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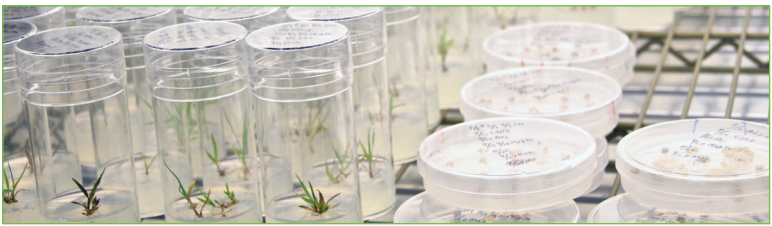

EPSCoR SCIENTISTS:
FORGING A RESEARCH PATH FOR RENEWABLE ENERGY DEVELOPMENT THROUGH CELLULOSIC BIOFUELS

OKLAHOMA EPSCoR

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Cellulosic Bioenergy Research Poster Session

Tuesday, April 23, 2013
Exhibit Hall West * Wes Watkins Conference Center
Oklahoma State University

Student Hybrid Poster Competition * Posters 1-12

Feedstock Development * Posters 13-27

Microbial Conversion * Posters 28-35

Chemical Conversion * Posters 36-38

Other/Misc. & Late Submissions * Posters 39-41 (& up)

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

STUDENT PRESENTERS

Hybrid Poster Competition

No.	Student Presenter	University	Scientific Poster Title
1	Laxman Adhikari	Oklahoma State University	Synchronization & Isolation of Switchgrass for Interecotypic Hybrid Development
2	Prakash R. Bhoi	Oklahoma State University	Equilibrium Based Process Modeling of a Packed Bed Scrubbing System for the Removal of Model Tar Compounds
3	Matthew B. Couger	Oklahoma State University	Genome Sequence of the Anaerobic Gut Fungi *Orpinomyces* sp. Strain C1A
4	Miguel A. Gonzalez Borja	University of Oklahoma	Alkylation Reactions for the Upgrading of Bio-Oil in the Presence of LIquid Water Using Hydrophobic Zeolites
5	Fan Lin	University of Oklahoma	Identification of Grass Cell Wall Synthesis Genes from Correlations Between Gene Expression & Cell Wall Composition in Rice
6	Lei Nie	University of Oklahoma	Improving Carbon Retention in Biomass Conversion by Alkylation of Phenolics with Small Oxygenates
7	Taiwo Omotoso	University of Oklahoma	Mechanism of Methoxybenzene Conversion on Ruthenium Titania Catalysts
8	Naveen Pessani	Oklahoma State University	Multi-Stage Counter Current Diffusion of Sugar from Sweet Sorghum for Use in a Dual Feedstocks Process
9	Ashokkumar M. Sharma	Oklahoma State University	Reaction Kinetics-Based Biomass Gasification Model to Predict Syngas Quality Suitable for Biofuel Production
10	Christopher Waters	University of Oklahoma	Deactivation of Zeolite Catalysts During Upgrading of Pyrolysis Vapors
11	Tyler Weirick	Oklahoma State University	Ligpred: Comprehensive Prediction System for the ID & Classification of Enzymes Related to Synthesis & Degradation of Lignin
12	Tao Xu	University of Oklahoma	Dockerin-Containing Protease Inhibitor Protects Key Cellulosomal Components from Proteolysis in *Clostridium Cellulolyticum*

Cellulosic Biofuels Research

No.	Primary Presenter	University	Scientific Poster Title
13	Hongxu Dong	Oklahoma State University	QTL Mapping for Reproductive Maturity in Lowland Switchgrass Populations
14	A. J. Foster	Oklahoma State University	Use of Hyperspectral Remote Sensing to Discriminate Nitrogen Treatments in Switchgrass and Biomass Sorghum
15	Upinder Gill	Samuel Roberts Noble Foundation	A Multifaceted Approach to Control Switchgrass Rust by Employing Non-Host Resistance and Chemical Control
16	Yong-Fang Li	Oklahoma State University	Transcriptome Analysis of Heat Stress Response in Switchgrass *(Panicum Virgatum L.)*
17	Hao Lin	Oklahoma State University	Overexpression of Medicago *Wox* Gene *Stenofolia* Improves Biomass Production by Promoting Switchgrass Cell Proliferation
18	Linglong Liu	Oklahoma State University	Two Self-Compatible Lowland Switchgrass Plants Set Completely Outcrossed Seeds Under the Field Conditions
19	S. O. Makaju	Oklahoma State University	Genetic Diversity Among 5 Lowland Switch-grass Cultivars Detected by AFLP Markers
20	Anuradha Mukherjee	Oklahoma State University	Ethanol from Sweet Sorghum: Role of the Alcohol Separation Unit at OSU
21	Lifang Niu	Oklahoma State University	Control of Flowering Time in Switchgrass for Improvement of Biomass Yield
22	Gabriela K. Orquera	Oklahoma State University	Three DNA Barcode Genes from Switchgrass Rust Fungus *Puccinia Emaculata*
23	David Ponder	University of Oklahoma	Investigating Cell Wall Changes During Grass Lateral Root Emergence
24	Vince Schielack III	Oklahoma State University	Moisture Content Sampling Variance with Large Format, Square Hay Bales: Evaluation of Sampling Protocol
25	Ramanjulu Sunkar	Oklahoma State University	Identification of Conserved Novel & Stress-Responsive MicroRNAs in Switchgrass
26	Pradeep Wagle	Oklahoma State University	Comparison of Net Ecosystem Exchange of $C0_2$ & H_2O Between High Biomass Sorghum & Switchgrass in U.S. S. Great Plains
27	Chengcheng Zhang	University of Oklahoma	Identification of OsAT10 Interactors by Yeast Two-Hybrid

Cellulosic Biofuels Research

No.	Primary Presenter	University	Scientific Poster Title
28	Mamatha Devarapalli	Oklahoma State University	Syngas Fermentation in a Trickle Bed Reactor Using *Clostridium Ragsdalei*
29	Babu Z. Fathepure	Oklahoma State University	Isolation & Characterization of Bacteria that Degrade Lignin in Plant Biomass
30	Yong Jin Lee	University of Oklahoma	Analysis of Cellulosomal Gene Expression in Cellulolytic Clostridia Using a Multi-Genome Array
31	Yongchao Li	University of Oklahoma	Combination of Sporulation Disruption & Carbon Overload Alleviation Helps Increase Cellulose Catabolism in Clostridium Cellulolyticum
32	Yuan Li	University of Oklahoma	Microarray & Network Analysis Reveals Gene Expression Profiles of Carbohydrate Active Enzymes in *Penicillum Expansum* YT02
33	Audra S. Liggenstoffer	Oklahoma State University	The Anaerobic Fungal Isolate *Orpinomyces* SP. Strain C1A is an Efficient Plant Biomass Degrader Under Anaerobic Conditions
34	Kan Liu	Oklahoma State University	Drop-In Biofuels Production by a Mixed Culture Fermentation of Syngas
35	Michael Ukpong	University of Oklahoma	Investigating the Role of Duplicated Alcohol Dehydrogenase Genes from *Clostridium Carboxidivorans* Strain P7 in Alcohol Production

CHEMICAL CONVERSION

Cellulosic Biofuels Research

No.	Primary Presenter	University	Scientific Poster Title
36	Camille Boucher-Jacobs	University of Oklahoma	Oxorhenium Catalyzed Deoxydehydration of Polyols Using Benzl Alcohols as Reductant
37	Garry Chapman, Jr.	University of Oklahoma	Vanadium (V) - Catalyzed Deoxy-dehydration of Glycols & Epoxides
38	J. Michael McClain	University of Oklahoma	Mechanistic Investigation of Oxorhenium Catalyzed Deoxydehydration of Polyols

OTHER RESEARCH

39	M. D. Buser	Oklahoma State University	Performance of Large Bale Switchgrass & Energy Sorghum Storage Stacks: OSU BRDI Project Overview
40	Xuesong Li	OKlahoma State University	An Efficient Statistical Method for QTL Mapping in Tetraploid Organisms
41	E. A. Miller	Oklahoma State University	Field-Scale Switchgrass & Energy Sorghum Harvesting: OSU BRDI Project Update

POSTER 1

SYNCHRONIZATION AND ISOLATION OF SWITCHGRASS FOR INTERECOTYPIC HYBRID DEVELOPMENT

STUDENT PRESENTER: LAXMAN ADHIKARI

Laxman Adhikari andYanqi Wu
Department of Plant and Soil Sciences
Oklahoma State University, Stillwater, OK

Interecotypic hybrid in switchgrass refers to the plants derived from crossing between genotypes of upland and lowland ecotypes. These hybrids are the possible sources of male sterile traits which can be used in different breeding activities, generating female lines in crossing, facilitating heterosis utilization and hybrid seed production. Proper hybridization can only be achieved when both pollinator and seed parent flower at the same time. Objective of this experiment was to synchronize plants of upland and lowland ecotypes and see its effects in hybrid development. The other objective of this study was to determine the significance of isolation distance in obtaining pure breeding progenies. Therefore, the flowering activities of nine upland and lowland plants were observed in the greenhouse in early 2012.The earlier headed upland plants were trimmed about 3 inches below from the base of seed head and each shoot was observed individually. In March 2012, the first pair of synchronized upland and lowland ecotypes, Dacotah-14 X NL225/7, was obtained. The synchronization process continued to last week of April when we synchronized all 9 combinations. Six plant pairs were isolated in one greenhouse and remaining three crossing combinations were isolated in next greenhouse providing relatively better isolation. Seeds were collected from these crossing pairs and analyzed for genetic origin using simple sequence repeated (SSR) markers; we used two SSRs for each genomic DNA. Well isolated and perfect synchronized plant pairs produced higher percentage of hybrid seeds (up to 100 %) and very low selfed seeds (about zero %) with negligible contamination, while the reverse condition was observed in the weakly synchronized and nearer isolated crossing combinations.

