



Projects Awarded 2007 - 2014



Research & Innovative Technology Administration



United States Department of Agriculture National Institute of Food and Agriculture

South Central Sun Grant Program Funded Projects (2007-2014)

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Project Title: Cofiring Animal Waste in Low NO_x Burners for NO_x and Hg Reduction in Coal Fired Plants

DR. KALYAN ANNAMALAI

Project Goals

The specific goal of current proposed research is to demonstrate the use of CB as a co-fired fuel in Low NOx burners and demonstrate the new technology in reducing NOx

Objectives:

- Obtain CB fuel and coal characteristics.
- Modify Facility and conduct cofiring experiments.
- Conduct economic analysis on fuel collection, transportation, and processing .

Project Outcomes

 This project completed construction of a small scale LNB furnace and determined the effects of cofiring Wyoming coal (WYO):DB) fuel blends in a 29.31kW (100,000BTU/hr) LNB furnace.

Note: Experiments were performed using WYO as the base case coal. These fuels were used because of lower ash to reduce ash fouling issues. The cattle biomass (CB) represent both FB and DB fuels. Low ash , high ash, separated solids were abbreviated as LA,HA and Sep Sol.

- WYO contained 3.10 kg of ash/GJ, 15.66 kg of VM/GJ, 0.36 kg of N/GJ, and 6.21 kg of O/GJ.
- LA- PC-DB-SepSol contained 11.57 kg of Ash/GJ, 36.50 kg of VM/GJ, 1.50 kg of N/GJ, and 14.48 kg of O/GJ.
- For unstaged combustion for constant fuel feed rate/heat output cases and constant air flow cases, cofiring resulted in most fuel blends showing similar NOx emissions to WYO.
- Staged cofiring resulted in a slight increase of NO_x in rich regions while producing similar to slightly lower amounts of NO_x in lean regions.
- Inverse relationship between NO_x and CO was identified: as NO_x decreased, CO increased.
- Recently computational model was used to modify the reactor for the location of tertiary air injector which revealed better performance of LNB in reducing NOx with coal:CB cofiring
- Respiratory Quotient (RQ= CO2 mols/O₂ mols) method was introduced to rank fossil fuels for ranking coal and biomass based on warming potential.



PI: Dr. Kalyan Annamalai Texas A&M University Paul Pepper Prof of *Mechanical Engineering*

Co-PI: Dr. John M. Sweeten Texas A&M University Texas AgriLife Research-Amarillo

Funded: \$70,000 Start Date: 07/01/2007 End Date: 12/31/2009

Other Sources of Funding:

The PI and Co-PI cost shared a portion of their time each year. The PI's time and applicable fringe benefits and 25% indirect rate made up the 20% cost share requirement. Partial support was requested for two graduate students.



Project Title: Cofiring Cattle Biomass (CB) and Agricultural Biomass (AB) Fuels in Low NO_x Burners

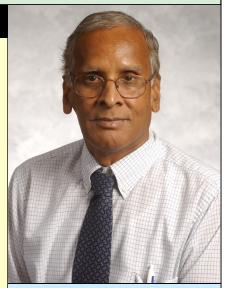
Dr. Kalyan Annamalai

Rationale

Due to restrictions regarding maximum total daily loads of phosphorus, animal waste can no longer be disposed of by land application and an alternate, cost effective disposal method must be developed. Simultaneously, NO_x emission restrictions for fossil fueled power plants are becoming increasingly stricter. Cofiring coal with the high N animal waste, termed as dairy biomass (DB), was done as the thermo-chemical method to address the concerns. DB wass evaluated as a cofiring fuel with Wyoming Powder River Basin (PRB) subbituminous coal in a small scale 29 kWt low NO_x burner (LNB) boiler facility equipped with air staged combustion for NO_x control.

Project Outcomes

- Proximate and ultimate analyses performed on coal and DB revealed the following: nitrogen and sulfur loading on heat basis: 0.15 to 0.48 kg/GJ for the coal and 0.33 to 2.67 kg/GJ for the dairy biomass; ash loading : 3.10 to 8.02 kg/GJ for the coals and 1.57 to 139 kg/GJ for the dairy biomass.
- The cofiring experiments were performed with 85:15, 90:10, 95:5 (by mass percent) coal:dairy biomass blended fuels as well as pure coal. Standard emissions from solid fuel combustion (O2, CO2, CO, NO_x, and SO₂) were measured in addition to the temperature profile along the axial length of the furnace.
- A new method called Respiratory Quotient, used widely in biology is introduced to engineering literature to rank global warming potential (GWP) of various fossil and biomass fuels; the higher the RQ higher CO₂ emission and higher the GWP. They were estimated as 0.92 and 0.94 for PRB and DB while for CH₄ it was 0.5
- Standard emissions from solid fuel combustion (O_2 , CO_2 , CO, NO_x , and SO_2) were measured and NO_x on a heat basis (g/GJ), fuel burnt fraction, and fuel nitrogen conversion percentage were estimated .The gas analyses yielded burnt fraction ranging from 100% to 89% and gas analyses confirmed RQ of 0.9 to 0.94 which is almost same as RQ based on fuel composition.
- By staging 20% of the total combustion air as tertiary air (1.12 initial equivalence ratio), NO_x was reduced to 545 ppm (304 g/GJ) for the 90-10 blended fuel which is a 16.5% reduction from the unstaged pure coal. In addition to these emissions measurements, zero dimensional modeling of the combustion within the low NO_x burner and economic analysis were performed.



PI: Dr. Kalyan Annamalai, Texas A&M University *Mechanical Engineering*

Co-PI:

Dr. John M. Sweeten Texas A&M University Agriculture Research & Extension Center Texas AgriLife Research— Amarillo

Funded: \$70,000

Start Date: 07/01/2009 End Date: 12/31/2012



Project Title: Critical analysis of syngas fermentation reactors for biological alcohol production

DR. Hasan Atiyeh

Project Goal:

- The goal of this research is to expand our understanding of reactors used in hybrid gasification-syngas fermentation technology.
- To achieve this, we measured and compared the mass transfer coefficients (k_La/V_L) of trickle-bed reactor (TBR), various types of hollow fiber reactor (HFR), and continuous stirred tank reactor (CSTR). The effect of agitation speed, gas and liquid flow rates on the mass transfer capabilities of the three reactors was studied.

Project Outcomes:

- Evaluation of the enhancement of mass transfer and associated alcohol productivity in various types and modifications of reactor designs. The three reactor designs are trickle-bed reactor (TBR), continuous stirred tank reactor (CSTR), and hollow fiber membrane reactor (HFR). Five different types of HFRs were examined. We found that the reactor geometry, material of construction, volume, and operating conditions affect the mass transfer and associated gas utilization and biofuel productivity. These findings are critical in designing reactors for efficient biofuels and chemicals production from syngas.
- Development of a novel method to sustain culture activity, gas uptake and improve selectivity for ethanol production during syngas fermentation in the CSTR. This method produced 26 times the ethanol concentration compared to the standard method.
- Development of a new technique to measure the mass transfer coefficients for H₂ and CO using gas chromatography and gas flow rate measurements. A mass transfer model was derived and found to be in very good agreement with the experimental data for CO and H₂.



PI: Dr. Hasan Atiyeh Oklahoma State University Biosystems and Agricultural Engineering

Co-PI: Dr. Randy Lewis Brigham Young University Chemical Engineering

Funded: \$ 374,999.00 Start Date: 12/1/2009 End Date: 11/30/2013

Other Sources of

Funding: Oklahoma State University and Brigham Young University respectively covered the cost sharing portion of this award.



Title: Conversion of Eastern Redcedar to Butanol using a Novel Biocatalytic Process

DR. HASAN ATIYEH

Rationale

Butanol is a more desirable fuel than ethanol due to its higher energy density and compatibility with gasoline and existing fuel infrastructure. Butanol has been produced by the traditional acetone-butanolethanol (ABE) fermentation using molasses, hydrolyzed starches and recently from lignocellulosic biomass. The ABE process has low conversion yields, prohibiting commercialization. It is critical that the conversion efficiency is increased to make butanol production viable. To address this issue, the research team is developing a novel biocatalytic process with butanol yields 20% higher than traditional ABE technology. The overall goal is to develop sustainable and feasible bioconversion of Eastern redcedar into butanol. Their two objectives are determining conditions favorable for butanol production and process development, and optimizing reactor conditions to maximize butanol productivity and yield.

Expected Outcomes

Successful completion of this project will involve development of a novel biocatalytic process with increased conversion efficiency to make biofuels. At the conclusion of this project, Atiyeh's research team will identify the factors that result in maximum butanol productivity and yield. Increased butanol and alcohol yields from the developed process in a facility that utilizes 2,000 dry metric tons of Eastern redcedar per day is expected to result in an increase in net revenues of over \$33 million per year when compared to traditional sugar fermentation. Additionally, an attractive opportunity exists for Oklahoma in the use of Eastern redcedar, which is a tree that is regarded as a pest by most ranchers and rural landowners. It is estimated that there are over 11 million metric tons of redcedar in northwestern Oklahoma. The infestation of redcedars across grasslands in the Central Plains has resulted in lost pasture for cattle grazing, water use by the trees and allergy problems during pollination. This project will not only allow reduction of the redcedar infestation problem, but also convert them into transportation fuels.



PI: Dr. Hasan Atiyeh Oklahoma State University Biosystems & Agricultural Engineering

Co-PI: Dr. Mark Wilkins Oklahoma State University *Biosystems & Agricultural Engineering*

Funded: \$149,999 Start Date: 01/01/13 End Date: 12/31/15

Other Sources of Funding:

Oklahoma State University covered the cost sharing portion of this award.



Project Title: Improving Conversion of Syngas to Biofuels via Direct Monitoring and Control of CO/H₂ in Bioreactors

DR. HASAN ATIYEH

Rationale

Hybrid gasification-syngas fermentation technology for conversion of biomass to biofuels is on the verge of commercialization. Mass transfer is a critical challenge for advancing syngas fermentation because it is associated with low solubility of CO and H₂ in the fermentation medium. We are developing a novel method to increase culture activity, ethanol production, and ethanol to acetic acid selectivity during syngas fermentation. However, the method could not be applied because it was based on indirect CO/H₂ measurements and predictions from our model to provide strategies to control the bioreactor operation. To address these issues, this team will investigate the feasibility of direct monitoring and control of CO/H₂ in the bioreactor. This will allow efficient bioreactor control at conditions that maximize gas conversion and ethanol productivity and yield.



Successful completion of this project will involve the development of the CO/H₂monitoring system for biofuels production in syngas fermentation reactors. This team also plans to identify the operating conditions that maximize gas conversion and ethanol productivity and yield. The Energy Independence and Security Act of 2007 mandates that U.S. transportation fuels contain at least 16 billion gallons of cellulosic biofuels, including ethanol and butanol, by 2022. This shows the potential for a huge market for cellulosic ethanol. At an 80% ethanol theoretical yield from both H₂ and CO, about 109 gallons of ethanol can be produced from one ton of biomass. The theoretical ethanol can be made at an estimated production cost of \$1.41 per gallon, even though it is typically sold at prices above \$2.00 per gallon.



PI: Dr. Hasan Atiyeh Oklahoma State University Biosystems & Agricultural Engineering

Co-PI: Dr. Ning Wang Oklahoma State University *Biosystems & Agricultural Engineering*

Funded: \$140,509 Start Date: 01/01/2013 End Date: 12/31/2015

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Title: Development of Sustainable Hybrid Gasification-Syngas Fermentation Process for Alcohol Production

DR. HASAN ATIYEH

Project Goal

Hybrid gasification-syngas fermentation technology is promising for cellulosic alcohol production from all organic biomass content, including lignin. However, high medium cost and mass transfer limitation are challenges for this process that reduce its efficiency and feasibility. A novel integrated and sustainable process is proposed to address these issues and move the hybrid technology towards commercialization. The overall goal of the project is to develop sustainable and feasible bioconversion of switchgrass into ethanol. The objectives are:

- (1) Generation and characterization of gasification products.
- (2) Development and optimization of syngas fermentation medium and operation parameters.
- (3) Estimation of production cost and evaluation of a proof of concept for the integrated process.

Expected Outcomes

The hybrid technology when further developed with Sun Grant Program support, is expected to reduce the production cost of cellulosic ethanol by \$0.16 per gallon compared to saccharification-fermentation. This will increase the profitability of biorefineries. If biofuel producers adopt the hybrid technology to produce 25% of the mandated 16 billion GPY renewable transportation fuels (i.e., 4 billion GPY), a projected savings of over \$650 million per year can be achieved due to the use of 13.1 million tons less biomass with the hybrid technology.



PI: Dr. Hasan Atiyeh Co-PI: Dr. Ajay Kumar Dr. Mark Wilkins

All personel are from Oklahoma State University Biosystems and Agricultural Engineering

Funded: \$136,000 Start Date: 01/01/2014 End Date: 12/31/2015





Title: Feedback Control of Gas Supply for Alcohol Production via Syngas Fermentation

DR. HASAN ATIYEH

Project Goal

The project implements and tests a novel control method to optimize gas feed rate for production of ethanol and other alcohols from syngas via fermentation conducted by acetogenic bacteria. The method leverages advanced concepts of fermentation developed at OSU and uses common sensors and control equipment used in commercial fermentation.

The goal of the method is to optimize fermentation productivity, selectivity for the alcohol product, and conservation of energy from the feed gas into the product. This optimization is necessary to ensure process profitability.

Expected Outcomes

Our research is expected to further develop our knowledge of the hybrid gasification-syngas fermentation conversion of biomass to liquid fuel and chemicals. Models of fermentation will be extended to support design and control planning, and a specific method for automated control of fermentation will be developed. Implementation of the control algorithm in a commercial control system will allow stable operation of fermentation by moderately skilled personnel and promote expansion of commercial application. Expected outcomes include a patent and licensing in support of a growing industry that will serve an existing market over \$1,000,000 per day in Oklahoma and \$60,000,000 per day nationally for ethanol blending in motor fuel.



PI: Dr. Hasan Atiyeh Oklahoma State University *Biosystems and Agricultural Engineering*

Funded: \$114,000 Start Date: 1/1/2014 End Date: 12/31/2015





Project Title: Enhanced Biofuels Production with Genetically Optimized Feedstocks by Multistage Pyrolysis with Catalytic Upgrading

DR. LAURA BARTLEY

Project Goal

The long-term aim of this program is to develop an optimal thermochemical biorefinery platform by optimizing biomass feedstock characteristics, with switchgrass and sorghum as the primary foci of this project.

Specifically, we will: a) identify biomass compositional features that improve pyrolytic conversion (product quality and yield) and design strategies for engineering grasses (switchgrass and sorghum) to enhance these traits, b) conduct multi-temperature (staged) pyrolysis experiments, and c) use selective upgrading reactions (light oxygenate condensation, furan, anhydro sugar condensation, mild deoxygenation) along with extension of the pyrolysis process to two stages.

Expected Project Outcomes

The projected outcomes from this project will be understanding of the relationships between plant structure and composition with yield, quality and refinability of bio-oil produced from diverse switchgrass and sorghum genotypes. This understanding will direct research toward the development of optimal feedstock-conversion biorefineries. Optimal includes not only economics, but as importantly, sustainability. This will be achieved by maximizing the production of biofuels while minimizing the required agricultural resource inputs, acreage, water, fertilizer, etc.

Other Sources of Funding:

The University of Oklahoma and Texas A&M University covered the matching requirements.



PI: Dr. Laura Bartley University of Oklahoma Microbiology & Plant Biology

Co-PI: Dr. Richard Mallinson University of Oklahoma *Chemical Engineering*

Co-PI: Dr. John Mullet Texas A&M University *Biochemistry & Biophysics*

Funded: \$162,000 Start Date: 04/30/2013 End Date: 04/29/2015



Project Title: Simultaneous Starch and Cellulose Hydrolysis for Whole Stalk Processing of Sweet Sorghum

DR. DANI BELLMER

Summary:

Sweet sorghum's high productivity, low input requirements, and versatility make it an attractive feedstock for energy production. It contains high levels of carbohydrates in the form of directly fermentable sugars, starch, and cellulose. In order to maximize carbohydrate production from all three forms, a whole stalk process will be evaluated for sweet sorghum.

The proposed process includes physical pretreatment of the stalks by either a twin screw press or a mechanical refiner for fiber development followed by simultaneous hydrolysis of starch and cellulose.

Specific objectives include

- 1) Evaluate a twin screw press in a dual role of juice extraction and bagasse pretreatment
- 2) Optimize conditions for simultaneous hydrolysis of starch and cellulose in the bagasse and
- 3) Evaluate the use of a mechanical refiner for sweet sorghum bagasse pretreatment.

