal patterns in WUE were observed with smaller WUE during drought due to more rapid reduction in carbon assimilation than ET. These results establish the major role of precipitation in determining water and energy balances in switchgrass.

# POSTER 43

# HARVESTING MICROALGAL BIOMASS BY FLOCCULATION

**Yan Zhu[1], Nurhan Turgut Dunford[1]* and Carla Goad[2]**

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Algal biomass shows significant promise as feedstock for biofuel and bio-based product manufacturing. One of the major challenges involved in large scale algal biomass production is the difficulty of harvesting microalgal cells from the dilute suspensions of a culture. In this study, three flocculation methods, pH adjustment, biopolymer (chitosan) addition, and electro-flocculation were examined for their efficiency to recover biomass produced by *Picochlorum oklahomensis* (PO), a microalgae strain native to Oklahoma. A generalized linear mixed model using a beta distribution for response was utilized for optimization of the process variables. The pH flocculation efficiency was below 10% between pH 4 and 10. A sharp increase in flocculation efficiency was observed over pH 10. The highest flocculation efficiency, 97%, was achieved at pH 13. Three process parameters, chitosan: algal biomass ratio (CAR), pH, and settling time (ST) were investigated as the independent variables for optimization of a biopolymer flocculation process. There were significant 3-way interactions among the process variables. The highest chitosan flocculation efficiency, 98.4%, was obtained at CAR of 0.36, pH 9 and ST of 12 h. The independent variables for the electro-flocculation were current, operation time (OT) and settling time (ST). A factorial experimental design was used for the optimization study. The electro-flocculation efficiency improved with increasing current, OT and ST. The highest electro-flocculation efficiency, 99.7%, was obtained at current of 0.8 A, OT of 15 min and ST of 12 h. This study demonstrated that pH adjustment, chitosan addition and electro-flocculation were effective methods to flocculate PO cells under the conditions examined in this study. The scalability of these flocculation techniques for commercial algal biomass production needs to be further examined. The selection of the best flocculation technique for a given application would be based on the economic as well as the technical feasibility of these processes.

# POSTER 44

# UTILIZATION OF SOFT DRINK WASTE FOR PRODUCTION OF ETHANOL

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Food processing industries generate large amounts of waste, including both liquid and solid waste, which represents a significant opportunity to reclaim valuable products and byproducts. Bottling plants generate soft drink waste streams containing carbohydrates, which have potential value for production of ethanol. Currently the waste product is sent to wastewater treatment plants for disposal, in limited quantities.

The overall goal of the project is to determine the ease of fermentation of waste soft drinks into ethanol. The specific objectives were to evaluate the sugar conversion efficiencies of several common soft drinks and to determine the effects of temperature and yeast inoculation level on fermentation rate. Four common soft drinks, including Coke®, Mountain Dew® (Mt. Dew), Pepsi® and Sprite® were compared during fermentation in 400 ml plastic containers. Two levels of pH adjustment were tested, including no adjustment and pH adjustment to approximately 5.0. Fermentation at three different temperatures was evaluated, including 25, 31, and 37$^{o}$C. Three different levels of yeast inoculation (ranging from 0.2 to 1.0 g/L) were also investigated. In each experiment, fermentation samples were taken daily for ten days and analyzed for ethanol, sugars (glucose, and fructose), and fermentation by-products using an HPLC. Results showed that with both pH adjustment and nutrient addition, all 4 samples exhibited very high sugar conversion efficiency (>90%). The increase in fermentation temperature from 25 to 37$^{o}$C showed a significantly increased rate of fermentation for the first 48 hours. Also, increasing the yeast inoculation level from 0.2 to 1.0 g/L increased the ethanol productivity from 0.31 g/L/h to 0.76 g/L/h at 48 hours. Similar trends were seen among all 4 soft drinks tested. Conversion of waste soft drink products to ethanol is one example where utilization of waste products can be used to help industries meet future energy and resource needs.