POSTER 2

AN EQUILIBRIUM BASED PROCESS MODELING OF A PACKED BED SCRUBBING SYSTEM FOR THE REMOVAL OF MODEL TAR COMPOUNDS

STUDENT PRESENTER: PRAKASH R. BHOI

Prakash R. Bhoi, Krushna N. Patil, Ajay Kumar, and Raymond L. Huhnke

Department of Biosystems & Agricultural Engineering

Oklahoma State University, Stillwater, OK

Wet scrubbing systems often use water as a solvent for producer gas tar removal. The major drawbacks with the water-based wet scrubbing systems are poor solubility of tar compounds and the costly wastewater treatment. Vegetable oils can be considered as a potential solvent for the removal of producer gas tars. This study considers soybean oil as a solvent for the removal of model tar compounds. Soybean oil is one of the largest sources of vegetable oil in the United States. In addition, soybean oil is renewable, plant based, CO2 neutral, and hazard free. The aim of this study is to develop an equilibrium based process model of a wet packed bed scrubbing system. The wet packed bed column is modeled using "RadFrac" block of the Aspen PlusTM software. Raschig rings 6 mm in size are used as the packing media. A model tar compound mixture consists of benzene, toluene and ethyl benzene. Because the model tar compounds are non-polar, Peng-Robinson and RK-Soave thermodynamic property methods are considered for the process modeling. The effect of packing bed height, solvent temperature, and liquid-to-gas ratio on the tar removal efficiency will be presented. The process model will be useful in the design and optimization of a laboratory and pilot scale oil-based packed bed scrubbing system for removal of biomass producer gas tars.

POSTER 3

GENOME SEQUENCE OF THE ANAEROBIC GUT FUNGI *ORPINOMYCES* SP. STRAIN C1A

STUDENT PRESENTER: MATTHEW BRIAN COUGER

M. B. Couger, Noha H. Youssef, Audra S. Liggenstoffer, and Mostafa Elshahed

Department of Microbiology and Molecular Genetics

Oklahoma State University, Stillwater, OK

Anaerobic gut fungi (AF) represent a distinct basal fungal phylum (Neocallimastigomycota), and reside in the rumen and gut of herbivores. AF play an important role in plant biomass degradation in ruminant herbivores, represent the only strictly anaerobic fungal group, and are efficient colonizers of ingested plant materials in cow rumens. Here, we report on the sequencing and analysis of he genome of the AF *Orpinomyces* sp. strain C1A. The genome was sequenced using a combination of Illumina and PacBio SMRT technologies. The large fungal genome (100.95 Mb, 16,347 genes) displayed extremely low G+C content (17.0%), large non-coding intergenic regions (73.1%) and proliferation of microsatellite repeats (4.9%). Analysis of the lignocellulolytic machinery revealed an extremely rich repertoire of CAZy enzymes, with evidence of horizontal gene acquisition from multiple rumen prokaryotic lineages. The genome contained all genes required for the degradation of cellulose, as well as xylans mannans, and mixed β-glucans. The genome appears to be highly adapted to the degradation of xylans, the prevalent hemicelluloses in grasses (order Poales). We argue that the unique features in the genome, when compared to other members of the Mycota, is a reflection of their distinct phylogenetic position and unique evolutionary trajectory.

POSTER 4

ALKYLATION REACTIONS FOR THE UPGRADING OF BIO-OIL IN THE PRESENCE OF LIQUID WATER USING HYDROPHOBIC ZEOLITES

STUDENT PRESENTER: MIGUEL A. GONZALEZ BORJA

Miguel A. Gonzalez Borja and Daniel E. Resasco
School of Chemical, Biological and Materials Engineering
University of Oklahoma, Norman, OK

One challenge in the upgrading of bio-oil is to minimize the loss of carbon from small oxygenates such as acetic acid, acetol, acetaldehyde, among others. These are usually lost as light gases during conventional hydrodeoxygenation (HDO) processes and cannot be recovered as liquid products. We have proposed an upgrading strategy that incorporates small oxygenates into larger molecules prior to HDO. In a multistage pyrolysis unit, three different fractions of bio-oil can be obtained: a low temperature cut containing small oxygenates together with water; a medium temperature cut with sugars derived compounds; and a higher temperature cut composed of phenolics. The first cut can undergo coupling reactions to yield acetone, which is then hydrogenated to 2-propanol, a very effective alkylating agent. We studied the alkylation of the phenolic compound m-cresol with 2-propanol in a single oil phase reactor using HY zeolite. We were able to synthesize phenolics in the C10 – C16 range, which upon hydrodeoxygenation could yield hydrocarbons suitable for diesel fuel. On the other hand, when the reaction was performed in the presence of water, the HY zeolite rapidly lost its alkylation activity. It is known that hot liquid water can severely affect the structure of zeolites, causing a fast deactivation of the catalyst in an aqueous environment. To address this problem our group synthesized a hydrophobic HY zeolite, which has higher stability due to the prevention of contact between bulk water and the zeolite. This catalyst showed exceptional performance in the biphasic system when compared to the untreated HY zeolite. A kinetic model was used to estimate the rates of reaction and catalyst deactivation for both untreated and hydrophobic zeolites. In this way it was possible to propose a reaction pathway and to quantify the difference between untreated and hydrophobic zeolite, confirming the great advantage of the latter material.

POSTER 5

IDENTIFICATION OF GRASS CELL WALL SYNTHESIS GENES FROM CORRELATIONS BETWEEN GENE EXPRESSION AND CELL WALL COMPOSITION IN RICE

STUDENT PRESENTER: FAN LIN

F. Lin[1], C. Manisseri[2], A. Fagerstrom[4], B. Williams[3], D. M. Chiniquy[2,3], M. L. Peck[1], P. Saha[1], M. Vega-Sanchez[2,3], J. U. Fangel[4], W. T. Willats[4], H. V. Scheller[2], P. C. Ronald[2,3], L. E. Bartley[1,2,3]

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[2]Joint BioEnergy Institute, Emeryville, CA and Lawrence Berkeley National Laboratory, Berkeley, CA

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[4]Department of Plant and Environmental Sciences, University of Copenhagen, Denmark

Grass cell walls serve as animal feed and are a potential sustainable feedstock for biofuel production. Glycosyltransferases (GTs) and acyltransferases (ATs) perform cell wall synthesis but the roles of many GT and AT families/members remain undetermined. We are identifying the correlations between the putative cell wall synthesis genes and cell wall components to establish testable hypotheses of gene function. In this study, we took 30 samples from different organs and different developmental stages of rice (Oryza sativa) and measured their chemical cell wall composition and properties including monosaccharide components, phenolics, and enzymatic digestibility. With Comprehensive Microarray Polymer Profiling (CoMPP), we measured cell wall epitopes in the same samples. We also used qPCR to measure the expression of grass-diverged genes that are putatively involved in cell wall synthesis. We analyzed the data with both Pearson's and Gini correlations, the latter of which is a hybrid of parametric and non-parametric methods. We identified 107 significant correlations between cell wall components and GTs and ATs in whole data set ($q<0.05$, correlation coefficient>0.6). For example, among expected correlations, we found a correlation between p-coumarate and the expression of a recently characterized p-coumarate monolignol transferase gene. We will also discuss a number of novel correlations that provide leads for better understanding cell wall synthesis in grasses and improving cell walls for economic uses.