A whole stalk process is potentially more beneficial because carbohydrate yields can be doubled when the conversion of cellulose and starch is considered in addition to the directly fermentable sugar, and because the process is more versatile and can be used with other complementary feedstocks in a dual feedstock system.



PI: Dr. Dani Bellmer Co-PI: Dr. Hasan Atiyeh Oklahoma State University Biosystems & Ag. Engineering

Co-PI: Dr. Gopal Kakani Oklahoma State University *Plant & Soil Sciences*

Funded: \$149,766 Start Date: 01/01/13 End Date: 12/31/15

Other Sources of Funding: Oklahoma State University covered the cost sharing portion of this award.



Project Title: Advanced Technologies for Biodiesel Production using a Microwave Extraction System

DR. DORIN BOLDOR

Project Goal

Current fossil fuel prices make biodiesel produced from vegetable oil a viable renewable energy source. Notwithstanding feedstock prices, a major component of the biodiesel price is its production costs, higher than petroleum diesel. This project addresses the critical need to develop cost-saving processing technologies to assist biodiesel producers in reducing energy consumption and increasing yields. The overall objectives for the project were: a) To optimize the oil extraction process from rice bran and soybean flour using a batch microwave process; b) To design, develop and optimize a continuous microwave system for oil extraction using the optimization results obtained from the batch microwave process for soybean and rice bran flour; c) To use the batch and continuous microwave oil extraction optimization results obtained for soybean and rice bran flour and extend it to study the oil extraction from Chinese tallow tree (CTT) seeds; and d) To achieve microwave-assisted trans-esterification of these lipids into biodiesel.

Project Outcomes

The use of microwaves as energy source (batch and continuous) for oil extraction from various oil containing agricultural feedstocks (soybean flour, rice bran and Chinese tallow tree seeds [CTT]) and as a heating source for transesterification of the extracted oils into biodiesel was investigated. The microwave assisted oil extraction (MAE) was compared with conventional (CE) and Soxhlet extraction (SE) methods. Results indicate that the MAE process was faster than CE and oil yields comparable to SE were obtained. The quality of the oil extracted also adhered to biodiesel specifications and oil conversion into biodiesel rates of > 99% were obtained indicating the feasibility of using microwaves for oil extraction and transesterification. Future research will focus on scaleup of the continuous microwave process and a detailed study on the cost economics involved for introducing the process on an industrial scale. Use of other alternative energy efficient technologies in conjunction with microwave process to further reduce the operating costs involved and produce competitively priced biodiesel will also be investigated.



PI: Dr. Dorin Boldor Louisiana State University AgCenter Biological and Ag Engineering

Co-PI: Dr. Vadim Kochergin Louisiana State University AgCenter Audubon Sugar Institute

Co-PI: Dr. Cristina Sabliov Louisiana State University Biological and Ag Engineering

Co-PI: Dr. Marybeth Lima Louisiana State University Biological and Ag Engineering

Funded: \$70,000 Start Date: 07/01/2007 End Date: 06/30/2010



Project Title: Development of a Skid-Mounted Gasification System for On-Site Heat, Fuel, and Power Production

DR. SERGIO CAPAREDA

Project Goal

The goal of the project was to develop modular biomass thermal conversion systems (for heat and power) for the different agricultural industries in the region that generate enormous amounts of residues and wastes. The specific objectives include the following: a) Investigate the technical feasibility of on-site thermal gasification systems for different unique biomass wastes in the region and evaluate the gas fuel quality, composition, heat energy and power output, b) Evaluate the economic feasibility of decentralized thermal gasification systems for different on-site applications conduct systems analysis for the different applications within the region and lay-out strategies to reduce barriers for commercialization, d) Evaluate the environmental and air quality implications of the systems including permitting procedures.



PI: Dr. Sergio Capareda Texas A&M University Texas Agri-Life Research

Co-PI: Dr. Michael Mailander* Louisiana State University *Biological & Ag Engineering*

Co-PI: Dr. Chandra Theegala Louisiana State University *Biological & Ag Engineering*

Associate PI: Dr. Michael Farmer Texas Tech University Agricultural & Applied Economics

Funded: \$279,380 Start Date: 07/01/2007 End Date: 06/30/2011

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Project Outcomes

The project has developed the TAMU fluidized bed and the LSU downdraft gasifier. The individual performances have been evaluated for small and modular applications in bench and pilot scale studies.

The economic and systems analysis studies have shown numerous potential applications in several key industries in the regions as follows: cotton gins in Texas and animal farms in both Texas and Louisiana. Preliminary sizing has been made and suited to the different industries in the region. For example, numerous cotton gins in Texas have the potential to generate between 1-3MW of electrical power from gasification of cotton gin trash.

The fluidized bed technology developed at Texas A&M University (TAMU) was licensed by SDL Citadel Global (Dallas, Texas) and in the process of commercialization.

Other Sources of Funding:

The collaborating private organization contributed their time and personnel during the project implementation and through Texas A&M University's cost share.



Project Title: Structure Dynamics-Guided Biocatalyst Improvement

DR. SUSIE DAI

Project Goals

The project aimed to develop a novel structure dynamics-guided platform for biocatalyst improvement and employ the platform to improve the biocatalysts like cellulases for higher efficiency and better inhibitor resistance. The ultimate goal for this research is to produce the cost-effective biocatalysts with higher efficiency, more inhibitor resistance, improved thermostability, better substrate specificity, enhanced acidic tolerance, and superior compatibility for expression in different systems. The biocatalyst improvement will help to improve the biomass conversion efficiency and revolutionize the biorefinery procedures. The two major objectives are: I) Comparative structure dynamics profiling for three cellulases upon binding with different substrates and inhibitors and II) Developing improved cellulases based on the structure dynamics analysis.

Project Outcomes

- Performed the HDX analysis of both CBHI and EGI enzymes and applied the HDX analysis to xylanase enzymes. The HDX mass spectrometry data was combined with comparative genome analysis and identified important regions for enzyme functions. The novel approach will enable the finding of new important regions for enzyme improvement and proprietary enzyme improvement strategies.
- Successfully engineered a *T. reesei* xylanase to allow the expression in *E. coli* to reach similar activity as in *T. reesei*. Traditionally, the expression of *T. reesei* enzymes in *E. coli* and yeast resulted in extremely low activity (1/30th). The xylanase expressed in *E. coli* showed much lower activity than the wild type xylanase expressed in *T. reesei*. However, with the mutant that was engineered, SprF xylanase expressed in *E. coli* showed comparable activity to that of wild type enzyme expressed in *T. reesei* at 50 degree. The activity is also retained at higher temperature of 70 degree. These results have overcome the problem and enabled the T. reesei and other fungal enzymes to be used for en-



Dr. Susie Dai Texas A&M University Office of the Texas State Chemist

Co-PI: Dr. Joshua Yuan Texas A&M University *Plant Pathology & Microbiology*

Funded: \$63,436 Start Date: 07/01/09 End Date: 06/30/11

> Other Sources of Funding: The Texas AgriLife Research Center.



Project Title: Vibrio Furnissii - A Biotechnology Platform for Biomass Conversion

DR. PAUL DE FIGUEIREDO

Project Goals

The long-term aim of the project is developing an efficient and economical platform for the direct bioconversion of biomass into kerosene and other long-chain alkanes. The following objectives are : 1) To generate *V. furnissii* strains with altered hydrocarbon biosynthesis capacities, 2) To characterize the hydrocarbon profiles of *V. furnissii* strains with altered hydrocarbon-producing capacities, and 3) To characterize the genetic lesions in *V. furnissii* strains harboring altered hydrocarbon biosynthesis and accumulation phenotypes.

Expected Outcomes

It was originally proposed to study Vibrio furnissii, a gram-negative bacterium that purportedly possessed superb oleagenic properties. However, early in the course of our investigation, we learned that other investigators had failed to reproduce the oleaginous phenotype, raising serious questions about the use of this microbe as a biofuel platform. With this fact in mind, we turned our attention to D. hansenii. D. hansenii has several attractive features for biofuel production. First, the organism is genetically tractable. Therefore, it is amenable to genetic engineering. Second, it mediates the biotransformation of diverse feedstocks into the high-energy, long-chain hydrocarbons (C16-C18) commonly found in biodiesel. These hydrocarbons accumu late in large quantities when the organism is grown under defined conditions, approaching 50% of its dry weight. Third, the organism is halotolerant, and can grow in high salt conditions where the risk of contamination to industrial-scale bioreactors is limited. Finally, the organism synthesizes a variety of hydrocarbon structures under assorted culture conditions. Therefore, microbial metabolism can likely be fine tuned to address varied biofuel and co-product demands. Taken together, the organism under development constitutes a potentially attractive tool for converting diverse bioenergy feedstocks into high-energy fuels.



PI: Dr. Paul de Figueiredo Texas A&M University Plant Pathology and Microbiology

Funded: \$70,000

Start Date: 07/01/2007 **End Date:**

06/30/2009

Other Sources of Funding:

Texas A&M University Department of Plant Pathology & Microbiology contributed to graduate student's salary (as shown in the budget) to meet the

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Title: Effect of Growth Media Chemical Composition on Algal Biomass Properties

DR. NURHAN DUNFORD

Project Goals

The main objective of this project was to optimize a closed photobioreactor system that will maximize oil accumulation in microalgae cells growing in swine lagoon waste water and vinasse from sweet sorghum to ethanol production process while maintaining a high rate of biomass production, carbon dioxide capture and waste water remediation. Specific objectives include to: 1) Select high performance microalgae strains that are suitable for waste water remediation and high oil accumulation, 2) Design, construct and optimize operating conditions for a 25 L microalgae photo-bioreactor system to maximize oil and biomass production yield, 3) Test and improve the non-destructive realtime process monitoring and control system that has been developed at OSU and develop a mathematical model for calibration of sensors to be used to monitor biomass production in 25 L reactor, 4) Study chemical and physical properties of biomass and oil produced by different microalgae strains grown on swine lagoon waste water and vinasse. Examine the efficiency of nutrient removal and carbon dioxide capture during microalgae growth, and 5) Train graduate and undergraduate students and extension personnel on microalgae production.

Project Outcomes

- Six commercial microalgae strains [*Botryococcus braunii*, *Nannochlo-ropsis oculata* and *Dunaliella tertiolecta*, and three strains native to Oklahoma-*Picochlorum Oklahomensis* (PO), *Dunaliella* species SP19 and SP20] were used for the screening studies.
- One of the Oklahoma native algae strains, PO has the highest biomass productivity, grows very well in swine waste water & accumulates significant amount of oil, about 30% of dry biomass.
- PO has a good potential for producing algal biomass that can be used in production of various bioproducts including biofuels while removing nitrogen and potassium from animal waste water.
- Higher biomass concentration in the media (g/L) and lower ash content in the algal biomass were achieved using animal waste water as compared to regular media.
- A non-destructive real-time process monitoring and control system was developed and tested successfully.



PI: Dr. Nurhan Dunford Co-PIs:

Drs. Dani Bellmer, Doug Hamilton, and Ning Wang

All Senior Personnel are located at Oklahoma State University - *Biosystems & Agricultural Engineering*

Funded: \$74,608 Start Date: 07/01/11 End Date: 06/30/13

Other Sources of

Funding: Oklahoma State University covered the cost sharing portion of this award.



Project Title: Development of a customized enzyme cocktail for corn stover and switchgrass saccharification

DR. MOSTAFA ELSHADED

Project Goal:

- Identify genes belonging to 12 distinct enzymatic categories that exhibited highest levels of expression on corn stover and switchgrass, respectively.
- Synthesize, clone, and express target genes in an expression vector.
- Purify products and confirm their enzymatic activity and specificity.

Project Outcomes:

- We have identified target genes belonging to 12 distinct enzymatic categories that exhibited high levels of expression on corn stover and switchgrass, respectively.
- Synthesizing, cloning, and expressing target genes in an expression vector are nearly complete. Genes with the following activities have successfully been targeted for cloning: β -glucosidase, xylanases, cellobiohydrolases, carbohydrate esterases, endogluconases. Xylosidases, mannanases. Cloning of genes with the following activities is underway: mannosidases, α -glucuronidase, and α -N- arabinofuranosidase. In addition, enzyme assays for all 12 targeted activities has been established.
- We initially began to purify products and confirm their enzymatic activity and specificity for a few enzymes (endogluconases and cellobiohydrolases). Purification of three distinct cellobiohydrolases through affinity chromatography is underway. Current results indicate that multiple purification steps may be necessary to purify such enzymes.



Dr. Mostafa Elshaded Oklahoma State University Microbiology and Molecular Genetics

Co-PI:

Dr. Noha Youssef Oklahoma State University *Microbiology and Molecular Genetics*

Funded: \$135,000 Start Date: 1/1/14 End Date: 12/31/15

Other Sources of Funding:

Oklahoma State University contributed cost share to meet matching requirement.



Project Title: Development of Enrichment Cultures that Degrade Lignin for Enhanced Biofuel Production

DR. BABU FATHEPURE

Project Goals

The overall objective of this project was to develop bacterial enrichment cultures and assess their potential to degrade lignin for enhanced bioavailability of cellulose and hemicellulose in plant residues. The long -term goal of this study is to identify novel enzymes and express them in a heterologous system.

Project Outcomes

- Four enrichment cultures including the Cow creek (CC), Yellowstone (YS), Greenstream (GS), and Rumen-Termite (RT) were developed that degraded lignin as the sole source of carbon.
- Analysis of 16S rRNA clone libraries generated from genomic DNA of the enrichments showed a large percentage of clones belonging organisms known to degrade aromatic compounds including members of the order Pseudomonadales, Burkholderiales, Bacillales, Xanthomonadales, Spingomonadales, and Actinobacteria.
- All enrichment cultures grew well on lignin as well as lignin-derived monomers and dimers as the sole sources of carbon.
- Ring-cleaving genes: catechol 1, 2- dioxygenase, catechol 2, 3dioxygenase, and protocatechuate 3, 4 dioxygenase were amplified from the genomic DNA using degenerate primers & confirmed by cloning and sequencing PCR products.
- Cow Creek and Yellowstone enrichments degraded significant amount of lignin in alfalfa within 30 days and almost complete degradation within 80 days. On the other hand, degradation of lignin in switchgrass was minimal (9 to 15%) and occurred at slow rates.

Conclusion:

Bacterial enrichments established from various decaying biomass sources were dominated with aromatic compound degrading bacteria.





PI: Dr. Babu Fatherpure Oklahoma State University Microbiology & Molecular Genetics

Co-PI:

Dr. Mark Wilkins & Dr. Marthah DeLorme Oklahoma State University Biosystems & Ag Engineering

Funded: \$70,000 Start Date: 3/1/2009 End Date: 8/31/2010

Other Sources of Funding:

Oklahoma State University contributed cost share to meet the matching requirement.



Title: Discovery of Novel Lignin-Degrading Genes in Bacteria Using Metagenomic and Proteomic Approach for Enhanced Saccharification of Plant Biomass

DR. BABU FATHEPURE

Project Goal

The primary goal of this research is to identify novel genes and enzymes in bacteria that have high potential to be useful for lignocellulose bioconversion when introduced into an appropriate expression system for enzyme production. The objectives are: (i) Perform in-silico analysis of Yellowstone metagenome and generate a protein database, (ii) Identify extracellular proteins in cultures grown on lignin as the sole carbon source, and (iii) Express and purify lignin-degrading enzymes using a recombinant production system.

Project Outcomes

- A lignin degrading bacterial consortium was developed from decaying wood from a thermal pond in Yellowstone National Park (YS-Enrichment). This enrichment is capable of degrading native lignin in alfalfa and switch grass.
- YS Metagenome revealed an inventory of lignin degrading genes including laccase, peroxidase, chloroperoxidase, C-alpha dehydrogenase, beta-etherase, feruloyl esterase, carboxylesterase, vanillate demethylase, vanillyl-alchol oxidase, vanillin dehydrogenase, syringate O-demthylase, and many genes for ring oxidation and cleavage.
- YS Metagenome revealed a high abundance of sequences related to Proteobacteria, Actinobacteria, and Firmicutes.
- *Pseudomonas* sp., *Rhizobium* sp., *Acromobacter* sp.,*Bravibacillus* sp., and *Novospingobium* sp., capable of growing solely on lignin and lignin-derived compounds were isolated from the enrichments.
- Genome analysis of the pure cultures revealed the presence of lignin degrading genes
- Secretome analysis of YS-enrichment as well as pure cultures grown on switchgrass or alfalfa detected lignin degrading extracellular enzymes.
- *Conclusion*: Bacteria have the genes/enzymes that degrade lignocellulose.