POSTER 6

IMPROVING CARBON RETENTION IN BIOMASS CONVERSION BY ALKYLATION OF PHENOLICS WITH SMALL OXYGENATES

STUDENT PRESENTER: LEI NIE

Lei Nie and Daniel Resasco
Center for Biomass Refining
University of Oklahoma, Norman, OK

Phenolics and alcohols derived from pyrolysis oil can be alkylated to retain the carbon from small oxygenates in the gasoline/diesel range liquid products. A two-stage process has been designed to convert light fractions of bio-oil (rich in small oxygenates) together with phenolics. In the first stage, acetone, produced by ketonization of acetic acid, a major component of bio-oil, is selectively hydrogenated to alcohol. In this stage, Pt-Fe/SiO2 was found to be effective for converting the ketone while preserving the aromatic ring of the phenolic. In the second stage, the resulting alcohols alkylate the phenolic compounds over an H-Beta zeolite. We have compared the effectiveness of three C3 alkylating agents. The m-cresol alkylation activity was found to follow the trend 2-propanol > propylene > 1-propanol. While in all cases, propylene is the real alkylating agent, the water produced during dehydration of the alcohol was found to play a positive role in maintaining the activity.

POSTER 7

MECHANISM OF METHOXYBENZENE CONVERSION ON RUTHENIUM TITANIA CATALYSTS

STUDENT PRESENTER: TAIWO OMOTOSO

Taiwo Omotoso and Steven Crossley
School of Chemical, Biological and Materials Engineering
University of Oklahoma, Norman, OK

Renewable fuels and chemicals can be obtained from fast pyrolysis of a wide range of biomass sources and this process is attractive because of its relative cost effectiveness when compared with other thermal methods. However characteristics of the liquid bio-oil product obtained such as low heating value, high oxygen content and thermal instability makes it a poor candidate for use as transportation fuels. In this study methoxybenzene, which is a simple model compound derived from the pyrolysis of the lignin fraction of biomass, is employed to understand the mechanism of conversion of methoxy groups on aromatic rings over a bifunctional Ru/TiO2 catalyst. Vapor phase flow reactions were conducted at 400°C under atmospheric pressure of hydrogen. The reaction pathway and product distribution for this simple molecule were used to propose a mechanism for the more complex molecules derived from lignin, such as guaiacol, which have both methoxy and hydroxyl functions. A sequential mechanism for the conversion of methoxybenzene to benzene has been proposed drawing from the reaction data with the evolution of phenol as a primary product. Also it has been shown that catalyst pre-treatment conditions, more specifically calcination temperature, has an important effect on both the activity and selectivity of the Ru/TiO2 system.

POSTER 8

MULTI-STAGE COUNTER CURRENT DIFFUSION OF SUGAR FROM SWEET SORGHUM FOR USE IN A DUAL FEEDSTOCK PROCESS: THE EFFECT OF TEMPERATURE, LIQUID-SOLID RATIO AND PARTICLE SIZE

STUDENT PRESENTER: NAVEEN PESSANI

N. Pessani, P. Venkata, D. Bellmer, H. Atiyeh, and G. Kakani

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Sweet sorghum is a promising bioenergy crop due to its high productivity, low input requirements and versatility. Due to a relatively short harvest window and a lack of storability of the directly fermentable sugars, a processing plant dedicated to sweet sorghum in temperate climates may be operated only seasonally. In order to maintain a more continual feedstock supply and improve economic viability, a dual feedstock process utilizing sweet sorghum and sugar beets is being investigated. In the Southern Great Plains, winter beets and sweet sorghum would complement each other well in a dual processing scenario. Ideally, the same sugar extraction process could be used for both crops. The objective of this project was to evaluate a diffusion process for extraction of sugar from sweet sorghum.

Diffusion experiments were conducted using a four stage countercurrent diffusion process. Sweet sorghum stalks were chopped to coarse and fine particle sizes using a Seydelman bowl chopper. Samples of 200 g were used to test the effects of process temperature and liquid-to-solid ratio on sugar extraction efficiency. Three different temperatures (60, 70, 80 °C), three different liquid-to-solid ratios (1:0.75, 1:1 and 1:1.5), and two different contact times (10 and 15 min) were evaluated. Results showed that liquid-to-solid ratio is critically important in the sugar extraction efficiency, and temperature seems much less important. Sugar extraction efficiencies ranged from 45-85%, with the highest extraction occurring at the highest liquid-to-solid ratio.

Keywords: Sweet sorghum, diffusion, counter current, sugar, extraction

POSTER 9

REACTION KINETICS-BASED BIOMASS GASIFICATION MODEL TO PREDICT SYNGAS QUALITY SUITABLE FOR BIOFUEL PRODUCTION

STUDENT PRESENTER: ASHOKKUMAR M. SHARMA

Ashokkumar M. Sharma[1], Ajay Kumar[1], Sundar Madihally[2], Rob Whiteley[2], Raymond L. Huhnke[1]

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[1,2]Oklahoma State University, Stillwater, OK

Syngas, the main gasification product, is a well-known intermediate for making biofuels, biochemicals and biopower. Literature shows several modeling studies on biomass gasification using the Gibbs equilibrium approach. However, the assumption made in the Gibbs equilibrium model that the gasification reactions reach equilibrium condition does not occur in application due to the short residence time and multiphase reactions. The objective of this study was to develop a reaction kinetics-based gasification model using a continuous stirred-tank reactor (CSTR) to predict syngas composition and yield, and validate the model using experimental results. Kinetics of gasification reactions were obtained from the literature. The mass balance and fundamental design equations of CSTR were used to determine the extents of gasification reactions. The gasification model was studied by varying air and biomass flowrates between 6.4 to 6.8 kg/h and 2.9 to 4.2 dry kg/h, respectively. Gasification temperature ranged from 809 to 893°C at a constant operating pressure of 1 atm. The equilibrium-based gasification model was also developed using Aspen Plus and used as a baseline to evaluate the improvement in prediction of the reaction kinetics-based gasification model. Results showed that the Gibbs equilibrium -based gasification model predicted CO and H2 yields 78% and 180%, respectively, higher than the corresponding yields obtained through experiment. On the other hand, the reaction kinetics based gasification model predicted yields of CO and H2 within 28% and 21%, respectively, of the corresponding yields obtained through experiment.

Keywords: Gasification, biomass, syngas, Gibbs equilibrium model, CSTR

POSTER 10

DEACTIVATION OF ZEOLITE CATALYSTS DURING UPGRADING OF PYROLYSIS VAPORS

STUDENT PRESENTER: CHRISTOPHER WATERS

Shaolong Wan, Christopher Waters, Rolf Jentoft, Steven Crossley, Lance Lobban, Daniel Resasco, and Richard Mallinson
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University of Oklahoma, Norman, OK

Decentralized renewable fuel production can become a reality with sweet sorghum feedstock. Sweet sorghum requires lower water and nutrient input, it is easy to obtain and process the fermentable sugars in its juice, and requires little attention in terms of pH or temperature control during fermentation. However, without a technically robust downstream separation system, the success of sweet sorghum based ethanol can be compromised.

The Alcohol Separation Unit at Oklahoma State University has been constructed as both, a research and demonstration facility, for sweet sorghum bioethanol production. Located at the Bioenergy Laboratory, on an off-campus outdoor location, the pilot plant features two distillation columns, both taller than 35 ft, which form the heart of this separation process. The facility features state-of-the-art process technology – column internals, heat transfer equipment, instrumentation and software for measurement, monitoring and control.

Upon operation outstanding issues of operating cost, maintenance, specifically in terms of water use, waste water generation, process fouling, and project economics are expected to be resolved. For the purpose of this presentation, we will focus on the technical features of the Alcohol Separation Unit, and how the design caters to sweet sorghum bioethanol production on a small, on-farm scale.

POSTER 11

LIGPRED A COMPREHENSIVE PREDICTION SYSTEM FOR THE IDENTIFICATION AND CLASSIFICATION OF ENZYMES RELATED TO THE SYNTHESIS AND DEGRADATION OF LIGNIN

STUDENT PRESENTER: TYLER WEIRICK

Tyler Weirick[1], Babu Z. Fathepure[2],Ramamurthy Mahalingam[3], and Rakesh Kaundal[1]

[1]National Institute for Mocrobial Forensics & Food and Agricultural Biosecurity

[1,3]Department of Biochemistry & Molecular Biology, [2]Department of Microbiology & Molecular Genetics

[1,2,3]Oklahoma State University, Stillwater, OK

Lignin is a major component of plant cell walls and the second most abundant organic polymer on earth. This heterogeneous aromatic polymer, despite having significantly higher energy density than cellulose, is extremely recalcitrant presenting a major challenge to the biofuels industry. Research has looked to plants to reduce the recalcitrance and quantity of lignin produced and to lignin degraders for more efficient enzymes as solutions to reducing the negative impact of lignin in biofuel production. Next-generation sequencing offers a powerful tool for the study of the genetics of lignin synthesis and degradation though the study of plant genomes and metagenomes of lignin degrading environments. However, many proteins is cannot be accurately predicted with general identification techniques such as sequence similarity, clustering, motifs, or evolutionary relationships. Machine learning offers a solution to the shortcomings of these approaches at the cost of generalization. Thus to abet lignin research we developed support vector machine models to identify novel enzymes related to the synthesis and degradation of lignin, analyzed genomes and metagenomes for novel lignin related enzymes, and have released a web tool and the novel sequences found on the website http://bioinfo.okstate.edu/ligpred/.

POSTER 12

DOCKERIN-CONTAINING PROTEASE INHIBITOR PROTECTS KEY CELLULOSOMAL COMPONENTS FROM PROTEOLYSIS IN *CLOSTRIDIUM CELLULOLYTICUM*

STUDENT PRESENTER: TAO XU

Tao Xu[1,2], Yongchao Li[1,2], and Jizhong Zhou[1,2,3,4]

[1]Institute for Environmental Genomics, [2]Department of Microbiology and Plant Biology, University of Oklahoma, Norman, OK; [3]Earth Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA; [4]Department of Environmental Science and Engineering, Tsinghua University, Beijing, China

Cellulosome plays key roles in lignocellulosic biomass conversion into biofuels in cellulolytic *Clostridia*. Enzymatic characterization of several cellulosomal protease inhibitors revealed their inhibition against proteolysis, but little is known about their physiological functions. Here a dockerin-containing protease inhibitor (dpi) gene in *Clostridium cellulolyticium* was characterized by mutagenesis. The dpi mutant decreased cell yield on glucose, cellulose and xylan with a lower efficiency of cellulose utilization. The protein abundance of cellulosomal components was obviously altered with Cel48F and Cel9E decreased by 70% and 52%, respectively. However, Quantitative RT-PCR showed transcripts of cipC, cel48F and cel9E were at the similar level, even though all of them decreased about 40% in the mutant. Therefore, our data suggest that the decreased cellulose degradation efficiency in the mutant may be caused by both lower level expression of the cip-cel cluster and higher proteolysis of cellulosomal components compared to wild type. Disruption of cel48F and cel9E severely impaired cell growth on cellulose but the former could completely abolish cellulolytic feature. The recombinant Dpi presented effective inhibitory activity against cysteine protease. Taken together, Dpi as a cysteine protease inhibitor protects key cellulosomal cellulases from proteolysis in *C. cellulolyticium*. This study appears to be the first effort to identify the important *in vivo* function of cellulosome-localized protease inhibitors.

POSTER #13

QTL MAPPING FOR REPRODUCTIVE MATURITY IN LOWLAND SWITCHGRASS POPULATIONS

Hongxu Dong, Linglong Liu, and Yanqi Wu
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Switchgrass is developed as a promising cellulosic bioenergy crop due to its multiple agronomic advantages, such as longevity, drought and flooding tolerance, low fertilizer requirement, and relatively high yielding potential on marginal land. Reproductive stage is a key trait in the determining developmental process, consequently affecting biomass production in switchgrass. Accordingly, the objective of this study was to identify genomic regions responsible for reproductive stages in lowland switchgrass. A hybrid population consisting of 179 progeny lines derived from a cross between parents NL94 (♀) × SL93 (♂) and a self-pollinated population of 277 progeny lines from first generation (S1) progeny of NL94, were tested in this study. A numerical scale ranging from 1 to 7 was used to evaluate maturity stages of the two populations. Phenotypes were characterized in September 2012. A total of 150 simple sequence repeat (SSR) markers were genotyped in the hybrid population. Genotypic data of more than 500 SSR markers in the S1 population collected in two previous experiments will be used as well. The genotypic and phenotypic data will be analyzed to locate QTLs for reproductive stages. Results from the experiment will be valuable in marker assisted selection and elucidation of genetic mechanisms for reproductive development in lowland switchgrass.

POSTER #14

USE OF HYPERSPECTRAL REMOTE SENSING TO DISCRIMINATE NITROGEN TREATMENTS IN SWITCHGRASS AND HIGH BIOMASS SORGHUM

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, Ardmore, OK

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

The recent advances in hyperspectral remote sensing provide a unique opportunity and critical information needed for understanding nitrogen management in bioenergy feedstock production systems. Therefore, the objectives of this study were to determine the optimal hyperspectral narrowbands for discriminating nitrogen treatments at different times throughout the growing season. The feedstocks evaluated in the study were switchgrass (Alamo) and high biomass sorghum (ES 5200) grown in a research experiment to evaluate N applications rates on biomass yield and quality. Canopy hyperspectral data was collected using ASD FieldSpec FR spectroradiometer (350-2500 nm) at monthly intervals from June to August in 2011 and May to September in 2012. Principal component analysis (PCA) was used to determine optimal hyperspectral narrowbands and the separation of the N treatments was done using stepwise discriminant analysis (SDA). The most frequently occurring wavebands identified from the PCA and SDA for separating the N treatments were mostly in the visible (520-560 and 650-690 nm) and red edge (710-730 nm) region of the spectrum. The N treatments in high biomass sorghum were best separated early in the season, while the N treatments in switchgrass were best separated late in the season. In general, the results indicate that hyperspectral reflectance is a viable tool that could be used to estimate biomass yield and quality in bioenergy crop production systems.

POSTER #15

A MULTIFACETED APPROACH TO CONTROL SWITCHGRASS RUST (*PUCCINIA EMACULATA*) BY EMPLOYING NON-HOST RESISTANCE AND CHEMICAL CONTROL

Upinder Gill, Srinivasa R. Uppalapati, and Kirankumar S. Mysore

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Switchgrass rust, caused by *Puccinia emaculata*, has the potential to severely impact biomass yield of switchgrass (*Penicum virgatum* L.), an important biofuel crop. During the past several years, severity of rust has been reported on switchgrass cultivation across Oklahoma. Nonhost disease resistance is the most common form of plant defense mechanism exhibited by all plants towards a majority of plant pathogens. In an effort to improve disease resistance in switchgrass, we have characterized *Brachypodium dystachion* as a nonhost model plant to identify novel sources of resistance against *P. emaculata*. After screening ~50 *Brachypodium* inbred accessions, two *Brachypodium* inbred accessions, Bd3-1 and Bd30-1, showed natural variations for rust resistance. Bd30-1 showed enhanced urediniospores germination and penetration compared to Bd3-1 but none of the accessions showed any visual symptoms of susceptibility in terms of sporulation/rust pustules development. Defense related gene expression in both inbred accessions at different time points after rust inoculations identified initial high basal levels of salicylic acid (SA) pathway related gene expression in Bd3-1 and initial high levels of jasmonic acid biosynthesis and ethylene response factor gene expression in Bd30-1. As a chemical control, phosphite (phi), which is known to enhance resistance against oomycetes, was tested for its role in switchgrass rust resistance. Results indicated that phi at different concentrations (10 mM to 100 mM) inhibits the germination and germ tube growth of *P. emaculata* at varying degree under *in vitro* and *in vivo* conditions. Phi sprayed switchgrass plants also exhibited reduced disease symptoms. Washing off phi from leaves 24 hours after application could partially recover disease susceptibility. Phi primed plants also showed up-regulation of chitinases and phenylpropanoid pathway genes. In summary, phi enhanced rust resistance by inhibiting pathogen growth as well as priming plant defense responses.

POSTER #16

TRANSCRIPTOME ANALYSIS OF HEAT STRESS RESPONSE IN SWITCHGRASS (*PANICUM VIRGATUM L.*)

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Global warming predictions indicate that temperatures will increase by another 4 to 11°F over the next century. The increasing temperatures will have major consequences for all life forms. Switchgrass (*Panicum virgatum* L.) has been selected as a model herbaceous bioenergy crop, due to its rapid growth rate, reliable biomass yield across locations, minimal requirements of water and nutrients, adaptability to growth on marginal lands and widespread distribution throughout North America. We conducted transcriptome analysis using Affymetrix gene chips to elucidate the transcriptional changes in response to heat stress in Switchgrass. Switchgrass plants were maintained in a growth chamber at 28º/20ºC (day/night) or were subjected to heat stress 38º/30ºC (day/night) for 50 days. A significant decrease in the plant height and total biomass was evident at the end of the heat treatment. Total RNA from control and heat stress samples were extracted and used for microarray analysis. Following normalization and pre-processing, 2076 probesets were identified as up-regulated, and 3088 probesets were down-regulated using a 2-fold change cutoff. Differential expression of 45 genes from this list was validated using semi-quantitative PCR. Rice orthologs were retrieved for 78.7 % (4062) of the heat stress responsive probesets. Gene ontology (GOs) analysis using AgriGO program showed that genes related to ATPase regulator, chaperone binding, and protein folding was significantly up-regulated. GOs associated with protein modification, phosphorus metabolic process, and protein kinase activity was significantly down-regulated. These results show that heat stress affects multiple biological processes leading to reduction in switchgrass biomass.