PI: Dr. Babu Fathepure Co-PIs: Drs. Patricia Canaan and Rolf Prade Oklahoma State University - *Microbiology* and Molecular Genetics

Funded: \$75,000 Start Date: 07/01/11 End Date: 06/30/13

Other Sources of Funding: Oklahoma State University covered the cost sharing portion of this award.



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U.S. Department of Agriculture-NIFA

> Project Title: Building the Basis for the Improvement of Microalgae Oil Production Systems Efficiency Through the Quantification and Model Integration of Fundamental Biological and Physiological Processes

DR. CARLOS FERNANDEZ

Project Goal

The goal of his project is to build the basis for the improvement of the efficiency of microalgae oil production systems through the quantification and integration by mechanistic modeling of fundamental processes governing the growth and lipid production of microalgae cultures. This project specifically aimed: 1) to quantify fundamental responses of selected microalgae strains during the phases of growth and oil biosynthesis, including carbon assimilation (photosynthesis), carbon losses (growth and maintenance respiration), biomass conversion efficiency, biomass production, and population dynamics, to various levels of primary environmental factors(i.e. water temperature, quantum flux interception , supply of CO2), and 2) to integrate the fundamental biological and physiological responses to environmental factors quantified in the first objective into a mechanistic simulation tool for gaining understanding and assisting the analysis of microalgae-based oil production systems and improving their production efficiency.

Project Outcomes

- The light attenuation coefficient obtained for N. salina growing in the bioreactors ranged from 0.0098 cm⁻¹ &0.5385 cm⁻¹, depending on the culture biomass density, with lowest value corresponding to only saline water and higher value to a culture with a biomass density of 783 g m⁻³. A linear regression with an intercept of 0.0326 cm⁻¹ and slope of 0.0007 m³g⁻¹cm⁻¹ was obtained to numerically represent the effect of biomass concentration (in g m⁻³) on the light attenuation coefficient (in cm⁻¹).
- Quantification of growth responses (cell population growth) of *N. salina* as affected by the combination of four temperature levels ranging from 15 to 27 °C and five levels of incident irradiance (on lit bioreactor side) of 893, 512, 358, 220, and 133μE m⁻² s⁻¹ showed a significant interaction between these two environmental factors. A maximum cell population was obtained at average incident PPFD of 512μE m⁻² s⁻¹ in combination with a culture temperature of 15°C.
- A numerical mechanistic model was developed to simulate experimental growth data collected with the bioreactors. Outputs produced from this model demonstrated its capability for generating the basic trends of light dynamics, growth kinetics, nutrient uptake, and lipid production in the bioreactors. The model suggests that lipid production could be optimized to as much as 3.2724 g d⁻¹ or 50.42 g m⁻³ d⁻¹.



PI: Dr. Carlos Fernandez Texas A & M University *Texas AgriLife Research & Ext. Center Corpus Christi*

Co-PI: Dr. Joe Fox Texas A&M Univ. Corpus Christi Life Sciences

Co-PI: Dr. Barbara Benson Univ. of Louisiana-Lafayette *Environmental Science*

Funded: \$160,000.00 Start Date: 11/01/2011 End Date: 10/31/2014



Project Title: Improving Biofuel Conversion from Animal Waste

DR. ZHIQIANG HU

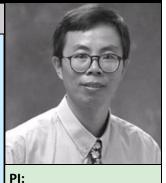
Project Goal

Animal waste and other agricultural waste streams are important biomass resources for renewable energy production. The current animal waste treatment and management systems are often inadequate. A common collection and disposal system in the United States is an anaerobic lagoon/spray irrigation system. Anaerobic digestion is the most common technology applied for sludge stabilization and is now an encouraged method for animal waste management. However, farmers are reluctant to use traditional anaerobic digestion systems due to the high construction costs and the skill and experience requirements in operation and management. A major technical problem involved in anaerobic digestion is the slow growth of methanogens that are susceptible to inhibition by a variety of factors. Of all known methanogens, aceticlastic methanogens such as Methanosaeta grow slowly by using only acetic acid as the electron donor and carbon source. For comparison, H₂-oxidizing methanogens have much faster growth rate and high affinity for hydrogen.

This research project aimed to use electrochemical and biological augmentation processes to improve biogas (both in yield and rate) production from animal waste. The objectives of this project are: 1) to use a robust electrochemically-assisted anaerobic digestion process to improve biogas production with less maintenance need and (2) to enrich fast-growing hydrogen-oxidizing methanogens for rapid and efficient methane production.

Project Outcomes

The propionic acid concentration profiles and the cumulative methane gas production curves showed significant difference in VFA production during anaerobic digestion and improved methane production in the MEC-based anaerobic digestion systems. This suggests that a hybrid system by combining a MEC with an anaerobic digestion system has the potential to produce more methane as a fuel gas. However it is still unclear as to whether the bioenergy in the form of methane being generated would offset the electric energy investigated. Further studies are needed to address issues such as energy balance, effect of different substrates, process complexity and stability before a complete success of the MEC-based anaerobic digestion can be achieved.



Dr. Zhiqiang Hu University of Missouri *Civil & Environmental Engineering*

Co-PI:

Dr. Robert Reed University of Missouri Water Resources Research Center

Funded: \$70,000 Start Date: 07/01/2009 End Date: 12/31/2011

Other Sources of Funding: University of Missouri-Columbia funded the cost of the PI's salary via cost share.



U.S. Department of Agriculture National Institute of Food & Agriculture



Project Title: Torrefaction and Densification of Switchgrass to Improve Syngas Quality and Transportation Logistics

DR. AJAY KUMAR

Project Goal

The overall goal is to evaluate torrefaction and densification as preprocessing options prior to switchgrass gasification.

The specific objectives are to: 1) Study and analyze the selected preprocessing methods, 2) Gasify preprocessed samples and evaluate syngas quantity and quality, and 3) Evaluate mass and energy balances and system logistics for each case.

Project Outcomes

- A graduate student (Ms. Madhura Sarkar) focused on the analysis of preprocessing on biomass properties and syngas obtained through gasification. The team investigated effects of four pretreatments (torrefaction at 230°C, torrefaction at 270°C, densification, and combined torrefaction and densification) on the thermal devolatilization characteristics, gasification and pyrolysis of switchgrass.
- Switchgrass pretreated with combined torrefaction and densification had the highest rate of devolatilization, followed by switchgrass pretreated with densification, switchgrass pretreated with torrefaction at 230°C, switchgrass pretreated with torrefaction at 270°C, and raw switchgrass.
- The pretreatment significantly effected gasification performance. Combined torrefaction and densification had the highest gas yield and efficiencies.
- Torrefaction and densification affected bio-oil composition. Increased torrefaction temperature favored the production of sugars and phenols.
- High pyrolysis temperature favored the production of aromatic compounds.
- Densification favors degradation of cellulose and hemicellulose during pyrolysis.



PI: Dr. Ajay Kumar Co-PIs: Drs. Danielle Bellmer, Carol Jones, and Krushna Patil Oklahoma State University *Biosystems & Agricultural* Engineering

Co-Pls:

Dr. Jaya Shankar Tumuluru Idaho National Laboratory Biofuels & Renewable Energy Technologies Department

Funded: \$83, 287 Start Date: 01/13/2012 End Date: 01/12/2014



Project Title: Developing a Cost-Effective Technology for Conditioning Biomass-Generated Syngas with In-Situ Bed of Biochar-Based Catalysts

DR. Ajay Kumar

Project Goal

Kumar's long-term goal is to break barriers of converting biomass resources into fuels, chemicals and power through gasification process so that a successful bio-based economy can be feasible. The project main objectives is to develop a novel patentable technology to produce syngas with low tar and high hydrogen to carbon monoxide ratio. His rationale is that in-situ syngas conditioning, if effective, can drastically reduce the technical and economical penalty associated with conditioning (upgrading) syngas for production of fungible fuels. To accomplish the goal, the three specific objectives are to: (1) identify key factors for design and operation of the novel reactor, (2) determine effects of catalysts and operating conditions on syngas composition and yield, and (3) compare the cost and sustainability indices of the technology developed with the conventional technology.

Project Outcomes

- Biochar supported nickel catalysts have been developed through different preparation methods. The catalysts are also characterized.
- Effects of preparation methods on properties of biochar supported catalysts were studied. The properties of catalysts include surface textural properties (surface area and surface functional groups), nickel dispersions.
- Surface areas and pore distributions were determined using N2 isotherm method.
- Surface functional groups on biochar supports were analyzed using temperature controlled desorption and FTIR.
- The nickel dispersion and nickel particle sizes on the biochar supports were analyzed using transmission electronic microscopy.
- The model tar reforming experiment using biochar based catalyst has been carried out in a pyroprobe.



PI: Dr. Ajay Kumar Co-PIs: Dr. Robert Scott Frazier Dr. Krushna Patil

All senior personnel are employed by Oklahoma State University in the *Biosystems & Agricultural Engineering Department*

Funded: \$149,381 Start Date: 01/01/13 End Date: 12/31/14

Other Sources of

Funding: Oklahoma State University covered the cost sharing for this award.



Project Title: Cellulase Immobilization on Nano-Carriers for Reuse in Cellulose Hydrolysis

DR. YU "JESSIE" MAO

Project Goal

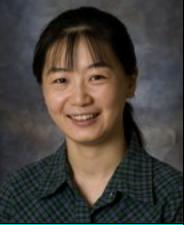
The project goal was to investigate immobilization of both cellobiohydrolase and β -glucosidase on custom-synthesized magnetic nanoparticles in order to reduce enzyme cost during cellulose hydrolysis.

The objectives were:

- 1. Develop methods to immobilize cellulases on magnetic nanoparticles.
- 2. Investigate the activities of immobilized cellulases in cellulose hydrolysis.
- 3. Evaluate the reusability of immobilized cellulases in cellulose hydrolysis.

Project Outcome(s)

- 1) The nanoparticles demonstrated strong magnetization per particle that allows fast separation using a medium magnetic field.
- Immobilized cellobiohydrolase and β-glucosidase retained 44.4% and 67.6% of the enzyme activity of their non-immobilized counterparts, respectively.
- The immobilized cellobiohydrolase and β-glucosidase showed improved thermal stability and similar pH stability compared with their non-immobilized counterparts.
- We demonstrated the reuse of the immobilized β-glucosidase and cellobiohydrolase with the retention of enzyme activity to 43.9% and 46.1% of the corresponding initial enzyme activity after being reused for 10 times.
- 5) Reuse of cellulases is expected to significantly reduce enzyme cost during the process of cellulose hydrolysis for cellulosic ethanol production and facilitate biofuel product purification. This benefits starting cellulosic ethanol plants and the industrial conversion of cellulosic biomass to fuels.



PI: Dr. Yu "Jessie" Mao Oklahoma State University *Biosystems & Ag Engineering*

Co-PI: Dr. Mark Wilkins Oklahoma State University *Biosystems & Ag Engineering*

Funded: \$70,000

Start Date: 12/1/2009 End Date: 11/30/2012

Other Sources of Funding:

Oklahoma State University: A portion of Dr. Mao's salary for each year, fringe benefits on that salary, and waived indirect costs at a rate of 47.7% of total direct costs.

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U.S. Department of Transportation

Project Title: Hydrogen Fuel Production from Microalgae

DR. SVETLANA OARD

Project Goal

The long term goal of the research is development of economically viable hydrogen (H_2) biofuels. We aimed to develop renewable H_2 fuels utilizing solar-powered CO₂-sequestering microalgae. Three components of an economical process were developed: scalable and economical algae cultivation, improved microalgae capable of producing H₂ in the presence of O_2 , and H_2 utilization as transportation fuels.

Project Outcomes

- This project completed the development of microalgae/H₂ /fuel cell systems.
- A reliable system for microalgae transformation was selected, and stable genetic transformation of microalgae *Chlamydomonas* was demonstrated in the PI's laboratory. The best strain for genetic engineering of Chlamydomonas was selected after testing eight candidate strains. A chimeric gene cassette for inducible expression of the algal hydrogenase with the highest H₂ forming activity was constructed. This chimeric gene enables regulation of gene induction that will allow tuning of metabolic balance in algal cells.
- An advanced computational methodology that combined structure homology analysis, three dimensional mapping, and molecular dvnamics simulations resulted in discovery of three functional networks in the hydrogenase protein matrix. Each network is linked to the catalytic site. Discovery of these networks will greatly facilitate design of mutations to increase hydrogenase resistance to O₂.
- Components of an economical process for scalable manufacturing of H₂ were analyzed. These included methods for scalable and economical algae cultivation, H₂ separation/purification, and storage/ utilization.
- Preliminary analysis of process economics was performed and the rates of H2 yield for commercial manufacturing were estimated.



PI: Dr. Svetlana Oard Louisiana State University AgCenter Biotechnology Lab

Co-PI: Dr. Michael Mailander Louisiana State University AqCenter BAE

Co-PI: Dr. Chandra Theegala Louisiana State University AgCenter BAE

Funded: \$69,925 Start Date: 7/01/2009 End Date: 6/30/2011

> **Other Sources** of Funding: Louisiana State University AgCenter



Project Title: Optimizing a New Downdraft Gasification System for Synthesis Gas Production from Low Bulk Density Biomass Material

DR. KRUSHNA PATIL

Rationale

A unique biomass downdraft gasification design was developed at OSU. The new gasifier has the potential for heat and power applications and for generating high quality syngas which is the basic building block for generating chemicals and liquid fuels. One of the main requirements for the unique design was an ability to convert low bulk density feedstocks. In initial tests using wood pellets, a high density biomass, the laboratory-scale gasifier produced very good quality syngas with relatively high concentrations of CO and H₂. The primary aim of this project was to test and evaluate the gasifier using low bulk density biomass materials like switchgrass, wheat straw and wood-based feedstocks which constitute the major biomass resource base in Oklahoma and South Central region. Once the gasifier is tested using low bulk density feedstocks, it will be demonstrated to potential industries in Oklahoma and South Central region to creating awareness about the fossil fuel saving potential.

Project Outcomes

- The main results for wood pellets gasification include tar cracking temperatures close to 1000⁰C; the average volumetric concentration levels of 20% CO and 13% H₂ in the gas, and hot and cold gas efficiencies of 78 and 64%, respectively.
- A series of research trials were conducted on low bulk density biomass materials including wood shavings, switchgrass and sweet sorghum bagasse. The maximum tar cracking temperatures exceeded 1100[°]C. Among all the low bulk density materials, switchgrass generated syngas with highest lower heating value of 1448 kcal/Nm³ and 24% CO concentration level by volume. The tar content was 18 g/Nm³. Sweet sorghum showed syngas generation with lowest lower heating value of 1086 kcal/Nm³ while the tar content in the gas was 58 g/Nm³.
- The hot and cold gas efficiencies for low bulk density biomass materials varied from 60 to 79%, and 52 to 72%, respectively.
- A gasifier design patent was generated: U.S. Patent 8,657,892 "Downdraft Gasifier with Internal Cyclonic Combustion Chamber" issued 02/25/2014.
- The gasifier system was successfully demonstrated to numerous companies. Unfortunately, the downturn of the economy prevented any of these companies to invest in this technology during the project duration. Technology update (January 2015): A license agreement has been established with a company intending to use this technology in a decentralized electricity generation system. A scaled-up version (60 kWe) is currently being fabricated at OSU with testing to



PI: Dr. Krushna Patil Oklahoma State University Biosystems & Ag Engineering

Co-PI: Dr. Ray Huhnke Oklahoma State University Biosystems & Ag Engineering

Co-PI: Dr.Danielle Bellmer Oklahoma State University Biosystems & Ag Engineering

Funded: \$69,997

Start Date: 07/01/2007 End Date: 06/30/2010



Project Title: Saline Extractive Distillation for Ethanol Separation

DR. PETER PFROMM

Rationale

The thermal energy demand for producing fuel ethanol from the fermentation broth of a state of the art corn-to-fuel ethanol plant in the U.S. is largely satisfied by combustion of fossil fuels, which impacts the possible economical and environmental advantages of bio-ethanol over fossil fuels. To reduce the thermal energy demand for producing fuel ethanol, a process integrating salt extractive distillation – enabled by a new scheme of electrodialysis and spray drying for salt recovery – in the water-ethanol separation train of a state of the art corn-to-fuel ethanol plant is investigated. Process simulation using Aspen Plus[®] 2006.5, with the ENRTL-RK property method to model the vapor liquid equilibrium of the water-ethanol-salt system, was carried out.

Project Outcomes

- The integrated salt extractive distillation process resulted in a thermal energy savings of 30%, when compared with the state of the art process for separating fuel ethanol from the beer column distillate.
- A thermal energy savings potential of 8.1*10^13 J (as natural gas HHV) per year with an operating cost savings potential on the order of 500,000\$ per year can be estimated for producing 151.4 ML of fuel ethanol (99.95 wt%) per year. An overall maximum energy savings potential of 5.9*10^16 J (as natural gas HHV) per year could be realized for the targeted 117.4 GL of fuel ethanol to be produced in the U.S in 2022, if fermentation is the process of choice.

Other Sources of Funding:

Salaries and benefits cost-shared by Kansas State University.



PI: Dr. Peter Pfromm Kansas State University Chemical Engineering

Funded: \$69,988 Start Date: 07/01/2007 End Date: 06/30/2010





Project Title: Breaking the Cost Barrier for Bio-Ethanol; Reactive Adsorption of Fermentation Broth

DR. MARY REZAC

Project Goal

• To develop reactive adsorption technology for the efficient technical-scale recovery of ethanol from fermentation broths.

Project Outcomes

- Unlike conventional ethanol recovery systems, where separation of ethanol from water relies upon differences in the boiling points of the components, in the study system, we attempted to achieve separation by selectively reacting ethanol with a chemical moiety tethered to the surface of a solid support and subsequently reversing the reaction to recover purified product.
- This study demonstrated that several chemical species could be used for selective and reversible reaction with ethanol to form stable products.
- An increase in temperature of less than 30°C was sufficient to reverse the reaction.
- Detailed engineering design analysis indicated that the use of distillation to recover dilute ethanol from the fermentation broth consumes nearly 40% of the total energy of the ethanol production process. The beer column consumes approximately 60% of the total energy required by the separation system (or about 24% of the total used in the plant); the ethanol concentration to the rectifying column is nearly 58 wt%.
- Replacement of the distillation column with ethanol selective membranes operated in a pervaporation mode has the potential to significantly reduce the overall energy demand.



PI: Dr. Mary Rezac Kansas State University *Chemical Engineering*

Funded: \$70,000

Start Date: 07/01/2007

End Date: 12/31/2009

Other Sources of Funding:

Indirect costs are charged at a rate of 25% to all the total direct costs. Cost share is paid by Kansas State University.

Project Title: Nanoparticle Systems for Delivery of Biological Antimicrobial Compounds to Limit Microbial Contamination in Industrial Yeast Fermentation

DR. STEVEN RICKE

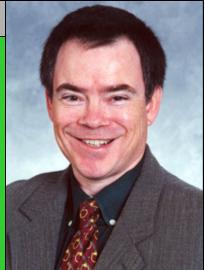
Project Outcomes

- Lactobacillus delbrueckii subsp Lactis ATCC479 exhibited maximal MICs with chitosan (1.87 mg/mL), ε-polylysine (0.3125 mg/mL) and nisin (0.05 mg/mL), respectively.
- *L. plantarum* ATCC 8014 had a minimal MICs with CS, ε-polylysine and nisin (1.25 mg, 0.156 mg and 0,00156 mg, respectively).
- Nisin significantly reduced most Lactobacillus strains by 6 log CFU/mL after 48 hours with the exception of *L. casei* which remained viable.
- From the wide range of the antimicrobials tested against the Lactobacillus strains, nisin was the most successful. The nisin alone was effective against most of the Lactobacillus strains except *L. casei*.
- EDTA chelator had a synergistic effect on the nisin activity. The EDTA alone was not significantly effective against the *L. casei*. However, the EDTA had a synergistic effect with nisin against the *L. casei* strain in this study (no detectable cells after 24 and 48 hours). The synergistic activity of the EDTA with the nisin might be explained by the action of the EDTA on the cell wall first allowing the nisin to enter the cytoplasmic membrane causing leaks and then cell damage.
- The synergistic effect of nisin with EDTA chelator inhibited nisinresistant potential contaminant bacteria such as *L. casei*. An MIC of 0.4 mg/mL of nisin combined with the EDTA at an MIC of 1 mg/ml markedly suppressed *L. casei* by 6 log CFU/mL.



Other Sources of Funding:

University of Arkansas: PI provided Cost Share at Sun Grant determined rate of 20% or more from his available fund - Donald "Buddy" Wray Food Safety Endowment Fund, University of Arkansas. This Cost Share amount was used to cover salary, fringe benefits, supplies, and facilities and administrative costs.



PI: Dr. Steven Ricke University of Arkansas Food Science

Co-PI: Dr. Michael Johnson University of Arkansas Food Science, IFSE

Co-PI: Dr. Jin-Woo Kim University of Arkansas *Biological and Ag Engineering*

Funded: \$70,000

Start Date: 07/01/2007

End Date: 06/30/2009



Project Title: Screening and Assessing Growth Kinetics of High Lipid Microalgal Strains DR. CHANDRA THEEGALA

Project Goal:

The project goal was to comprehensively screen microalgal species for sustainability to produce biodiesel feedstock. The objectives were: 1) A shortlist of algal strains suitable for outdoor cultivation in Louisiana for high and moderate temperature seasons, 2)Lipid content for all strains suitable for outdoor cultivation, 3) Kinetic parameters for the best strains (8 high and 8 moderate temperature strains), 4) Mathematical models for estimating lipid productivities.

Project Outcomes:

- A total of 34 species of microalgae were successfully screened for growth rates and non-polar lipid contents (NPLC) at two different water temperatures of 35 and 25 ^oC, representing the average local water temperatures for high and moderate temperature production periods, respectively.
- Top 8 algal sp. for the best lipid yields for warmer water temperatures (simulating outdoor conditions during summer): *Dunaliella tertiolecta, Nannochloris* sp., *N. oculata, Neobloris oleabundans, Selenastrum capriconutum, Scenedesmus dimorphus, Scenedesmus* sp., *and Thalassiosira* sp.
- Top 8 algal sp. for the best lipid yields for moderate water temperatures (simulating outdoor conditions during moderate months): *Dunaliella tertio-lecta, Dunaliella sp. 1, Nannochloris sp., N. oculata, Neobloris oleabundans, Selenastrum capriconutum, Scenedesmus dimorphus, and Thalassiosira sp.*
- The kinetic parameters (maximum net specific growth rate, half saturation constants, biomass production factors, lipid production factor, etc.) were computed for the 16 short-listed species/strains for the two culture temperatures, under outdoor field conditions.
- Mathematical models were developed to predict the lipid productivities from the top 3 finalist species from outdoor cultures (at each culture temperatures).



Dr. Chandra Theegala Louisiana State University AgCenter *Biological and Agricultural Engineering*

Co-PI:

PI:

Dr. Ronald Malone Louisiana State University *Civil and Environmental Engineering*

Co-PI: Dr. Eric Achberger Louisiana State University Biology

Funded: \$69,674 Start Date: 12/01/2009 End Date: 05/31/2012

Other Sources of Funding

Louisiana University funded the cost share regarding salaries and fringe benefits for the PI and Co-PI's.



Project Title: Bio-Oil from Fast Pyrolysis of Forage Sorghum Biomass

DR. DONGHAI WANG

Project Goal

Bio-oil production has great potential to produce high heat content fuels including higher alcohols, "green" gasoline and diesel, and aviation fuels. The goal of this proposed research was to develop a comprehensive understanding of the use of sorghum biomass for bio-oil production through fast pyrolysis. The specific objectives are: 1) Characterize the chemical composition and physical and thermal properties of forage sorghum biomass related to pyrolysis processing; 2) Develop a coordinated understanding of the relationship between composition, physical and thermal properties, processing condition, and bio-oil yield; and 3) Determine the optimum pyrolysis conditions for high bio-oil yield and low energy input.

Project Outcomes

- Chemical composition of the sorghum biomasses were analyzed following the NREL standard procedures and ranged from 38-44% cellulose, 18-26% hemicellulose, 13-20% lignin, and 7.5-11% ash. The total carbohydrate composition ranged from 51-69%.
- Little biomass (<1%) was pyrolyzed at temperatures below 300 °C & a minimal amount (~2%) was left after a 600 °C pyrolysis, as well.
- The results also indicated bio-oil produced through catalytic fast pyrolysis of biomass were mainly hydrocarbons, which can be converted into fuels including "green" gasoline, diesel and jet fuels by utilizing the same refining technologies employed by the current petroleum and coal-based fuel industry.
- Results from this research may be used for the design and construction of catalytic fast pyrolysis reactors for the production of high quality bio-oils readily amenable for the existing fuel industries.

Other Sources of Funding:

Kansas State University paid faculty time, fringe benefits, and indirect costs via cost share.



PI: Dr. Donghai Wang Kansas State University Biological & Agricultural Engineering

Co-PI: Dr. Ron Madl Kansas State University *Grain Science and Industry*

Co-PI:

Dr. Scott Staggenborg Kansas State University Agronomy

Funded: \$70,000 Start Date: 12/01/2009 End Date: 5/31/2012





U.S. Department of Agriculture-NIFA

Project Title: Value-Added Utilization of Biochar in Syngas Cleanup and Conditioning

DR. DONGHAI WANG

Project Goal

The project goal was to improve the economic and environmental sustainability of biomass gasification through value-added utilization of biochar byproduct and effective syngas cleanup and upgrading.

The objectives were:

- Determine the effect of biomass type, gasification condition, and gasifier design on biochar characteristics.
- Evaluate and optimize the performace of biochar-supported Ni catalysts in syngas cleanup and conditioning in various types of gasifier systems.
- Conduct an economic justification and analysis of using biochar in biomass gasification.

Project Outcomes

- Biomass type, air flow rate and gasifier design (insulation) had significant effects on biochar. Wood chips has significantly lower biochar yield, but the surface area of the char was much higher, compared to rice hulls at the same operating conditions. Higher air flow rate led to lower biochar yield but larger surface area. Insulation increased the pyrolysis temperatures so larger biochar surface area was obtained.
- Performance of the Ni/char catalysts was found superior to that of commercial tar cracking catalysts such as Ni/γ-Al₂O₃. For example, more than 99% of tars were effectively removed using the Ni/char catalysts. H₂ and CO concentrations in syngas also went up significantly.
- Initial cost analysis indicated that chars are less expensive than metal oxides, olivine, or dolomite that are common catalyst supports. In addition, consumption of Ni can be reduced by using char



PI: Dr. Donghai Wang Kansas State University Biological and Ag Engineering

Co-PI: Dr. Wenquiao Yuan North Carolina State University *Biological and Ag. Engineering*

Co-PI: Dr. Ajay Kumar Oklahoma State University *Biosystems and Ag. Engineering*

Co-PI: Dr. Danielle Bellmer Oklahoma State University *Biosystems and Ag. Engineering*

Co-PI: Dr. Krushna Patil Oklahoma State University *Biosystems and Ag. Engineering*

Funded: \$159,954.00 Start Date: 11/01/2011 End Date: 10/31/2014

as the support. Ni particles stay only on the outer surface of the char support. In metal-oxide-supported catalysts, Ni is also formed inside the support via the impregnating and calcining methods. Moreover, mechanical mixing of Ni and char saves energy and time in catalyst preparation.

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Title: Multi-scale Fouling Characterization of Fermented/ Hydrolyzed Sweet Sorghum

DR. ROB WHITELEY

Project Goal

The goal of this project is to quantify the fouling characteristics of fermented/hydrolyzed sweet sorghum on multiple length-scales in a bioethanol recovery process. The research plan calls for testing in a lab-scale flow loop and in the OSU farm-scale Alcohol Separation Unit located at the Bioenergy Laboratory.

The objectives are to:

- 1. Develop a composite multi-component fouling model to predict the rate of fouling for fermented/hydrolyzed sweet sorghum on multiple length scales (small scale lab to large scale industrial equipment).
- 2. Document the actual fouling rates measured in the Alcohol Separation Unit as a function of feed composition, pretreatment and operating conditions.
- 3. Develop recommended practices for operation and cleaning of the Alcohol Separation Unit as related to equipment and instrument fouling.
- 4. Fully commission the Alcohol Separation Unit and document the capabilities of the world-class separation technology.

Expected Outcomes

Results of the work will be used to develop a fundamental fouling model and to develop optimal maintenance and equipment cleaning practices. The work will be performed in collaboration with Sulzer ChemTech, USA. This work will provide answers to a critical question (how to minimize/handle equipment fouling) regarding the ability of farmers to operate and maintain on-site alcohol separation processes on a commercial scale. Completion of this project combined with cost data from the recently constructed farm-scale Alcohol Separation Unit will allow economists to establish the economic viability of de-centralized ethanol production from sweet sorghum with much greater accuracy.



PI: Dr. Rob Whiteley Oklahoma State University *Chemical Engineering* Funded: \$150,000 Start Date: 01/01/13 End Date: 12/31/14

Other Sources of Funding: Oklahoma State University covered the cost sharing portion of this award.





Project Title: Effects of Syngas Sources on Ethanol Production via Fermentation

DR. MARK WILKINS

Project Outcomes:

- The potential impurities which can impact fermentation system have been identified. The effects of benzene and ammonia, ethane, acetylene, ethylene and acetone on *Clostridium ragsdalei* cell growth, enzymatic activities, and product distribution have been quantified.
- It was found that ammonia in producer gas can accumulate to high concentrations and raise the osmolarity of the media, which can inhibit cell growth. Ammonium ions non-competitively inhibit hydrogenase activity, and inhibition is significant for typical syngas ammonia concentrations. Higher levels of osmolarity will also alter the ratio of [EtOH] versus [HAc]. Thus, ammonia should be removed from syngas prior to fermentation.
- At benzene levels typically found in media (around 0.6 mM), there is minimal effect on cell growth and ethanol production.
- Hydrogenase activity was reduced by 5%. Therefore, benzene impurity within syngas does not need to be vigorously removed as in the case for ammonia.
- Ethane was not observed to affect ethanol formation.
- Acetylene at levels as low as 0.2% inhibit cell growth, ethanol and acetic acid formation, alcohol dehydrogenase (ADH) activity, and hydrogen consumption.
- Ethylene slightly inhibited ethanol formation.
- Acetone that is in producer gas after wet scrubbing to remove tars was converted to isopropanol at >90% yield by *C. ragsdalei*.
- Isopropanol is a valuable industrial compound with potential fuel value.

Other Sources of Funding:

Subcontract: Brigham Young University. The total sub-contract amount to BYU included graduate and undergraduate student stipends, equipment, materials and supplies and travel expenses. A cost share match was provided by BYU. The overall cost share match was met period-by-period by OSU and BYU (included indirect costs).

Oklahoma State University Cost Share: Salaries of Drs. Bellmer and Huhnke were provided as matching funds.



PI: Dr. Mark Wilkins Oklahoma State University *Biosystems & Ag Engineering*

Co-PI: Dr. Ray Huhnke Oklahoma State University *Biosystems & Ag Engineering*

Co-PI: Dr. Krushna Patil Oklahoma State University *Biosystems & Ag Engineering*

Co-PI: Dr. Danielle Bellmer Oklahoma State University *Biosystems & Ag Engineering*

Co-PI: Dr. Randy Lewis Brigham Young University *Chemical Engineering*

Funded: \$337,194

Start Date: 07/01/2007 End Date: 12/31/2011



Project Title: Development of Winter Safflower as a Biodiesel Feedstock

DR. DICK AULD

Project Goal

The overall goal of this proposal was to successfully introduce a fallseeded safflower crop that is well adapted to the Lower Great Plains Region.

- Objective 1: Genetic Enhancement: Research conducted under this objective will develop broad based F3 populations of winter safflower that are segregating for increased oil content (42-44%) and high concentrations of oleic acid in the oil (70%+C18:1).
- Objective 2: Agronomic Evaluation: This research will develop planting date, irrigation management, fertility and harvesting guidelines necessary for the successful production of winter safflower in this region.
- Objective 3: Economic and Life Cycle Analysis: This segment of the research will compare the economic, water use budget and carbon life cycle analysis of safflower in direct comparison with other crops grown in the region.

Project Outcomes

- At production sites above Interstate 20 and below Interstate 40 (Albuquerque to Oklahoma City), winter survival of existing genotype of winter hardy safflower was sporadic and limited by extended exposure to temperatures below -10° C. Above Interstate 40 there was no consistent survival of winter hardy safflower.
- Winter hardy genotypes appeared to be homozygous when fall planted. However, when spring seeded, these genotypes segregated for time to flower, oil content, and tolerance to the major foliar pathogens found here in Lubbock, TX.
- After allocating energy by co-products, the total energy required to produce a gallon of biodiesel is 22,414 Btu.
- Safflower farmers would produce safflower at any seed price above \$0.06/lbs. Safflower farmers maximize profits with irrigation (in an average year) of about 6.5 inches per acre, with minimal required fertilizer application (depending on nitrogen content of soil).