POSTER #17

OVEREXPRESSION OF THE MEDICAGO *WOX* GENE *STENOFOLIA* IMPROVES BIOMASS PRODUCTION BY PROMOTING CELL PROLIFERATION IN SWITCHGRASS

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Lignocellulosic biomass is a major target for feedstock improvement in switchgrass (*Panicum virgatum* L.), one of the leading dedicated bioenergy crops. The *Medicago WUSCHEL-related homeobox (WOX)* gene *STENOFOLIA (STF)* performs a conserved function during leaf lamina outgrowth. Loss of function of *STF* in *Medicago truncatula* results in dramatically reduced blade width and decreased total biomass. To test the hypothesis that *STF* can promote leaf blade outgrowth and regulates plant biomass in monocots, we constitutively expressed *STF* in switchgrass using the maize *Ubiquitin* promoter. *STF* transgenic switchgrass plants produced wider leaf blades and thicker stems, resulting in an almost two-fold increase in above ground dry biomass. Histological analysis through the leaf and stem of *STF* transgenic switchgrass revealed that the cell size is not affected, but the cell number is significantly increased, which led to bigger plant stature and increased biomass. Using transcript profiling analysis, we uncover that specific cell division marker gene *Histone H4* as well as plant cell wall loosening proteins Expansins are upregulated in the *STF* transgenic switchgrass plants. These results point to the potential utility of *STF*-related switchgrass genes as a tool in breeding programs for increasing biomass yield.

POSTER #18

TWO SELF-COMPATIBLE LOWLAND SWITCHGRASS PLANTS SET COMPLETELY OUTCROSSED SEEDS UNDER THE FIELD CONDITIONS

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Two lowland switchgrass (*Panicum virgatum L.*) plants, 'NL94 LYE 16x13' (NL94) and 'SL93 7x15' (SL93), were reported to set substantial seeds due to self-fertilization when both were grown in a large growth chamber. Consequently, the two genotypes were characterized to be self-compatible. However, self- versus cross-fertility of the two genotypes, when grown under the field conditions allowing free open-pollination, is unknown. Accordingly, this experiment was designed to quantify selfing vs. outcrossing progeny of the two genotypes under field conditions. The two tetraploid genotypes were planted in a field plot with two replications by clonal propagation on the OSU Agronomy Research Farm. They were grown along with two genetic populations, including one crossed population between NL94 × SL93 and a selfed progeny population of NL94. Sixty-four progeny derived from open-pollinated seeds of each genotype per replication per year were genotyped with four to 20 simple sequence repeat markers to test their breeding origin in 2010 and 2011. Despite significant weather differences over the two years, both genotypes demonstrated complete self-incompatibility (i.e., producing 100% outcrossed progeny) in the field. The identification of specific genotypes like NL94 and SL93, which are highly self-incompatible in the open-pollinated field but self-compatible under controlled conditions potentially enables the efficient production of F1 hybrid seed in switchgrass.

Keywords: Self-incompatibility (SI) – outcrossing – simple sequence repeat (SSR) – hybrid seed – switchgrass

POSTER #19

GENETIC DIVERSITY AMONG FIVE LOWLAND SWITCHGRASS CULTIVARS DETECTED BY AFLP MARKERS

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Switchgrass (*Panicum virgatum* L.) is a cellulosic herbaceous feedstock species for biofuels production. Five lowland cultivars, Alamo, BoMaster, Cimarron, Performer and Kanlow, were developed and released for feedstock production. However, genetic relatedness among them was unknown. Therefore, the objective of this experiment was designed to analyze genetic diversity among five lowland switchgrass cultivars. Thirteen individual DNA samples extracted from each of Alamo, BoMaster, Performer and Kanlow, and 12 from Cimarron were used in the experiment. AFLP bands throughout the gel profiles were visually scored and analyzed using Numerical Taxonomy System, version 2.0 (NTSYSpc 2) and GenAlex software. In a total of 642 AFLP bands generated in the experiment, 548 (85%) bands were polymorphic. Similarity coefficient values calculated in NTSYS ranged from 0.69 to 0.88. Goodness of fit test gave matrix correlation coefficient (r) as 0.8. The AFLP data grouped the plants into separate clusters corresponding to the cultivars. Alamo showed maximum variation while Kanlow showed the least. In pairwise, maximum variation was observed between Alamo and Kanlow, and minimum between Alamo and Cimarron. The information will be valuable in the development of new cultivars in lowland switchgrass.

POSTER #20

ETHANOL FROM SWEET SORGHUM: ROLE OF THE ALCOHOL SEPARATION UNIT AT OKLAHOMA STATE UNIVERSITY

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Decentralized renewable fuel production can become a reality with sweet sorghum feedstock. Sweet sorghum requires lower water and nutrient input, it is easy to obtain and process the fermentable sugars in its juice, and requires little attention in terms of pH or temperature control during fermentation. However, without a technically robust downstream separation system, the success of sweet sorghum based ethanol can be compromised.

The Alcohol Separation Unit at Oklahoma State University has been constructed as both, a research and demonstration facility, for sweet sorghum bioethanol production. Located at the Bioenergy Laboratory, on an off-campus outdoor location, the pilot plant features two distillation columns, both taller than 35 ft, which form the heart of this separation process. The facility features state-of-the-art process technology – column internals, heat transfer equipment, instrumentation and software for measurement, monitoring and control.

Upon operation outstanding issues of operating cost, maintenance, specifically in terms of water use, waste water generation, process fouling, and project economics are expected to be resolved. For the purpose of this presentation, we will focus on the technical features of the Alcohol Separation Unit, and how the design caters to sweet sorghum bioethanol production on a small, on-farm scale.

POSTER #21

CONTROL OF FLOWERING TIME IN SWITCHGRASS FOR IMPROVEMENT OF BIOMASS YIELD

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Lignocellulosic biomass is a renewable energy source, and high biomass yield is an important trait to increase biofuel production. The timing of the transition from vegetative to reproductive growth is a very important biomass yield parameter, as delaying flowering can yield greater vegetative biomass. Thus, controlling floral transition is a potential strategy to increase biomass yield. Switchgrass (*Panicum virgatum* L.) is a leading dedicated bioenergy crop in which understanding the regulation of flowering time will be useful for the ultimate goal to increasing the biomass yield through altering the flowering time. Using a reverse genetics approach, we identified three key flowering promoters *FT*, *SOC1*, and *AP1* homologues from switchgrass. Ectopic expression of *PvFTL1* complemented the late-flowering phenotype of the *Arabidopsis ft-1* mutant under LD conditions and *PvFTL1* interacts with AtFD in the nucleus using BiFC assay, suggesting that *PvFTL1* is an FT-like floral activator. Overexpression *PvFTL1* in switchgrass Alamo led to early flowering, and activation of the expression of FT downstream genes, *AP1* and *SOC1* like genes. However, Knockdown the expression of *PvFTL1* by *RNAi* did not show late flowering phenotype although the *PvFTL1* transcript level was reduced. This may be caused due to functional redundancies because there are around 14 **FT**-like genes in switchgrass. Our work contributes to the molecular understanding of the switchgrass flowering pathway. Further work is required to identify the major determinant of flowering in switchgrass or multiple *PvFTL* genes have to be simultaneously silenced to delay flowering and test the hypothesis that this would improve biomass yield by allowing further vegetative growth.

POSTER #22

THREE DNA BARCODE GENES FROM SWITCHGRASS RUST FUNGUS *PUCCINIA EMACULATA*: HAPLOTYPE DIVERSITY AND RESULTING PHYLOGENY

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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. Switchgrass rust caused by *Puccinia emaculata* can significantly reduce biomass yield and feedstock quality. Three other *Puccinia* species have been reported causing switchgrass leaf disease, but are now considered synonyms of *P. emaculata*. Currently, little is known about rust of switchgrass and its unclear etiology complicates development of effective management strategies. In order to assess the monophyly, genetic diversity and haplotype distribution of *P. emaculata*, urediniospores were collected from cultivated switchgrass grown in Iowa, Mississipi, Oklahoma, South Dakota, and Virginia. Three "DNA barcodes" ITS, TEF1a, and bTub were amplified and the PCR products subcloned and sequenced. At least 5 clones of each barcode were sequenced per spore collection. Phylogenetic analyses with each barcode strongly supported the monophyletic status of *P. emaculata*. Intraspecific variation among and within populations was observed, suggesting that several lineages are represented by different haplotypes. Barcodes differed in the number of haplotypes represented (ITS=13; bTub=24; TEF1a= 27) and their geographic distribution. bTub and TEF1a haplotypes displayed mostly local distributions; while ITS haplotypes were distributed either in multiple states or locally. Also, barcode haplotype diversity and distribution suggest prolonged propagation of urediniospores under growth chamber conditions may reduce variability. Future studies will examine the genetic diversity, phylogeography, population structure and pathogenicity variation within *P. emaculata*.