PI: Dr. Dick Auld Texas Tech University & Texas AgriLife Research—Lubbock Plant Genetics & Breeding

Co-PI: Dr. Calvin Trostle Texas A&M University AgriLife Extension & Agric. Research/Extension Center

Co-PI: Dr. Michael Foster Texas AgriLife Research *Pecos R&G Base*

Co-PI: Dr. Sangamesh Angadi New Mexico State University *Plant & Environmental Sciences*

Co-PI: Dr. Aaron Benson Texas Tech University Agricultural & Applied Economics

Funded: \$224,987 Start Date: 08/01/2009 End Date: 07/31/2012



U.S. Department of Agriculture National Institute of Food & Agriculture



Project Title: Development of Safflower as a New Biomass Energy Crop for the Lower Great Plains of North America

DR. DICK AULD

Project Goal

This project investigates on how to successfully introduce a frost-seeded safflower crop that is well adapted to the Lower Great Plains (LGP) region to ensure sustainable biodiesel production. The specific objectives are: 1) Genetic enhancement of cold tolerant safflower lines for increased oil content, 2) Agronomic research on planting date, irrigation management, fertility and havesting guidelines for production of cold tolerant safflower, and 3)Extension/Outreach to deliver the production guidelines to growers and processors in this region.

Project Outcomes

- Significant differences in irrigation response were not detected in cultivars, irrigation rates, or the interaction of cultivars and irrigation rates.
- The elite lines had seed yield which ranged from 985 to 1712 kg/ha in 2012 and from 2290 to 2838 kg/ ha in 2013.
- Six spring genotypes were evaluated at 0, 15, 30, and 45 kg ha⁻¹ N/ acre under drip irrigation to determine the optimum fertility management of short season safflower grown as a cover crop. Seed yield of the 4 rates of N fertilizer were not statistically different ranging from 1,330 to 1,484 kg ha⁻¹.
- 71 unique hybrid combinations which combine high seed yield potential with high oil content and high oleic acid composition were made. These populations will provide valuable germplasm resources for many years. The F1 generation of these crosses are currently being increased in the greenhouse at Texas Tech University to supply F2 populations for future research.
- Safflower responds to irrigation water linearly. The number of seeds not seed Dr. Jason Warren weight seems to drive the yield formation. Plant biomass was strongly related to seed yield.
- Safflower research at Clovis, NM focused on critical stage based irrigation management, safflower physiology, evaluating safflower breeding material and adoption on DSSAT for spring safflower. (DSSAT) is being assessed to simulate safflower growth and seed yield.
- Field studies for the 2013-2014 crop year were established in Stillwater, OK and Chickasha, OK and are still underway.



PI: Dr. Dick Auld Texas Tech University Plant & Soil Science

Co-Pls: Dr. Calvin Trostle Texas A&M University Texas Cooperative Extension

Dr. Sangamesh Angadi New Mexico State University Plant & Environ. Sciences

Oklahoma State University Plant & Soil Sciences

Funded: \$160,723 Start Date: 01/29/2013 End Date: 12/31/2015



Project Title: Can Understory from Managed Pine Forests be Used as Feedstock in the Biochemical Biorefinery?

DR. DANIELLE JULIE CARRIER

Project Goal

The overall goal of this project is to demonstrate the use of understory growth from southern pine forests as feedstock in the biochemical-based biorefinery.

The use of sweetgum from southern pine dominated forests as a biorefinery feedstock was investigated. The project also determined the effect of adding oak wood, sweetgum bark, or oak bark, to sweetgum wood on xylose and glucose yields, as addition of this material would be more representative of a natural harvesting operation.

Project Outcomes

- Results showed that oak and sweetgum wood yielded 35% and 65% of their theoretical xylose content. Both woody species resulted in higher glucose and lower formic acid recoveries than their respective bark material.
- Analysis of data with the Dunnett Control's test in JMP 10.0 showed contamination of sweetgum wood did not have a significant effect (P > 0.05) on hydrolysis except with sweetgum bark, which exhibited a significantly higher xylose concentration than the control.
- Results showed that the inclusion of bark resulted in the generation of saccharified streams that contained higher concentrations of inhibitory compounds. Mixing hardwood woods, such as oak and sweetgum, had no effect on saccharification yields.
- Sweetgum wood was a good source of carbohydrate for a biorefinery, but the removal of bark may be necessary to achieve desirable yields, as hydrolysis of bark material resulted in higher inhibitory compound concentrations.



PI: Dr. Julie Danielle Carrier University of Arkansas Biological and Agricultural Engineering

Co-PI: Dr. Matthew Pelkki University of Arkansas, Monticello *School of Forest Resources*

Funded: \$70,000 Start Date: 12/01/2009 End Date: 11/30/2012

Other Sources of Funding

Dr. Carrier's cost-share amount came from tuition paid by the Biological and Agricultural Engineering Department. Dr. Pelkki's cost-share came from Department of Forestry expenditures, which are state funds. 25% F&A was applied to the cost-share



Title: Assessing and Predicting Switchgrass and High-Biomass Sorghum Yields and Economic Viability

DR. FELIX FRITSCHI

Project Goal

The goals of this project are:

- 1) To predict and evaluate switchgrass and biomass sorghum biofuel feedstock productivity in diverse production environments using crop growth models
- 2) To incorporate, calibrate and validate algorithms to predict nitrogen and phosphorus removal in the harvested biomass
- 3) To develop a spreadsheet-based decision tool for calculating production costs and breakeven biomass prices.

Expected Outcomes

- Existing field sites located in a region spanning from northern Louisiana to central Missouri and from eastern Arkansas to Oklahoma, were leveraged for collection of crop growth and development, biomass yield, and nutrient removal data. The metadata generated based on these sites are used for model development, calibration, and validation.
- The models ALMANAC (switchgrass) and DSSAT (biomass sorghum) were calibrated and validated based on these sites and used in prediction of yields and nutrient removal in a sitespecific manner across this region.
- The output from the crop models were linked to an economic model that calculates annualized, prorated breakeven prices for the two species across a range of weather and soil conditions. The team is currently developing decision support tools to facilitate the selection between switchgrass and biomass sorghum based on economic evaluation of yields and input costs.
- By the end of this project, producers, consultants, industry representatives and policy makers will be able to access and run crop and economic models to aid them in their decision-making processes.



PI: Dr. Felix Fritschi University of Missouri *Plant Sciences*

Co-Pls: Dr. Montgomery Alison Louisiana State University AgCenter

Dr. Vijaya Gopal Kakani Oklahoma State University *Plant and Soil Science*

Dr. Michael Popp University of Arkansas *Ag Economics*

Dr. Chuck West Texas Tech University - Crop, Soil & Environmental Sciences

Associate PI: Dr. Jim Kiniry USDA-ARS - Grassland, Soil, and Water Research

Funded: \$367,158 Start Date: 07/01/11 End Date: 06/30/15

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Title: Use of Alternative Water Sources for Bioenergy Crops

Dr. Girisha Ganjegunte

Project Goal

The main goal project is to develop alternative irrigation water sources and suitable salinity management practices to ensure long -term sustainability of bioenergy crop production and improve farm profitability in the arid southwest region.

This project used greenhouse and bench top experiments to evaluate the feasibility of using marginal quality water for producing selected bioenergy crops on salt affected land and its effects on soil salinity.

Project Outcomes

- Jatropha and camelina were not tolerant to salinity, whereas the cultivars castor-Memphis; switchgrass-Alamo, NSL2009-1 & 2; sorghum-ES-5200, Shallu, Desert Maize and 1790E; and canola-DKW 47-15 were relatively salt tolerant.
- Average biomass yields for switchgrass and sorghum were 7.4 and 21.6 Mg ha⁻¹, respectively. Canola seed yield was 917 kg ha⁻¹. There were no significant differences for biomass/oil seed yield between wastewater (EC~ 2.6 dS m-1) and freshwater (EC~ 1.4 dS m-1) irrigated columns.
- Soil salinity increased compared to pre-study levels, but, most of the increases in salinity came from the solubilization of Ca salts, which is not expected to affect soil productivity and soil sodicity.
- Economic feasibility study results indicated that fertilizer is a major production cost and that energy sorghum production under marginal quality water irrigation was highly profitable when no amendments were added. Irrigation with reclaimed municipal wastewater improved soil nutrient levels that may have important implications for the cost of production and farm profitability.

Other Sources of Funding: Each respective institution covered the cost sharing portion of this award.



PI: Dr. Girisha Ganjegunte Texas AgriLife Research Soil & Crop Sciences

Co-PIs: Dr. Genhua Niu Texas AgriLife Research *Horticulture*

Dr. April Ulery New Mexico State University Plant & Environ. Science

Dr. Chenggang Wang Texas Tech University -Agricultural Economics

Dr. Yanqi Wu Oklahoma State University Plant & Soil Science

Funded: \$74,999 Start Date: 07/01/11 End Date: 06/30/13



Project Title: Designer Sorghums: Development of High Yielding Sorghum Cultivars with Modified Endosperm Matrices for Optimized Low Energy Input Ethanol Production and High Nutrition Feed

DR. DIRK HAYS

Project Goal

The overall goal was to develop a systems approach for designer sorghum hybrids that optimizes the grain's endosperm matrix for low energy bioethanol conversion and grain distiller's feed value. This approach included the development of sorghum cultivars that combine a high endosperm protein digestibility (HD) trait with a high amylopectin (waxy) starch trait.



PI: Dr. Dirk Hays Texas A&M University Soil and Crop Sciences

Co-PI: Dr. Donghai Wang Kansas State University *Biological and Agricultural Engineering*

Co-PI: Dr. William Rooney *Texas A&M University Soil and Crop Sciences*

Co-PI: Dr. Gary Peterson Texas Agrilife Research Center

Co-PI: Dr. Don Vietor *Texas A&M University Soil and Crop Sciences*

Co-PI: Dr. Sergio Capareda *Texas A&M University Biological and Ag Engineering*

Co-PI: Dr. Joe Hancock Kansas State University Animal Science

Funded: \$337,500 Start Date: 07/01/2007 End Date: 06/30/2010

Project Outcomes

- The team had optimized the sorghum grain for bioethanol conversion by combining the high protein digestible (HD) trait with the high amylopectin starch endosperm trait (waxy). This combination improves the bioethanol efficiency by 65% higher (at 24 hrs) and by 14% higher (at 72 hrs) of fermentation versus wild-type sorghum or current commercial grain.
- Bioethanol systems often sell the remaining grain by-products, dry distillers grains (DDGs) as a high protein feed supplement, but, sorghum and corn grain are low in essential amino acids such as lysine. The combination of the HD by waxy trait also optimized the DDG feed by-products by increasing protein bioavailablity and the limiting amino acid lysine by 100% versus wild-type sorghum or corn DDGs. So, in the bioethanol-feed supplement system, the grain is optimized for feed.
- Therefore, this project has provided a systems package of optimized grain sorghum for high efficiency low energy bio-ethanol production while providing additional revenue streams via improved DDG animal feed value.



Title: Commercialization of Identity Preserved Grain Sorghum with Optimized Endosperm Matrices for Enhanced Bioethanol Conversion and High Lysine DDG Feed and Food Value

DR. Dirk Hays

Project Goal

The goal of the completed 2007 South Central Sun Grant award was to develop designer sorghum hybrids that optimize the grain's endosperm matrix for low energy bioethanol conversion and improved grain distiller's feed value. This project carries on that work to include development of sorghum that combines a high endosperm protein digestibility (HD) trait (removes the inhibitory protein matrix surrounding endosperm starch) with the high amylopectin (waxy) starch trait. The HD trait also confers 60% higher lysine content compared to wildtype corn or sorghum.

Expected Outcomes

- The outcomes/products derived from this research will be new sorghum hybrids ideally optimized for the bioethanol/DDG animal feed system.
- The grain endosperm will be optimized for low energy input cooking, and gelatinization, and higher efficiency fermentation. Additionally, the DDG will be optimized as a high lysine DDG/DWG feed.
- This project will improve the value chain bioethanol refineries' economic return by reducing energy cost, improving ethanol yield per raw input, and feed value return.
- Hays' team will also show that the HD x waxy grain traits improves other sorghum-based products. These include healthier high lysine, and Celiac friendly beers, breads, pancakes, cakes, North Africa Injera's and Kisra's, as well as whole grain breakfast cereals, and porridges. These will have a significant impact for previous USAID INTSORMIL collaboration and current USAID-SMIL collaboration in Africa and Central America.



PI: Dr. Dirk Hays Texas A&M University Soil and Crop Sciences

Co-PIs: Dr. Joseph Awika Texas A&M University *Soil and Crop Sciences*

Dr. Gary Peterson Texas AgriLife Research

Dr. William Rooney Texas Ag Experiment Station Soil and Crop Sciences

Dr. Donghai Wang

Kansas State University Biosystems & Ag Eng. Funded: \$341,922 Start Date: 07/01/11 End Date: 06/30/15



Project Title: Evaluate CRP Land Potential for Biomass Feedstock Production

DR. GOPAL KAKANI

Rationale

The Sun Grant Initiative (SGI) is working with the Department of Energy- Energy Efficiency and Renewable Energy Office of Biomass Program to develop and implement a Regional Biomass Partnership to address barriers associated with the development of a sustainable and predictable supply of biomass feedstocks. Close to million acres of CRP land in the State of Oklahoma offer potential land for growing bioenergy feedstocks for cellulosic ethanol industry.

There have been concerns from environmental groups about possible impact these minimally managed CRP ecosystems have on the natural habitat for birds and other organisms acclimated to the land. Timing and management of biomass harvest so as to avoid the nesting and breeding seasons of the wildlife in these areas or provide a continued habitat would be beneficial. This can be achieved by strip harvesting or staggering the harvest. In this study, we propose two harvest timings: one at peak standing crop and the other after frost kill. These harvest times would be useful to monitor the changes in the native habitat during the five year period of the project.

Project Goal

- 1) Replicate the SGI/DOE project at Altus, OK to evaluate biomass potential across locations.
- 2) Evaluate changes in soil's physical and chemical properties of CRP land during the feedstock production period.

Project Outcomes

Addition of nitrogen to the CRP land increased biomass production at a rate of 15 kg biomass per kg of N added. The dry biomass yields were 1884, 3072 and 3559 kg ha⁻¹ at 0, 55 and 110 kg N ha⁻¹.



PI: Dr. Gopal Kakani Oklahoma State University *Plant and Soil Sciences*

Co-PI: Dr. Rod Wanger United States Department of Agriculture *Oklahoma Farm Service Agency*

Funded: \$20,000 Start Date: 04/01/2008 End Date: 09/30/2009

Other Sources of Funding:

Oklahoma State University: A portion of Dr. Kakani's salary for each year, fringe benefits on that salary, and waived indirect costs at a rate of 47.7% of total direct costs.

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U.S. Department of Transportation

Project Title: Geographic Potential and Environmental Sustainability of Bioenergy Production in Oklahoma

South Central Region

DR. GOPAL KAKANI

Project Goal

The goal of this project is to develop practices and technologies necessary to ensure efficient, sustainable, and profitable production of cellulosic biomass.

Three main objectives are to (1) evaluate biomass production potential of lignocellulosic feedstocks, (2) increase the understanding of environmental sustainability, and (3) develop spatial maps to identify bioenergy hotspots.

Project Outcome(s)

The results will provide information to production scientists, county and area extension specialists, and farmers and landowers about the best establishment, maintenance plus management, and harvesting. Project will also provide regional biorefineries with the break-even farm gate price for several switchgrass, sorghum, and mixed grass species.

Publications and Presentations

- Haankuku, C., F.M. Epplin, and V.G. Kakani. 2014. Forage sorghum response to nitrogen fertilization and estimation of production cost. Agronomy Journal. 106: 1659-1666. doi:10.2134/agronj 14.0078; Published online 22 May 2014.
- Kakani, V.G., K. Dhakal, P. Wagle. 2013. Big Data for Big Solutions: Eddy Flux, Mesonet and C Sequestration. Fourth Annual AgMIP Global Workshop, Oct 28-30, New York, NY.