POSTER #23

INVESTIGATING CELL WALL CHANGES DURING GRASS LATERAL ROOT EMERGENCE

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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. Root tissue sectioning has confirmed the density improvement and the synchronicity of our treatment system. Roots sampled from three separate time points throughout the developmental time course of our experiment show that the lateral roots occur along the length of the root at regular intervals and are synchronous in their temporospatial location across different roots and plants. Using this system our goal is now to analyze the changes in cell wall chemical content and identify possible regulators of this process. This will allow for a better understanding of plant cell wall rearrangement.

POSTER #24

MOISTURE CONTENT SAMPLING VARIANCE WITH LARGE FORMAT, SQUARE HAY BALES: EVALUATION OF SAMPLING PROTOCOL

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Moisture content impacts every aspect of the cellulosic bioenergy feedstock supply chain. Research is currently being conducted to determine the economic advantages and disadvantages of moisture at the various nodes of the supply chain, such as baling, storing, pre-process, transportation, and conversion. A key factor affecting this research is moisture variability and migration. Sampling protocols that are currently being used throughout the country to draw conclusions about the effects of moisture on material quality and costs are being questioned because of the potential moisture variability within a bale and within a storage stack. The study is the first in a series aimed at quantifying the moisture variability within bale and within stack based on currently used sampling protocol. When baling, the stock, is not completely dried causing a portion of the weight of the bale to be from water contained within the bale. Moisture content varies between bales such that the dry weight of a bale cannot be estimated without a measurement of the moisture content. Thus the accuracy of the estimation of dry weight depends on the accuracy and precision of a moisture content measurement. From 2011 to the present, samples have been extracted from large format bales and tested for moisture content. Testing so far has shown that moisture content varies within each bale, making moisture content sampling dependent on sampling location. The goal of this research is to identify a sampling location, or system of a minimal number of sampling locations, that provides a moisture content resembling the average moisture content of the bale.

POSTER #25

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 most important bioenergy crops in the United States. 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 biomass accumulation and stress tolerance in this plant species. 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 environmental stresses. To gain an insight into miRNA networks that control such traits in switchgrass, several small RNA libraries were sequenced from flowers, emerging tillers as well as from untreated seedlings (control) and seedlings exposed to drought and heat stresses. Together, more than 100 million small RNA reads were generated for switchgrass. Sequence analyses revealed the identification of ~30 conserved miRNA families. More importantly, small RNA reads analysis has revealed approximately 50 novel miRNAs in switchgrass. Most conserved miRNA families are significantly altered in response to drought or heat stress. Similarly, some of the novel miRNAs also differentially regulated by these stresses. Taken together, miRNA analysis helped us to better understand post-transcriptional gene regulation critical for various biological processes including biomass accumulation and tolerance to stress conditions in switchgrass.

POSTER #26

COMPARISON OF NET ECOSYSTEM EXCHANGE OF CO2 AND H2O BETWEEN HIGH BIOMASS SORGHUM AND SWITCHGRASS IN THE US SOUTHERN GREAT PLAINS

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Switchgrass (*Panicum virgatum* L.) and high biomass sorghum (*Sorghum bicolor* L. Moench) are two major dedicated lignocellulosic feedstocks for biofuel production in Oklahoma. There is a scarcity of comparative studies on co-located ecosystems which are experiencing the same climate and soil type but differing in land-use. We measured net ecosystem exchange (NEE) of CO2 and H2O from co-located plots of high biomass sorghum and switchgrass ecosystems using the eddy covariance technique during the 2012 growing season. The objective of this study was to compare the magnitude and seasonality of NEE, evaportranspiration (ET), and ecosystem water use efficiency (EWUE) in these two contrasting ecosystems. The magnitudes of maximum monthly average NEE were -33.02 ± 1.96 (May) and -35.86 ± 2.32 µmol m-2 s-1 (June) in switchgrass and sorghum, respectively. However, the switchgrass ecosystem was a larger carbon sink with a cumulative seasonal carbon uptake of -490 g C m-2 (Mar – Oct) compared to -262 g C m-2 by sorghum (May – Oct). Similar seasonal trends of ET were observed in both ecosystems, with maximum rates during the peak growth period. The sorghum ecosystem had slightly higher peak ET (weekly average of daily ET = 6.7 mm day-1) than switchgrass ecosystem (6.2 mm day-1). However, total seasonal ET was higher in switchgrass (653 mm) than in sorghum (465 mm) due to longer growing season of switchgrass. The EWUE reached a maximum of 16 g CO2 mm-1 ET in switchgrass and 14 g CO2 mm-1 ET in sorghum during peak growth (June), with seasonal averages of 13.5 and 12 g CO2 mm-1 ET in switchgrass and sorghum, respectively. The results illustrated the benefits of these two important lignocellulosic feedstocks in this region.

POSTER #27

IDENTIFICATION OF OsAT10 INTERACTORS BY YEAST TWO-HYBRID

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Grasses are one of the most promising types of feedstocks for biofuel production. The crosslinked structure of grass cell walls is a main obstacle for biomass conversion. Ferulic acid (FA) is a crosslinking agent that esterifies with the hemicellulose, arabinoxylan. Para-coumaric acid (p-CA) is similarly esterified to arabinoxylan, though its function in cell walls is uncertain. Our group has found that OsAT10, a BAHD acyltransferase, functions in modification of arabinoxylan with p-CA. We hypothesize that OsAT10 and related proteins physically interact with other proteins like sugar nucleotide mutases or transporters to realize the incorporation of p-CA and FA into arabinoxylan. To test this, we are identifying potential interactors with OsAT10 by screening a *Brachypodium distachyon* cDNA library. Selection from 1.5×107 colonies for survival on His- plate gave 220 potential interactors. Of 39 clones that we rescreened with an X-gal assay so far, 36 (92%) showed a signal that is consistent with strong interactions with OsAT10. This suggests that the original screen worked well. Results of further analysis of the clones that putatively interact with OsAT10 will be discussed. The discovery of interactors of OsAT10 will be helpful for illustrating the molecular mechanism and function of the grass arabinoxylan modification and for creating feedstocks with more digestible cell walls for biofuel production.

POSTER #28

SYNGAS FERMENTATION IN A TRICKLE BED REACTOR USING *CLOSTRIDIUM RAGSDALEI*

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An efficient syngas fermentation bioreactor should provide a mass transfer capability that matches the kinetics of the microorganism used in order to obtain high gas conversion efficiency and productivity. The objective of the present study is to assess mass transfer and gas utilization efficiencies of a trickle bed reactor (TBR) for ethanol production by *Clostridium ragsdalei* P11. The fermentation was performed at 37oC using a syngas mixture of 38% CO, 28.5% CO2, 28.5% H2 and 5% N2. P11 was inoculated in the TBR following triple passaging when cells were in the growth stage. The effects of various gas and liquid flow rates on ethanol productivity and gas conversion efficiencies were examined. Additionally, cell mass, ethanol and acetic acid concentrations, and CO and H2 consumption rates were determined. Results showed that over 90% CO and 80% H2 conversion efficiencies were achieved at 2.3 sccm gas flow rate, regardless of the changes in the liquid flow rates. About 3.2 g/L ethanol and 5.5 g/L acetic acid were produced. Syngas fermentations and analysis of mass transfer in the TBR at 4.6 sccm are ongoing.

Keywords: Syngas fermentation Clostridium ragsdalei P11, Ethanol, Trickle bed reactor, mass transfer

POSTER #29

ISOLATION AND CHARACTERIZATION OF BACTERIA THAT DEGRADE LIGNIN IN PLANT BIOMASS

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Lignin is a major bottleneck for efficient saccharification of plant biomass into the individual component sugars. Several mechanical and chemical procedures for saccharification have been established; however, all are relatively expensive, inefficient and generate toxic intermediates. The search for efficient technique to breakdown of plant biomass has therefore attracted considerable interest. Biological pretreatment is an attractive alternative to the current pretreatment options. Although, to date many studies have focused on lignin degradation by fungi, a number of bacteria show a great potential for the degradation of lignin and lignin-like aromatic compounds. Hence more studies; especially involving pure cultures of bacteria are needed to better understand the physiology and the underlying molecular mechanism of lignin degradation.