Dr. Gopal Kakani Oklahoma State University *Plant and Soil Sciences*

Co-PI: Dr. Tyson Ochsner Oklahoma State University Plant and Soil Sciences

Dr. Francis Epplin Oklahoma State University *Agricultural Economics*

Dr. Jason Warren Oklahoma State University *Plant and Soil Sciences*

Start Date:1/1/14 End Date: 12/31/15 Funded: 131,000

Other Sources of Funding: Oklahoma State University



U.S. Department of Agriculture National Institute of Food & Agriculture



Project Title: Miscanthus and Switchgrass Bioenergy Production and Soil Remediation on Marginal and Vulnerable Landscapes

DR. NEWELL KITCHEN

Project Goal

The long-term goal of this research project is to determine the production capacity and remediation potential when growing bioenergy crops on marginal and vulnerable soil landscapes. Objectives include:

- 1) Measure and model *Miscanthus* and switchgrass production on marginal and vulnerable soil landscapes, and develop management practices for producing optimal yield.
- 2) Measure the soil remediation potential of *Miscanthus* and switchgrass production systems as compared to grain cropping on marginal and vulnerable soil landscapes.
- 3) Determine the profitability and equivalent foreign oil displacement of *Miscanthus* and switchgrass bioenergy crops on marginal and vulnerable soil

Project Outcomes

- N fertilizer correlated with in-season plant greenness (as measured with chlorophyll content readings) early in the growing season but the effect of N fertilizer rate diminished by September.
- Only one site responded to N fertilization in 2013. The lack of response to N fertilization could be due to the ability of *Miscanthus* to recycle nutrients from previous year's growth, along with its ability to re-partition N into the most photosynthetic-active upper leaves throughout the growing season.
- Preliminary results suggest little to no N fertilizer is needed when growing *Miscanthus* on claypan soils. Additional years of study are needed to confirm this finding.
- Switchgrass has been found to have 185-700% higher water use efficiency and 51% less surface runoff than corn production.
- Averaged across landscape positions, agronomic nitrogen use efficiency of switchgrass was double of that found for corn production.
- Using the ALMANAC model switchgrass yields were underestimated for claypan soils for most individual years compared to actual yield measurements. The model did not show variation in average yield with respect to differing depths to claypan, while measured yields increased with depth to clay for years with limited rainfall.



PI: Dr. Newell Kitchen USDA-ARS Cropping Systems and Water Quality Research Unit

Co-Pls: Dr. Ray Massey University of Missouri Agricultural Economics **Dr. Bob Kremer USDA-ARS** Soil, Environmental & Atmospheric Sciences Dr. Allen Thompson University of Missouri **Biological Engineering Dr. Ken Sudduth USDA-ARS** Cropping Systems and Water Quality **Research Unit Dr. Brent Myers** University of Missouri Columbia- Plant Science **Dr. Emily Heaton** Iowa State University Agronomy

Funded: \$160,000 Start Date: 04/30/2013 End Date: 04/29/2015



Project Title: Expanding Production Area and Alternative Energy Crop Market of Proso Millet for Water Deficient Lands

KEVIN LARSON

Project Goal

Kevin Larson's team looked at the feasibility of using proso millet as a viable biofuels crop for the Southern High Plains. The team: a) evaluated four cultivars to see which cultivars are adapted to the drier region, b) tested multiple planting dates to determine the optimum planting window, and c) developed crop enterprise budgets as production decision tools for proso millet as a birdseed crop compared to proso millet as an ethanol crop.

Project Outcomes

- Larson's team identified Huntsman and Sunrise as well-adapted, high ethanol-producing proso millet cultivars for the Southern High Plains.
- The team found mid-May to late June as the proso millet optimum planting date window in our region for high ethanol production. However, this planting date window was extrapolated from the Walsh, CO site only, as the monthly planting dates in Goodwell, OK were lost to bird damage.
- The team developed crop enterprise budgets for proso millet as an ethanol feedstock and as a birdseed crop. These crop enterprise budgets allow economic comparisons and provide cropping decision tools for growers. Unless the demand and price of proso millet increases, due to its acceptance as an ethanol grain, growers will not change their crops and cropping systems and proso millet will not capture additional acreage.
- They also found that proso millet is the least costly fermentable grain, currently worth \$2.35/bu more than its current price. As such, they anticipate a change from the traditional corn and grain sorghum ethanol feedstocks to include proso millet.



PI: Mr. Kevin Larson Colorado State University *Plainsman Research Center*

Co-PI: Mr. Rick Kochenower Oklahoma State University Panhandle Research & Extension Center

Co-PI: Mr. Jeffrey Tranel Colorado State University *Extension*

Funded: \$62,421 Start Date: 07/01/2009 End Date: 06/30/2011

Other Sources of Funding: Colorado State University: Salary & Fringe Benefits for Kevin Larson; Indirect Costs at CSU's federally-approved rate for off-campus research; Oklahoma State University: Salary & Fringe Benefits for Rick Kochenower and indirect costs.



U.S. Department of Agriculture National Institute of Food & Agriculture



Project Title: Sustainable Feedstock Production for Bioenergy

DR. CLYDE MUNSTER

Project Goal

The primary goal of this project was to utilize low productivity soils to grow feedstocks for bioenergy production. The specific objectives were:

- Methods and procedures to bind biochar with an inexpensive material to increase biochar density for easier handling.
- The ability to use existing farm equipment to handle and land apply aggregated biochar.
- Development of soil fertility BMPs for biochar nutrient recycling on low-productivity soils in the South Central region for the production of perennial grass feedstocks for bioenergy production.
- Improvements of soil tilth and water holding capacity for low productivity soils through applications of biochar.
- The opening of millions of acres of unproductive farmland to the production perennial grass feedstocks for sustainable bioenergy production in the South Central region.
- A comprehensive Extension program for the dissemination of BMPs for the production of bioenergy feedstocks on low productivity soils in SC region.

Project Outcomes

- Wind transport of inherently low-density biochar particles is a concern during application. Adding water in the amount of 80-110% by weight to the biochar produced mixtures with the best aggregation for handling and land application to prevent wind-induced loss biochar from target sites.
- There was a significant linear relationship between water holding capacity (0.1, 0.33, and 3 bar soil pressure) and runoff. Control plots (no biochar) produced the largest amount of runoff as expected. Biochar treated plots produced lower cumulative runoff values but a significant trend did not exist.
- There was a potential inverse relationship between the total biomass and runoff of rainfall, especially for the plots with the highest biochar applications. However, this trend was not statistically significant.
- There was a significant increase in grass production in research plots with the highest rates of incorporated biochar addition. Water holding capacity (at 3 bar soil pressure) also increased for the highest rate of incorporated biochar, likely leading to the observed increase in grass production. A linear relationship existed between water holding capacity (at 3 bar soil pressure) and multiple biomass harvests.
- Saturated hydrologic conductivity, bulk density, and porosity, of the field soil used in this study were not significantly influenced by biochar application.
- Nutrient testing of the research plots revealed significant increases in pH and potassium content of the soil with increasing rates of biochar application, regardless of whether the biochar was unincorporated or incorporated.
- There was a significant increase in nitrate-nitrogen content as unincorporated biochar rates increased.



PI: Dr. Clyde Munster Texas A&M University Biological and Agricultural Engineering

Co-PIs: Dr. Hailin Zhang Oklahoma State University Plant & Soil Sciences

Dr. Sergio Capareda Texas AgriLife Research *Biological & Agricultural Engineering*

Dr. Tony Provin Texas AgriLife Extension Service Soil & Crop Sciences

Dr. Kevin McInnes Texas A&M University Soil & Crop Sciences

Funded: \$162,000 Start Date: 01/29/2013 End Date: 07/31/2015



Title: Developing Low-Input, High-Biomass, Perennial Cropping Systems to Support a Bioenergy Economy on Marginal Land at Higher Elevations

DR. Calvin Pearson

Project Goal

The goal of the research project is to develop a sustainable high yield, low inputs (e.g. fertilizer, pesticides), and environmentally enhancing] biomass crop production system using adapted grasses that do not compete with food crops.

The project objectives of this project were to:

- Evaluate grasses and legume species as single and mixed specie plantings and production input levels to identify those that are productive and profitable for biomass in this region.
- 2) Assess carbon and nutrient cycling, carbon budgeting, and carbon sequestration in a biomass cropping system.
- Perform economic, energy, and life-cycle analyses of the treatment variables to identify a sustainable biomass production system for the region. 4) Conduct outreach, public education, and technology transfer regarding biomass crop production.

Project Outcomes

- Field performance of four grass and grass/legume mixtures:

 Native grass mixture ('Magnar' Great Basin Wildrye, 'San Luis' slender wheatgrass, 'Secar' bluebunch wheatgrass, 'Rosana' western wheatgrass);
 Switchgrass mix ('Dacotah' and 'Blackwell');
 Tall fescue ('Fawn'); and 4) Introduced pasture mix ('Cache' meadow brome, 'Fawn' tall fescue, 'Latar', 'Potomac', 'Paiute' orchardgrass, alfalfa) and four input levels (none/limited, sustainable, low and high commercial inputs)were evaluated to assess their effect on biomass production over a multi-year testing period beginning 2011.
- Extensive soil and agronomic field data at three locations –Fruita, Rifle, and Meeker, CO were collected beginning in 2011.
- The biomass budget generator that provides a valuable economic tool for producers and others when evaluating the potential to grow various grasses as biomass in the Intermountain West was developed. The biomass budget generator is available to the general public and was specifically made readily available to Extension personnel.



PI: Dr. Calvin Pearson Colorado State University Western Colorado Res. Center

Co-Pls:

Dr. Ron Follett USDA-ARS-Soil Plant Nutrient Res. Fort Collins, Colorado (retired)

Dr. Ardell Halvorson USDA-ARS-Soil Plant Nutrient Res. Fort Collins, Colorado (retired)

Dr. Catherine Keske University of Colorado

Dr. Steven Larson USDA-ARS, Forage and Range Research Laboratory, Logan, Utah

Dr. Kevin Jensen USDA-ARS, Forage and Range Research Laboratory, Logan, Utah

Funded: \$74,861 Start Date: 07/01/11 End Date: 07/30/14



Project Title: Cellulosic Feedstock Production and Environmental Benefit from Agroforest Systems Established on Marginal Lands

DR. MATTHEW PELKKI

Project Goal

To develop economically viable agroforest systems for producing cellulosic bioenergy crops that will also enhance water quality and provide wildlife habitat on low productivity agricultural land in the Lower Mississippi Alluvial Valley (LMAV).

The objectives were:

- Provide long-term biomass production estimates for cottonwood grown in mono-cultures or alley cropped with switchgrass.
- Calculate the amount of carbon and nitrogen sequestered by each of the cottonwood-switchgrass systems.
- Relate habitat utilization of small mammals to habitat characteristics associated with the various cottonwood-switchgrass agroforest sytems.
- Develop a complete life-cycle analysis, focusing on an energy balance for agroforest systems.

Project Outcomes:

- The marginal nature of the three sites used in this study is reflected in the production and economic data. For example, the costs of producing a ready to harvest ton of oven-dry biomass is \$65 to \$914 for cottonwood and \$13 to \$56 for switchgrass.
- The cost of producing cottonwood may decrease following the initial harvest. Increased cottonwood production and reduced establishment activities are likely to occur after the initial harvest and may lower woody biomass production costs.
- The addition of ecosystem service markets could further improve the economics of biomass production on marginal lands in the LMAV.
- The CN results indicate that switchgrass and cottonwood are better able to retain N on site than the typical row crops grown on marginal soils in the LMAV. Conversion to cottonwood or switchgrass bioenergy crops could reduce N losses to surface waters.
- Habitat characteristics varied greatly among seasons and study sites, which reflects the different success associated with crop establishment and the timing of crop establishment in the CT treatment.
- Carbon life-cycle ratios are positive for all three activities, and switchgrass has the best carbon ratios. More efficient tree harvesting equipment would reduce carbon emissions.



PI: Dr. Matthew Pelkki University of Arkansas-Monticello School of Forest Resources

Co-PI: Dr. Charles West University of Arkansas *Crop, Soil, & Environmental Sciences*

Co-PI: Dr. Phillip Tappe University of Arkansas *Division of Agriculture* Arkansas Forest Resources Center

Co-PI: Dr. Hal Liechty University of Arkansas *Division of Agriculture* Arkansas Forest Resources Center

Co-PI: Dr. Michael Blazier Louisiana State University AgCenter *Hill Farm Research Station*

Co-PI: Dr. Montgomery Alison Louisiana State University AgCenter *Macon Ridge Research Station*

Funded: \$350,000 Start Date: 07/01/2009 End Date: 06/30/2013



Title: Feasibility and Environmental Impact of Switchgrass Grown for Biofuel on Marginal Quality Lands

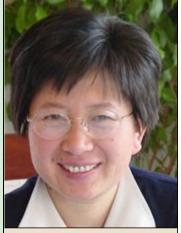
DR. YALING QIAN

Project Goal

The long-term goal of the project was to promote environmentally sustainable biofuel feedstock production on marginal quality land under rain-fed vs. irrigated conditions. The objectives were to: 1)Determine the impacts of different soil salinity levels on switchgrass germination, establishment, and yield; 2) Determine the relative production potential of different switchgrass varieties under rain-fed and irrigated conditions on marginally saline soil; 3) Assess carbon sequestration and management inputs of switchgrass grown on marginal quality land; and 4) Generate information on best management practices using ecosystem simulation models.

Project Outcomes

- Greenhouse experiment indicated that switchgrass had a moderate level of salinity tolerance. It would grow well without dramatic biomass reduction when soil salinity is below 7 dS m⁻¹.
- Switchgrass yield will be dramatically reduced when soil salinity exceeds 7 dS m⁻¹ or under drought conditions, since drought would concentrate soil salts.
- In Colorado, frequent irrigation was necessary during the first 2 months of establishment for successful establishment of switchgrass with N fertilization increasing weed pressure.
- Switchgrass grew well under limited supplemental irrigation on marginal quality soils.
- Significant differences in yield were observed among varieties. Blackwell, Pathfinder and Trailblazer have a higher biomass yield than other varieties. The average yields ranged from 8.6 to 10.3 Mg ha⁻¹. However, without supplemental irrigation, all varieties declined, showing decreased biomass production over time.
- According to the DAYCENT model, the best management should be irrigated every 14 days at 70% PET irrigation water and fertilizer at 67 Kg N ha⁻¹ yr⁻¹, producing biomass yield of 9.40 Mg/ha, with a high carbon sink potential at 2.04 Mg CO₂ equivalent ha⁻¹ yr⁻¹.



PI: Dr. Yaling Qian Colorado State University *Horticulture*

Co-PI: Dr. Joe Brummer Colorado State University *Soil and Crop Sciences*

Collaborator:

Dr. Ron Follett USDA-ARS *Soil Plant Nutrient Research*

Funded: \$75,000 Start Date: 07/01/11 End Date: 12/31/13

Other Sources of Funding: Colorado State University covered the cost sharing for this award.



Project Title: Evaluation of Sweet Sorghum Hybrids as a Bioenergy Feedstock; Germplasm Development, Agronomic Practices, and Conversion Efficiency

DR. WILLIAM ROONEY

Project Outcomes:

- Sweet sorghum elite hybrids produce fresh biomass and sugar yields similar to the traditional cultivars while overcoming the seed production limitations.
- Sweet sorghum seed parents have increased seed yields and reduced height which allows for higher yield of seed in seed production.
- Sweet sorghum hybrids perform comparably to, if not slightly better than existing sweet sorghum hybrids.
- Yields in more eastern regions of the testing were higher than those observed in more western region of the testing area especially in dryland conditions.
- Irrigated production was more stable over years and environments, indicating that the availability of irrigation may be important to insure productivity in all years.
- Given the inability to stabilize the juice for later processing, millers will require as long a harvest season as possible to facilitate extraction and conversion. Thus, while yields were certainly good enough in eastern Kansas, Oklahoma and Central Texas, the duration of harvest season drops as production is farther north. Thus, it is more likely that initial production will be in more Southern regions of the area rather than farther North.



Other Sources of Funding: Each of the PI and Co-PIs involved in this project had their respective institution (Texas A&M University, Oklahoma State University, Kansas State University, and New Mexico State University) matched salary and fringe benefits via cost share.



PI: Dr. William Rooney Texas Agricultural Experiment Station Soil and Crop Sciences

Co-PI: Dr. Jürg Blumenthal Texas Cooperative Extension Soil and Crop Sciences

Co-PI: Dr. Brent Bean Texas Cooperative Extension Texas Ag Experiment Station *Soil and Crop Sciences*

Co-PI: Dr. Danielle Bellmer Oklahoma State University *Biosystems and Ag Engineering*

Co-PI: Dr. Ray Huhnke Oklahoma State University *Biosystems and Ag Engineering*

Co-PI: Dr. Donghai Wang Kansas State University *Biological and Ag Engineering*

Co-PI: Dr. Mark Marsalis New Mexico State University *Extension Plant Sciences*

Co-PI: Mr. Rick Kochenower Oklahoma State University *Oklahoma Panhandle Research and Extension Center*

Co-PI: Dr. Scott Staggenborg Kansas State University Agronomy

Funded: \$327,125 Start Date: 07/01/2007 End Date: 06/30/2010



Project Title: Selection of Hybrids and Optimization of Planting to Facilitate Just-in-Time Harvest for Sweet and Energy Sorghum

DR. WILLIAM ROONEY

Project Goal

• The goal of this study was to identify the correct varieties and hybrids of energy and sweet sorghums to optimize production systems that span the complete harvest season.

The objectives of this study were:

- To establish yield potential for specific harvest times.
- To identify the correct hybrid(s) to optimize productivity throughout the harvest season for both biomass and sweet sorghums.

Project Outcomes

- Sweet sorghum hybrids can be continually harvested from late July to early November. Sugar yields are lowest early and peak between late August and early October.
- Sweet sorghum is complementary to sugarcane, and these two crops can be harvested in sequence to extend a mill season.
- Biomass sorghums were evaluated for yields and management system. The productivity of all types was strongly influenced by the environment, but single cut hybrids produced the highest biomass yields.
- Optimum yields were attained between 135 to 160 days but yields of 90% range from 100 to 200 days indicating that a significant harvest window does exist for biomass sorghum.





PI: Dr. William Rooney Texas Ag Experiment Station Soil and Crop Science

Co-PI: Dr. Jürg Blumenthal Texas Coop. Extension *Soil and Crop Sciences*

Co-PI: Dr. Howard Viator Louisiana State University *Iberia Research Station*

Funded: \$298,399Start Date: 12/01/2009End Date: 5/31/2013

Other Sources of Funding: Cost Share funding sources were combination of salary for faculty and staff associated with this program, and equipment, supplies, and land costs incurred during tests with collaborators: Louisiana GreenFuels LLC and Ceres, Inc.



Project Title: Biodiesel Feedstock Development for the Southern Great Plains

Michael Stamm

Rationale:

Winter canola is a desirable broadleaf crop that can be planted in rotation with winter wheat to improve the overall sustainability of production agriculture in the Southern Great Plains. Winter canola is adaptable to the region and has a life cycle similar to winter wheat. Specialty cultivars, with profiles having high oleic acid and low linoleic acid, produce more stable oils than conventional cultivars. Thus, wheat growers will garner the benefits of a crop rotation by including specialty winter canola cultivars in continuous wheat production systems. Rural communities will see an economic revitalization as new industry is established in the region to satisfy the demand for processing canola grain and creating biodiesel. Planted winter canola acres will likely increase as the processing infrastructure is developed. Following oil extraction from the grain, livestock producers will benefit from feeding a high protein meal. Industries will increase dependence upon diesel fuel blends with improved lubricity made from a biodegradable, renewable, and non-toxic feedstock. Consumers will rely upon a biodiesel fuel source with cleaner emissions rather than an unstable foreign petroleum supply.

PI: Mr. Michael Stamm Kansas State University Agronomy

Co-PI: Dr. Gary Pierzynski Kansas State University *Agronomy*

Funded: \$63,680 Start Date: 07/01/2007 End Date: 06/30/2011

Other Sources of Funding: Kansas State University covered salary and fringe benefits via cost sharing.

Project Outcomes:

The program has developed over 400 breeding populations with the potential for improved, highly-stable oil. F1 progeny ranged from 28.5 to 76.9% oleic acid, 10.1 to 25.2% linoleic acid, 3.4 to 9.4% linolenic acid, 0 to 30.6% erucic acid, and 3.5 to 7.5% saturated fats. As these populations are advanced through breeding and selection, more research will be performed to further biodiesel feedstock development in the Southern Great Plains.



Project Title: Develop Comprehensive Understanding and Utilization of Sorghum Stover and Brown Midrib Forage Sorghum for Ethanol Production

DR. DONGHAI WANG

Project Goal

The goal of this research was to develop comprehensive understanding and utilization of regular sorghum stover and bmr sorghum (sorghum biomass) for ethanol production.

The specific objectives were:

- 1) To characterize the physical properties and chemical composition of selected sorghum biomass
- 2) To develop chemical/physical pretreatment technologies to increase the fermentable sugars yields from sorghum biomass
- To increase the ethanol yields by identifying and reducing the effects of potential inhibitors formed during pretreatment of sorghum biomass
- 4) To investigate energy inputs and outputs associated with bioprocessing sorghum biomass.

Project Outcomes:

- Five varieties of forage sorghum (stems and leaves) including regular sorghum hybrids, forage sorghum, mid-rid (bmr) sorghum, photosensitive and sweet sorghum were characterized and evaluated as feedstock for fermentable sugar production.
- Chemical composition of the sorghum biomasses ranged from 28-44% cellulose, 18-24% hemicellulose, 14-20% lignin, 1.5-6.5% starch (as free sugars), and 4-10% ash. The total carbohydrate composition ranged from 51-69%. There is strong relationship among chemical structure, function, composition, and fermentable sugars yield.
- Forage sorghums with a high percentage of guaiacyl rings in their lignin structure were easy to hydrolyze after pretreatment. Pretreatment was more effective for forage sorghums with a low crystallinity index and easily transformed crystalline cellulose to amorphous cellulose, despite initial cellulose content.
- Results showed that SSF using *S. Cerevisiae* is an effective method to hydrolyze and ferment cellulose remaining in treated solids with high ethanol yields up to 88%. Input/output flows and estimated processing cost indicated that forage sorghum is an ideal feedstock for biofuel production with low energy input.



PI: Dr. Donghai Wang Kansas State University *Biological and Ag Engineering*

Co-PI: Dr. Ron Madl Kansas State University *Grain Science and Industry*

Co-PI: Dr. Scott Staggenborg Kansas State University *Agronomy*

Co-PI: Dr. Michael Woolverton Kansas State University *Agricultural Economics*

Funded: \$70,000 Start Date: 07/01/2007 End Date: 06/30/2009

Other Sources of Funding:

Kansas State University paid for partial salary and fringe benefits via cost share, and the Kansas Sorghum Commission provided in-kind.



Project Title: Evaluation of the Potential of Big Bluestem for Biofuel Production

Dr. Donghai Wang

Project Goal

The goal of this research was to develop a comprehensive understanding of the effects of ecotype and planting location on biofuel production potential from big bluestem. The specific objectives were:

- Characterize the chemical and elemental compositions of big bluestem and study the effects of ecotype and planting location on chemical and elemental compositions of big bluestem along the Great Plains precipitation gradient.
- 2) Evaluate what are the patterns of thermal properties of big bluestem across the ecotype and climate gradient.
- 3) Determine the optimum acid pretreatment conditions and investigate glucan yield variation from enzymatic hydrolysis in diverse big bluestem accessions and also assess the adaptive potential of different genotypes as potential bioenergy crops.

Project Outcomes

- •Three big bluestem ecotypes from central Kansas (Cedar Bluffs and Webster populations), eastern Kansas (Konza and Top of the World populations), and Illinois (12Mile and Fults populations), as well as the Kaw cultivar, were harvested and evaluated for their chemical and elemental compositions.
- •All populations revealed a large variation in cellulose (31.8–36.5%), hemicellulose (24.96–29.74%), lignin (14.4–18.0%), C (47.3–51.3%), &N (4.91–6.44%)
- •Planting location had significant effects on both chemical and elemental compositions of big bluestem. Ecotype had significant effects on glucan, xylan, lignin, and ash contents as well as on C, O2, & H elemental fractions.
- •The total sugar content of the big bluestem (regardless of ecotype) increased as the Great Plains precipitation gradient increased from west to east. Annual precipitation, growing degree days and potential evapotranspiration in 2010 explained up to 97%, 88% & 80% of the variation in compositions, respectively.
- •The populations varied widely in specific heat (2.35–2.62 kJ/kg/K), thermal conductivity (77.85–99.06 ×10-3 W/m/K), thermogravimetric property as weight loss during the heating process (71–73%), and high heating value (17.64–18.67 MJ/kg).
- •Glucan percentage in solid dropped from 98.4 to 77.3% with an increase in acid concentration. Glucan loss increased as acid concentration increased from 0.1 to 5.2%, whereas glucan content in liquid increased from 1.6 to 17.6%.
- •EEH (enzymatic hydrolysis efficiency) increased from 79.7 to 90.0% after 72 hours hydrolysis as acid concentration increased from 1.0 to 2.0%, which was significantly higher than control samples pretreated with water (18.1%).
- •All the populations revealed a large variation in mass recovery (52.0–59.7%) and glucan recovery (79.0–87.50%) after acid treatment, EEH (84.6–88.9%), and glucan mass yield (20.8–29.3%).



PI: Dr. Donghai Wang Kansas State University Biological & Agricultural Engineering

Co-Pls:

Dr. Wenqiao Yuan Kansas State University Biological & Agricultural Engineering

Dr. Richard Nelson Kansas State University Chemical Engineering

Dr. Loretta Johnson Kansas State University Biology

Funded: \$75,000

Start Date: 07/01/11 **End Date:** 06/30/13

Other Sources of Funding: *Kansas State University contributed cost share dollars.*



Title: Production of Lipids for Biofuels through Mixotrophic Growth of a Mixed Microalgae-Cyanobacteria Culture

Dr. Maria Teresa Gutierrez-Wing

Project Goal

The overall goal of this project was to determine the suitability of mixotrophic culture to produce biomass and lipids from a mixed culture of microalgae and cyanobacteria. The specific objectives were to: a) determine the suitability of a mixed culture composed of a species of *Chlorella* sp. and a cyanobacteria to grow in mixotrophic conditions; and b) determine the effect of different carbon sources on lipid production to explore the possibility of using agricultural waste streams rich in carbohydrates in the production of microalgal biofuels.

Project Outcomes

- Mixotrophic mixed culture of the microalgae *Chlorella vulgaris* and cyanobacteria *Leptolyngbia* sp. can grow and produce lipids with the addition of organic carbon.
- Biomass and lipid productivity of the co-cultures was higher in mixotrophic, compared with phototrophic cultures.
- Sodium acetate in the chemical media and sugar mill wastewater effluents resulted in the highest biomass and lipid productivity.
- Final dry mass concentration was higher in the cultures with higher proportion of sugar mill wastewater effluent, compared with those with higher proportion of evaporator effluent.
- Cultures maintained in 100% wastewater showed higher productivity and growth rate than the control.
- Total pigment content was higher in the treatment with 100% sugar mill wastewater effluent.
- Neutral lipids extracted from the biomass in all samples was composed mainly by C16 and C18 fatty acids.



PI: Dr. M. Teresa Gutierrez-Wing Louisiana State University Civil & Envir. Engineering

Co-PIs: Dr. Joan King LSU AgCenter Food Science

Dr. Kelly Rusch (formerly connected with LSU) *Engineering*

Funded: \$75,000 Start Date: 07/01/11 End Date: 06/30/13

Other Sources of Funding: Louisiana State University covered the cost sharing portion of this award.



Project Title: Breeding and Testing of New Switchgrass Cultivars for Increased Biomass Production in Oklahoma, Arkansas, Texas, and Kansas

DR. YANQI WU

Project Goal

The objectives of the project were to: (1) expand and improve an existing switchgrass working germplasm collection via further collection, evaluation, and genetic enhancement; (2) continue the long-term recurrent selection for general combining ability and breeding within upland and lowland ecotypic populations to effect incremental improvement in targeted traits; (3) develop switchgrass cultivars with increased biomass yield over major commercial cultivars for the south-central United States; and (4) establish a switchgrass cultivar testing network across a precipitation gradient from eastern Arkansas to western Oklahoma and across a temperature gradient from central Texas to northern Kansas.

Project Outcomes

- A new switchgrass cultivar, 'Cimarron' developed at the OSU breeding program, was released by Oklahoma Agricultural Experiment Station in 2008.
- Multi-test results indicated 'Cimarron' produced about 10% more biomass than the best commercial variety 'Alamo' and 25% more than 'Kanlow'.
- The new cultivar has been licensed to Johnston Seed Company at Enid, OK, for seed production and commercialization.
- Four switchgrass breeding populations of lowland and upland germplasm were advanced one cycle each.
- Nineteen new synthetic experimental cultivars were developed, which along with standard cultivars were included in a regional testing network across OK, AR, TX, and KS. The regional trials were established in 2010 and will be continued in three additional years testing biomass yield and persistence. Our molecular marker analysis indicated substantial genetic variation existed in the current switchgrass collections.

Other Sources of Funding

Oklahoma State University covered the salary of PI as cost share. University of Arkansas, Kansas State University and Texas Agricultural Experiment Station covered the salaries and fringe benefits of Co-PIs from their respective institutions.



PI: Dr. Yanqi Wu Oklahoma State University *Plant and Soil Sciences*

Co-PI: Dr. Charles West University of Arkansas *Crop, Soil, and Environmental Sciences*

Co-PI: Dr. Charles Taliaferro Oklahoma State University *Plant and Soil Sciences*

Co-PI: Dr. John Fritz Kansas State University *Agronomy*

Co-PI: Dr. James Muir Texas A&M University *Stephenville Agricultural Experiment Station*

Funded: \$200,000 Start Date: 07/01/2007 End Date: 06/30/2011

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Project Title: Testing and Breeding of New Switchgrass Cultivars for Increased Biomass Production in Oklahoma, Arkansas, Texas and Kansas

DR. YANQI WU

Rationale

Switchgrass has substantial potential to provide lignocellulosic feedstocks for conversion to biofuels. It is essential to develop new cultivars (synthetics and hybrids) with improved biomass yield by breeding and selection. Numerous investigations have indicated significant cultivar by location interactions for biomass yield in switchgrass. Multiple environment (location and year) evaluation of new cultivars is warranted.

Project Goal

To continue the established switchgrass regional trails of the OSU switchgrass breeding program.

Objectives

- 1. Continue five established field trials in the south-central United States.
- 2. Continue a switchgrass cultivar testing network across a precipitation gradient from eastern Arkansas to western Oklahoma and across a temperature gradient from Central Texas to northern Kansas.
- 3. Develop inbreds by selfing selected plants

Expected Outcomes

- a) Two breeding populations advanced from generation C3 to C4, and one from C0 to C1, respectively in 2014
- b) 10 SL-NL inbreeding and crosses made in 2011
- c) Data on persistence and biomass yield of new synthetics and major commercial cultivars at eight locations in 2011-13



PI: Dr. Yanqi Wu Oklahoma State University *Plant and Soil Sciences*

Co-PI: Dr. Charles West University of Arkansas *Crop, Soil, & Envi. Sciences*

Co-PI: Dr. Gopal Kakani Oklahoma State University *Plant and Soil Sciences*

Co-PI: Dr. Vara Prasad Kansas State University *Agronomy*

Co-PI: Dr. James Muir Texas A&M University *Stephenville Agricultural Experiment Station*

Funded: \$56,250

Start Date: 08/01/2011 End Date: 07/31/2015

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Project Title: A Novel Approach to Increase Biomass Yield through Altering Prohibitin Expression

Dr. Joshua Yuan

Project Goal

The goal of this project is to develop a new strategy for feedstock improvement to increase biomass accumulation. This research aims to translate the newest discovery of a novel mechanism for plant biomass regulation into bioenergy feedstock. The team discovered overexpression of one of the prohibitin genes (the PHB-8 homologs) in Arabidopsis that can significantly increase plant size and biomass accumulation, assumingly due to the preferred utilization lipid over sugar as energy source.

Objectives:

- 1) To investigate if the PHB-8 homologs in monocot have the same function for biomass regulation
- 2) To identify other genes in the PHB8 related pathway that could be used to increase biomass.

Project Outcomes

 Results showed that PHB8 can improve plant biomass and seed yield. The research has characterized the molecular function of PHB8 genes. The team discovered overexpression of one of the prohibitin genes (the PHB-8 homologs) in Arabidopsis that can significantly increase plant size and biomass accumulation, due to the stabilization of ATPase to promote the energy efficiency in plants.

• <u>Patent</u>

US Full patent applied by Joshua Yuan for 'Prohibitin genes to improve plant biomass and seed yield'.

Patent is licensed to Benson Hills Biosystems for engineering crops and bioenergy feedstock to improve seed and biomass yield.

• <u>Disclosure</u> Joshua Yuan. Prohibitin genes to improve plant biomass and seed yield.



PI: Dr. Joshua Yuan Texas A&M University *Plant Pathology and Microbiology*

Co-PI:

Dr. Susie Dai Texas A&M University *Office of the Texas State Chemist*

Funded: \$74,999 Start Date: 07/01/2011 End Date: 06/30/2013

Other Sources of Funding: Texas A&M University covered the cost sharing portion of this award.