We have isolated several bacteria in the phyla *Proteobacteria* and *Firmicutes* that degrade lignin (Sigma-Aldrich) as the sole source of carbon from highly enriched lignin metabolizing enrichments. All isolates, except *Brevibacillus* sp, grew rapidly from 103 CFU/ml to as high as 107 to 108 CFU/ml in a medium containing lignin as the sole source of carbon suggestig the isolates ability to mineralize lignin. Lignin peroxidase activity was measured in cell supernatant of the cultures grown on a variety of lignin substrate including anisoin, lignin, alfalfa, and switchgrass suggested the isolates ability to excrete lignin degrading enzyme. In addition, using LC-MS/MS we have identified feruloyl esterase, chloroperoxidase, 4-hydroxyphenylpyruvate dioxygenase, endo-1,3-1,4-beta-glycanase, and numerous sugar transporters in the secretome of alfalfa and switchgrass grown *Rhizobium* sp. strain YS-1r. We also determined the strain YS-1r's ability to degrade natural lignin in alfalfa and switchgrass. Our results showed that > 30% of the lignin in alfalfa and < 1% of lignin in switchgrass was degraded 30 days. Taken together, this study supports the notion that bacteria can play key role in the delignification of plant biomass.

POSTER #30

ANALYSIS OF CELLULOSOMAL GENE EXPRESSION IN CELLULOLYTIC CLOSTRIDIA USING A MULTI-GENOME ARRAY

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Clostridia species are metabolically versatile and have been employed for many industrial processes including biomass conversion and biofuel production. Many cellulolytic clostridia produce cellulosomes, a multienzyme complex for the breakdown of crystalline cellulose. Since the cellulosome is a highly complicated molecular machine, it requires significant characterization. Based on the genome sequences of cellulolytic clostridia, we first constructed a multi-genome array, the Cellulolytic Clostridia Array (CCA) to understand cellulose degradation at the transcriptomic level. The developed CCA contains a total of 124,891 probes including 117,102 probes targeting open reading frames (ORFs) of 11 cellulolytic clostridia genomes. The array also contained 8 degenerate probes targeting 16S rRNA sequences as positive controls, 563 strain-specific probes targeting seven hyperthermophile species as negative controls, and a common oligonucleotide reference standard for data normalization and comparison. We then applied CCA to investigate cellulosomal gene expression of cellulolytic clostridia growing on either cellulose or cellobiose. We extracted RNA from the biomass harvested from each strain, reverse-transcribed RNA to cDNA, and labeled and hybridized cDNA on CCA. The expression of cellulosomal genes varied between lineages and even within the same species (C. *thermocellum*). More cellulosomal genes were expressed in the strains growing on cellulose than on cellobiose, indicating coordinated substrate-specific regulation of cellulosomal gene expression. In this study we addressed changes of cellulosomal gene composition within and between lineages using a transcriptomics approach, which provides better insight into global changes in cellulosomal gene expression, the composition of the intact cellulosome, and the degree of cellulose degradation capacity.

POSTER #31

COMBINATION OF SPORULATION DISRUPTION AND CARBON OVERLOAD ALLEVIATION HELPS INCREASE CELLULOSE CATABOLISM IN CLOSTRIDIUM CELLULOLYTICUM

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Clostridium cellulolyticum can degrade lignocellulosic biomass, and ferment the soluble sugars to produce valuable chemicals such as lactate, acetate, ethanol and hydrogen. But its cellulose utilization efficiency still remains very low, impeding the application in consolidated bioprocessing for biofuels production. To improve its cellulose utilization efficiency, approaches were investigated based on manipulating the stress response due to heavy carbon load and high accumulation of metabolic products by inactivating *spo0A* gene and introducing heterologous synthetic pathway to consume extra pyruvate. The *spo0A* mutant abolished sporulation ability. In high concentration of cellulose (50 g/L), the performance of s*po0A* mutant increased dramatically in terms of the maximum growth, the final concentrations of three major metabolic products, and the cellulose catabolism. The GC-MS and microarray analyses showed that the valine, leucine and isoleucine biosynthesis pathways were up-regulated. Based on this information, a partial isobutanol producing pathway modified from valine biosynthesis was introduced into C. *cellulolyticum* strains to help further increase cellulose consumption by alleviating excessive pyruvate accumulation. With this synthetic pathway, wild-type strain improved the cellulose consumption from 17.6 g/l to 28.7 g/l with a production of 0.42 g/l isobutanol. However, spo0A mutant strain did not benefit much from this synthetic pathway and the cellulose utilization efficiency did not further increase. These findings here demonstrated one step forward to engineer C. *cellulolyticum* for the production of biocommodities and biofuels with high efficiency and at low cost directly from lignocellulosic biomass.

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POSTER #32

MICROARRAY & NETWORK ANALYSIS REVEALS GENE EXPRESSION PROFILES OF CARBOHYDRATE ACTIVE ENZYMES IN PENICILLIUM EXPANSUM YT02

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Penicillium species are common soil fungi and may contain novel lignocellulosic enzymes for biofuel production. We isolated a novel strain, *P. expansum* YT02, from a tropical soil. YT02 was able to release 35%-72% more reducing sugars than the commercial strain *Trichoderma reesei* ATCC 2449. To understand the mechanism of efficient lignocellulosic biomass degradation and saccharification of YT02, we analyzed its gene expression and enzyme production by microarray and proteomics, respectively on different substrates (corn stover, oak tree, switchgrass, and wheat straw) and at different cultivation times. The results revealed that at the early stage of cultivation (40 h), the gene expression profiles significantly differed among those substrates tested. However, the difference became smaller after long term cultivation (except on oak tree). The carbohydrate active enzyme genes that showed significant differences were found to be cellulase (GH12, GH3, GH5), hemicellulase (GH11, GH43), and pectinase (GH28, and PL1) as well as other functions (GH18, GH55, GH76, GH92 and GT2). CBH1, EG1, and CBH2 were the top secreted enzymes on all the substrates. However, YT02 produced more xylanases on oak tree than on other substrates. A gene co-expression network has been established from the microarray data (72 samples, 9,517 genes), which included 1,538 nodes and 4,577 edges and could be further separated into 192 modules. A hierarchical clustering structure was detected among those modules with the aggregation of similar functions. This network would be useful for identification of key functional genes and novel transcriptional regulators.

POSTER #33

THE ANAEROBIC FUNGAL ISOLATE ORPINOMYCES SP. STRAIN C1A IS AN EFFICIENT PLANT BIOMASS DEGRADER UNDER ANAEROBIC CONDITIONS

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Members of the gut fungi are strict anaerobes that belong to the basal fungal lineage *Neocallimastigomycota* and are present in the rumen, hindgut, and feces of ruminant and non-ruminant herbivorous mammals, as well as reptilian herbivores. Anaerobic fungi combine the resilience and invasiveness of filamentous fungi with the metabolic capabilities of anaerobic prokaryotes. We here provide evidence that an anaerobic fungal isolate, *Orpinomyces* species strain C1A is a remarkable biomass degrader. Strain C1A grew readily on untreated, as well as mild acid, mild alkali, and steam-explosion treated switchgrass, with the concurrent utilization of cellulose and hemicellulose, but not lignin, fractions. Dry weight loss ranged between 18.6% (28.9% of the non-lignin fraction) in untreated switchgrass, to 40.7 (51.8% of non lignin fraction) in NaOH treated switchgrass. Further, adjustments to the inoculum/substrate ratios resulted in an increase in the amount of switchgrass metabolized up to 57.8% and 70.3% of the non-lignin fraction in untreated and NaOH treated switchgrass, respectively. Patterns of product production showed an increase in the proportion of lactate, and to a lesser extend, ethanol in NaOH and steam-explosion treatments, and a reciprocal decrease in the proportion of formate and acetate when compared to untreated controls. Finally, in addition to switchgrass, we show that strain C1A is also capable of degrading multiple bioenergy crops and crop wastes such as corn stover, sorghum, energy cane, alfalfa, wheat bran, and Bermuda grass. The results demonstrate that *Orpinomyces* sp. strain C1A is an efficient biomass degrader that is capable of degradation, as well as simultaneous saccharification and fermentation of the cellulose and hemicellulose fractions in a large number of plants. Current strategies, and efforts to improve alcohol production and tolerance in *Orpinomyces* species are discussed.

POSTER #34

DROP-IN BIOFUELS PRODUCTION BY A MIXED CULTURE FERMENTATION OF SYNGAS

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”Drop-in” biofuels such as n-butanol have more energy density and are less hygroscopic compared to ethanol, making them more compatible with current fuel infrastructure. The production of biofuels through gasification-syngas fermentation begins with the gasification of biomass to syngas (CO, CO2 and H2) followed by the conversion of syngas to liquid fuels using acetogenic microorganisms. A monoculture of *Alkalibaculum bacchi* strain CP15 was observed to produce n-propanol and n-butanol during continuous syngas fermentation over 1000 h. These alcohols are not typically produced by strain CP15. An analysis of 16S rRNA genes obtained from this bioreactor revealed that this bioreactor contained a mixed culture consisting of strain CP15 and *Clostridium propionicum*. This finding explained the production of n-propanol and n-butanol and presented a new opportunity for production of higher alcohols from syngas. Syngas fermentations with only CP15 or the mixed culture were tested for their ability to produce higher alcohols from syngas (40% CO, 30% CO2 and 30% H2) and to convert carboxylic acids into corresponding alcohols. Fermentation bottles were fed fresh syngas and pressurized to 240 kPa every 24 h. Without the addition of carboxylic acids, the monoculture of CP15 only produced ethanol. However, the mixed culture produced ethanol, propanol and butanol. When propionic acid, butyric acid, hexanoic acid and lactic acid were added separately in the fermentation medium, the results showed that the mixed culture was 50% more efficient in converting the acids to their respective alcohols. Also, there was a synergy between strain CP15 and *C. propionicum* in which *C. propionicum* converted lactic acid and ethanol to propionic acid that was then reduced into propanol by strain CP15. These results show the advantage of using the mixed culture over the monoculture for the production of drop-in biofuels from syngas that could have a potential use in commercial applications.

Keywords: Syngas fermentation, Mixed culture, Alkalibaculum bacchi, Clostridium propionicum, Ethanol, n-Propanol, n-Butanol

POSTER #35

INVESTIGATING THE ROLE OF DUPLICATED ALCOHOL DEHYDROGENASE GENES FROM *CLOSTRIDIUM CARBOXIDIVORANS* STRAIN P7 IN ALCOHOL PRODUCTION

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Clostridium carboxidivorans strain P7 is one of only three microbial catalysts known to be capable of fermenting synthesis gas (mainly CO, CO2, and H2) to produce the liquid biofuels ethanol and butanol. Gasification of feedstocks to produce synthesis gas, followed by microbial conversion to solvents, greatly expands the diversity of suitable feedstocks beyond commonly used food and energy crops to include agricultural, industrial, and municipal waste streams. Strain P7 uses a variation of the classic Wood-Ljungdahl pathway, proposed or identified through genome sequence-enabled approaches but only limited direct metabolic analyses. The action of the enzyme alcohol dehydrogenase (ADH) is the rate limiting step of the production of solvents increased knowledge of this enzyme will aid in optimizing biofuel production. Strain P7 contains duplicate copies of this gene (*adh1* and *adh2*) with potentially different regulation and substrate specificity.

In this study both *adh* genes were clones and expressed in *E. coli*. Dehydrogenase activity for alcohol solvents was assayed. Ethanol, propanol, and butanol dehydrogenase activity have been detected for both gene products confirming their potential function in this organism. ADH1 possesses trace amounts of methanol dehydrogenase activity while ADH2 exhibits greater hexanol dehydrogenase activity. This indicates different metabolic roles within Strain P7. In order to investigate the *in vivo* role of these genes the second part of this study focused on developing a genetic system for the transformation of strain P7. Here a newly created *E. coli*- Clostridial shuttle vector plasmid pMM is described. Both adh1 and adh2 have been subcloned into this vector and used to transform strain P7. Plasmid pMM presents a possible mechanism for duplication of these ADH genes into strain p7 and measuring their effect on alcohol production.

POSTER #36

OXORHENIUM CATALYZED DEOXYDEHYDRATION OF POLYOLS USING BENZYL ALCOHOL AS REDUCTANT

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The potential for converting biomass-derived polyols and glycols to value-added chemicals stimulates the development of new, selective deoxygenation reactions. Recently, we and others have investigated the glycol-to-olefin deoxydehydration (DODH) reaction. Here we report on the scope, efficiency and selectivity using benzyl alcohol as reductant and commercial ammonium perrhenate as catalyst for the DODH of simple glycols, natural derivatives of glycerol and higher polyols for DODH.

POSTER #37

VANADIUM (V) - CATALYZED DEOXYDEHYDRATION OF GLYCOLS AND EPOXIDES

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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 high valent non-precious metal dioxo-vanadium(V) complexes catalyze the deoxydehydration (DODH) of glycols and the deoxygenation of epoxides generating olefins. The scope, efficiency, and selectivity of these reactions with respect to the polyol and epoxide substrates, the reducing agents, the catalyst, and the reaction conditions will be presented.

POSTER #38

MECHANISTIC INVESTIGATION OF OXORHENIUM CATALYZED DEOXYDEHYDRATION OF POLYOLS

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The deoxydehydration (DODH) of biomass derived polyols to value added chemicals such as olefins is of growing interest. The majority of the reported DODH systems have been oxorhenium catalyzed and there have been few mechanisms suggested. Aspiring to develop more efficient DODH catalysts, an improved understanding of the mechanism of DODH by oxo-metal catalysts would be a worthy endeavor. Since oxorhenium catalysts have been the most prevalent in the area of DODH, initial kinetic and mechanistic investigations using oxorhenium complexes will be discussed.

POSTER #39

PERFORMANCE OF LARGE BALE SWITCHGRASS AND ENERGY SORGHUM STORAGE STACKS: OSU BRDI PROJECT OVERVIEW

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Oklahoma State University was awarded a USDA Biomass Research and Development Initiative (BRDI) in 2009. The project objective was to develop practices and technologies necessary to ensure efficient, sustainable, and profitable production of cellulosic biomass. Using large-scale feedstock production research fields, economic and environmental sustainability of switchgrass, mixed-species perennial grasses, and forage sorghum will be evaluated. Feedstock quality and logistical performance characteristics will be assessed under various storage scenarios. Production and logistic economic models will use the data produced from the large-scale experiments to determine if an integrated landscape vision of diversified species can provide a flow of feedstock throughout the year to a cellulosic biorefinery at a cost that will enable cellulosic biofuel to compete with gasoline.

The field work will be completed in June 2014, so only preliminary data is available. The storage studies were large scale and included uncovered, tarped and fully wrapped treatments. Other variables included storage on well drained packed soil or gravel and storage location. Four Oklahoma storage sites were used. Based on the currently available data, dry matter losses are less than 3% which is much lower than losses attributed to bale and/or stack failures due to bale shrinkage. Bale shrinkage generally occurred in forage sorghum crops that were lightly conditioned and had initial moisture contents over 15%. The compositional analyses indicate that there are no feedstock quality losses when storing the material up to six months. Because the dry matter losses were relatively minor compared to other losses and no significant quality effects were detected in six months of storage, additional twelve and eighteen month storage periods were added to the study in 2012. Data from all aspects of the storage studies are still being generated and no final conclusions have been made.

POSTER #40

AN EFFICIENT STATISTICAL METHOD FOR QTL MAPPING IN TETRAPLOID ORGANISMS

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Mixed linear model is a popular method for quantitative trait loci mapping in all types of organisms including switchgrass, which is a potential source of biofuel. To identify trait associated markers, random sampling (independence among individuals) is a common assumption for linear model in general. In this study, we incorporate the relatedness among individuals in the mixed linear model and propose a novel penalized maximum likelihood method (PMLM) to estimate model parameters, with the special interest of applying our method to identify genomic regions that are associated with switchgrass cell wall quality. The performance of our proposed method is evaluated through simulation studies. We simulate tetraploid organisms under coalescence theory and sample individuals with given pedigree structure. We assess the power of our approach at different scenarios including combinations of the following factors: the sample size, the number of available markers and their minor allele frequencies, the recombination rate between markers, and the heritability of the trait. Results of simulation studies show that, our proposed method has great power (over 85% of correct identification rate) in detecting QTL or markers that are in strong linkage disequilibrium with QTL.

POSTER #41

FIELD-SCALE SWITCHGRASS AND ENERGY SORGHUM HARVESTING: OSU BRDI PROJECT UPDATE

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Oklahoma State University was awarded a USDA Biomass Research and Development Initiative (BRDI) in 2009. The project objective was to develop practices and technologies necessary to ensure efficient, sustainable, and profitable production of cellulosic biomass. Using large-scale feedstock production research fields, economic and environmental sustainability of switchgrass, mixed-species perennial grasses, and forage sorghum will be evaluated. Feedstock quality and logistical performance characteristics will be assessed under various harvest, handling and storage scenarios. Production and logistic economic models will use the data produced from the large-scale experiments to determine if an integrated landscape vision of diversified species can provide a flow of feedstock throughout the year to a cellulosic biorefinery at a cost that will enable cellulosic biofuel to compete with gasoline.

The harvesting field work was completed in February 2013. Preliminary outcomes/observations include:

- Harvesting, packaging, in-field transport, and stacking can be accomplished with currently available commercial equipment.
- Rotary disk blades dull faster when harvesting switchgrass after the first freeze as compared to before the first freeze.
- Forage sorghum can be naturally dried in the field and baled in Oklahoma with proper conditioning and cooperating weather conditions.
- Properly condition forage sorghum can improve bale density and uniformity.
- Raking is not generally required for harvests after freeze in mixed grass and switchgrass, unless it is economically feasible to pull windrows together.
- In-bale moisture variability is a major issue.
- Optimal harvesting would include: three windrowers for every high density baler and four balers for every in-field transport.