Project Title: Farmers' Willingness to Produce Cellulosic Biofuel Feedstocks Under Alternative Contractual, Pricing, and Harvesting Arrangements

DR. JASON BERGTOLD

Project Goal

The overall purpose of this project was to determine farmers' willingness to produce alternative sources of biofuel feedstocks in Kansas and surrounding states. Data was collected from commercial farm operations using enumerated surveys with stated choice experiments about their willingness to produce corn stover, sweet sorghum and switchgrass under contract.

Project Outcomes

- Feedstock adoption for value-added crop residues, dedicated annual bioenergy crops as well as dedicated perennial bioenergy crop by farmers is higher in the eastern Kansas and the surrounding area and declined going west.
- On average, farmers were willing to allocate 122 acres to try a dedicated annual bioenergy crop and 101 acres for a perennial bioenergy crop.
- The probabilistic models examining farmers' willingness to produce feedstocks under alternative contractual conditions found that the likelihood of producing a feedstock will increase: a) if the level of net returns per acre under contract is increased, b) having a biorefinery harvest option, c) availability of insurance especially for sweet sorghum and switchgrass, d) nutrient replacement if corn stover is harvested, and e) incentive payments and establishment cost share for bioenergy crops. On the other hand, likelihood of production for each feedstock will decrease if the length of contract increases.

Other Sources of Funding: Kansas State University: Cost share for salaries and benefits of PIs, as well as F&A. Oklahoma State University: Cost share is for academic salary and fringe benefits.



PI: Dr. Jason Bergtold Kansas State University Ag Econ

Co-PI: Dr. Richard Nelson Kansas State University *Engineering Extension*

Co-PI: Dr. Scott Staggenborg Kansas State University Agronomy

Co-PI: D. Jeffery Williams Kansas State University *Ag Econ*

Co-PI: Dr. Francis Epplin Oklahoma State University *Ag Econ*

Co-PI: Dr. Michael Langemeier Kansas State University *Ag Econ*

Funded: \$233,000 Start Date: 07/01/2009 End Date: 06/30/2013



Project Title: Investigation/Simulation of Environmental Impacts and Economic Feasibility for Sweet Sorghum as a Sustainable Bioenergy Crop in South Central United States to Help Meet EISA Goals

DR. Richard Nelson

Project Goal:

The goal of this project was to develop the capacity to simulate sweet sorghum biomass yields in order to help with strategy planning for renewable fuel development from biomass feedstocks.

The objectives of this project were:

- To select the most promising crop model with the potential to model sweet sorghum biomass production in the central US.
- To develop and test sweet sorghum crop parameters for the selected model.

Project Outcomes

- Out of the five crop models (CropSyst, CERE-Sorghum, APSIM, ALMANAC, and SORKAM), ALMANAC was selected as the best suited for the development and testing of sweet sorghum crop parameters.
- The combination of Saxton and Rawls (2006) and Priestley-Taylor (1972) methods has the potential for wide applicability in the US Central Plains for simulating grain yields using ALMANAC.
- ALMANAC modeled biomass yields from the development of sweet sorghum crop model parameters with reasonable accuracy. Differences from observed biomass values ranged from 0.89 –1.76 Mg ha⁻¹ (2.8 to 9.8%) in Kansas (Riley County), Oklahoma (Texas County), and Texas (Hale County).



PI: Dr. Richard Nelson Kansas State University Engineering Extension

Co-PI: Dr. Scott Staggenborg Kansas State University Agronomy

Co-PI: Dr. Michael Langemeier Kansas State University Agricultural Economics

Funded: \$60,952 Start Date: 12/01/2009 End Date: 5/31/2012

Other Sources of Funding

Kansas State University paid faculty time, fringe benefits, and indirect cost expenses via cost share.



Project Title: Online Courses on Biorenewable Resources and on Engineering Sustainability

DR. JOHN SCHLUP

Project Goal

The project goal was to deliver two courses developed to complement graduate and upper-level undergraduate education in biobased resources and sustainability.

The objectives of this project were:

- Deliver an overview course on Biorenewable Resources
- Deliver a course on Engineering Sustainability
- Develop Online Capstone Team Design Experience
- Offer the Capstone Team Design Experience
- Make a graduate certificate in Biobased Products and Biofuels available to non-resident students via distance education

Project Outcomes

- Two courses were developed to complement graduate and upperlevel undergraduate education in biobased resources and sustainability: 1) Once focused on an overview of biorenewable resource utilization; 2) The other emphasized quantitative tools to characterize the sustainability of materials and processes.
- The courses were delivered and well received by the students. The courses were delivered both on-campus and via distance education with the material recorded for future asynchronous delivery.
- The sustainability course has been offered as an asynchronous distance elective with KSU's senior chemical engineering students on several occasions .
- A multi-institutional graduate certificate program in Bioenergy and Sustainable Technologies involving Kansas State University, University of Arkansas, Oklahoma State University, and South Dakota State University is underway. Course and program approvals at each campus were completed and the approval process for the graduate certificate at each institution was completed.



PI: Dr. John Schlup Kansas State University Chemical Engineering

Funded: \$52,370 Start Date: 07/01/2009 End Date: 06/30/2011

Other Sources of Funding:

Kansas State University contributed faculty time and fringe benefits. Indirect rates on the faculty time was cost shared by KSU at the federally negotiated rate for research at 48% MTDC.



Project Title: Evaluation of the Energy and Cost Advantages of Modules for Packaging and Transporting Biomass Energy Crops

Dr. Stephen Searcy

Project Goal

This project evaluated the potential of a biomass logistics system based upon large packages of chopped biomass.

Project Outcomes

- Biomass modules would be a viable, and likely lowest cost, means for collecting, storing and transporting biomass feedstock to a biorefinery.
- This project demonstrated the ability to form modules of over to 7 Mg of dry matter, load that module in two minutes or less and store it up to 12 months without significant change in shape or dimension.
- Comparison of the logistics costs between the biomass module system silage and baling systems using IBSAL simulation software indicated the module system is the lowest cost alternative. These results provided a strong basis for continued development of this system.
- The laboratory and field data collected during these efforts have provided a set of machine performance parameters that must be achieved for this system to be successful. The parameters include the ability to compress the biomass into a package that will maintain an anaerobic environment for an extended period and will provide the residual compressive forces required to achieve the target density.



PI: Dr. Stephen Searcy Texas Agricultural Experiment Station *Biological and Ag Engineering*

Co-PI: Dr. Michael Popp University of Arkansas Agricultural Economics and Agribusiness

Funded: \$246,236 Start Date: 07/01/2007 End Date: 06/30/2011

Other Sources of Funding: Sun Grant funding was leveraged by obtaining in-kind support from several industrial partners including CNH Industrial, MacDon Industries, John Deere, Hlavinka Equipment and Vermeer Corp. TAMU provided funds for salaries and benefits via cost sharing.

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Project Title: Development of a Process for Bioethanol Production using Eastern Redcedar

DR. MARK WILKINS

Project Goal

The goal of this project was to develop a pretreatment and bioconversion process to use eastern red cedar to produce biofuels and commodity chemicals.

The main objectives were:

- Development of an effective pretreatment process for red cedar pretreatments.
- Observe the effect of red cedar oil during red cedar processing.

Project Outcomes

- A red cedar pretreatment procedure was developed and optimized for efficient production of fermentable glucose. We found 82% overall wood-glucan-to-glucose yield at 200°C, 10 min, 3.75 g sulfuric acid/100g of dry biomass and 20 g sodium bisulfite/100 g of dry wood.
- Hydrolysis at 16% solid loading (dry basis) produced 105 g/L of glucose in 120 h with a digestibility of 89%.
- A significant model was obtained from the statistical optimization, which predicted 91% overall wood-glucan-to-glucose yield at 200°C, 7.5 min, 3.75 g sulfuric acid/100g of dry biomass and 22.5 g sodium bisulfite/100 g of dry wood. Validation experiments supported the model by achieving 87% wood glucan-to-glucose yield.
- Red cedar oil was inhibitory to cellulolytic enzymes, but did not impact glucose fermentations using *S. cereviseae* D5A strain. The inhibitory effect of red cedar oil can be prevented by extracting cedar oil prior to conducting hydrolysis and/or fermentations.
- Chemical composition of the heartwood and sapwood fractions showed no significant difference between the glucan and lignin fractions, which indicated that both parts of the trunk could be used for fuel production.

Other Sources of Funding:

Oklahoma State University: A portion of one month's salary of Dr. Wilkins for each year, fringe benefits on that salary, & waived indirect costs at a rate of 47.7% of total direct costs.



PI: Dr. Mark Wilkins Oklahoma State University Biosystems & Ag Engineering

Co-PI: Dr. Nurhan Dunford Oklahoma State University *Biosystems & Ag Engineering*

Co-PI: Dr. Hasan Atiyeh Oklahoma State University *Biosystems & Ag Engineering*

Co-PI: Dr. Salim Hiziroglu Oklahoma State University *Food and Agricultural Products Center*

Funded: \$67,502 Start Date: 12/01/2009 End Date: 11/30/2012





U.S. Department of Agriculture National Institute of Food & Agriculture



Project Title: Decreasing Severity of Switchgrass Pretreatment through Wet Storage and Biological Pretreatment

DR. MARK WILKINS

Project Goal

Biorefineries face logistical issues due to the low bulk density of lignocellulosic biomass. Storage facilities must have large capacities to store feedstocks for a minimum of six months to ensure a continuous operation. Hence, the major goal of this project is to overcome the issues of physico-chemical pretreatment and reduce cost by adapting a holistic approach of exploiting a part of storage time for a biological pretreatment process.

Project Outcomes

- The team completed compositional analyses on all from square bales and 8 samples from round bales.
- In square bales, extractives content was greater at the bottom of the bales than in the middle and top of the bales, which was indicative of more fungal and other biological activity occurring in the top of the bale that was exposed to more air resulting in more activity. The extractives largely consist of sugars that are easily digested by fungi and other organisms.
- Glucan content was less in the bottom of the bale than at the top and middle.
- In round bales, extractives content was lower in mushroomtreated bales than in control bales, which was which was indicative of more fungal and other biological activity occurring in the mushroom treated bales, which was expected.
- Currently, the team is doing controlled studies in a laboratory environment to determine mushroom loading and moisture contents for a future bale study in a controlled environment.



PI: Dr. Mark Wilkins Oklahoma State University *Biosystems and Agricultural Engineering*

Co-PI:

Dr. Michael Buser Oklahoma State University *Biosystems and Agricultural Engineering*

Co-PI:

Dr. Danielle Julie Carrier University of Arkansas *Biological and Agricultural Engineering*

Funded: \$171,994 Start Date: 04/30/2013 End Date: 04/29/2015



Project Title: Using Crude Glycerin in High Forage Diets-A Way to Improve the Profitability of Biodiesel Production

DR. JIM MACDONALD

Project Goal

To determine the effects of crude glycerin (CG) concentration in steam-flaked corn (SFC)-based growing diets on animal performance, heath, ruminal fermentation, and diet digestibility.

The two experiments to achieve this were:

- 1. Four ruminally and duodenally cannulated steers were used using SFC-based diets with 0, 2.5, 5, or 10% CG replacing forage.
- 2. 309 crossbred steers were used which consisted of SFC-based diets with 0, 5, or 10% CG replacing forage.



PI: Dr. Jim Macdonald Texas A&M University *Texas AgriLife Research at Amarillo*

Co-PI: Dr. Mike Brown West Texas A&M University *Agricultural Science*

Funded: \$70,000

Start Date: 12/01/2009 End Date: 11/30/2011

Other Sources of Funding

Texas A&M University contributed the cost share regarding salaries and fringe benefits for PI Jim Macdonald and Co-PI Mike Brown.

Project Outcomes

- The addition of crude glycerin as a partial replacement for roughage in SFC-based diets appears to positively impact performance, nutrient digestibility and ruminal fermentation at levels up to 5% substitution for roughage. These data indicate crude glycerin may be an alternative lower-cost higher-yielding ration ingredient in receiving cattle diets.
- In Exp. 1, we found a large increase in propionate production and shift in the ratio of acetate to propionate produced, we observed no difference in fiber digestibility with increasing crude glycerin concentration, and we found crude glycerin increased microbial crude protein production when included at 5% of the diet.
- In Exp. 2, we observed no effects of crude glycerin on animal health, indicating crude glycerin can be safely incorporated into the diets of newly received calves without negatively impacting animal health.



Project Title: Evaluation of the Nutritional and Feeding Value of Ethanol By-Products for Animal Production

DR. TRAVIS WHITNEY

Project Outcomes:

- Substituting distillers dried grains for cottonseed meal in small ruminant finishing diets:
- As distiller's dried grains (DDG) increased in the diet, average daily gain (ADG) and gain to feed efficiency (G:F) decreased quadratically, but no difference in daily dry matter intake (DMI) was observed.
- Serum urea nitrogen (SUN) increased as DDG increasingly replaced cottonseed meal (CSM), which was attributed to an increase in degradable protein intake.
- Serum non-esterified fatty acids (NEFA) decreased linearly and serum IGF-1 decreased quadratically as DDG increasingly replaced CSM in the diets.
- Ruminal disappearance of fiber in the diet was not affected.
- Wool characteristics were not affected.
- DDG can replace all the CSM in small ruminant finishing diets without negatively affecting growth, efficiency of gain, or wool characteristics, and can potentially reduce cost of feed·kg⁻¹gain. As DDG increased in the diet, extracted fat from the longissimus muscle linearly increased.
- Meat from lambs fed diets with all CSM replaced by DDG, had less cook-loss and greater initial and sustained juiciness than meat from lambs fed 0DDG diet. Results indicated that partially or totally substituting DDG for CSM in lamb-finishing diets is acceptable and may enhance sensory traits.

Substituting distillers dried grains for cottonseed meal and milo in lamb finishing diets:

- As DDG increased in the diet (replaced 0% (0DDG), 25% (25DDG), 50% (50DDG), or 75% (75 DDG) of the milo and cottonseed meal (CSM), average daily gain quadratically increased and at times, DMI linear increased.
- Feed efficiency (gain:feed) linearly decreased as DDG increased in the diet, which may have been attributed to greater occurrence of urinary calculi in lambs fed diets containing DDG.
- Serum urea nitrogen and phosphorus linearly increased but IGF-1 was similar among lambs as DDG increased in the diet.
- Fecal P and N linearly increased but IGF-1 was similar among lambs as DDG increased in the diet.
- Increasing DDG in the diet quadratically increased average fiber diameter and average fiber curvature, but all other wool characteristics were similar among lambs.

Sun Grant funding on this project has led to additional feeding trials (funded by other sources) that used DDGS as the concentrate/protein source, and led to collaborations with industry such as POET nutrition.



PI: Dr. Travis Whitney Texas A&M University *Texas AgriLife Research, San Angelo*

Co-PI: Dr. James Muir Texas A&M University *Texas AgriLife Research, Stephenville*

Co-PI: Dr. Barry Lambert Tarleton State University *Animal Science*

Co-PI: Dr. Chris Lupton Texas A&M University *Texas AgriLife Research, San Angelo*

Co-PI: Dr. Mike Salisbury Angelo State University Animal Science

Co-PI: Dr. Kirk Braden Angelo State University *Animal Science*

Funded: \$116,103 Start Date: 07/01/2007 End Date: 06/30/2010



Project Title: A Multifunctional Frequency-Response Permittivity Sensor for Biofuel Concentration Measurement and Impurity Detection

DR. NAIQIAN ZHANG

Project Objectives

- 1. Develop a portable sensor for quick measurement of blend ratio and impurity concentrations for biodiesel.
- 2. Develop an embedded blend-ratio sensor to assist fuelinjection adjustment.
- 3. Prove the accuracy, reliability, and durability of the sensors through a well-designed experiment.

Project Outcomes

- A real-time control system for a frequency-response (FR) -based permittivity sensor was developed during the study. The system was improved from a previous design mainly on extended frequency range (0-400 MHz) and enhanced measurement resolution.
- Sensor probes of three sizes (2cm, 2.5cm, and 7.5cm) were fabricated and tested in biodiesel with different impurities, including water, glycerin, and glyceride.
- A study on the "signature frequencies" indicated that both the higher and lower frequency ranges were equally significant. The frequency that was selected most often was around 70 MHz.

Other Sources of Funding

Via cost sharing by Kansas State University, a Graduate Research Assistant was hired for hardware/software design and laboratory experiment.



PI: Dr. Naiqian Zhang Kansas State University Biological and Ag Engineering

Co-PI: Dr. Wenqiao Yuan Kansas State University *Biological and Ag Engineering*

Funded: \$69,770 Start Date: 07/01/2007 End Date: 06/30/2010