

South Central Region Sun Grant Initiative 2007 Awards DOT Biobased Transportation Research Program Regional Competitive Grants

INTEGRATED PROJECTS

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

Principal Investigators and affiliations:

PI: Mark Wilkins (Oklahoma State University, Biosystems & Agricultural Engineering)

Co-PI: Ray Huhnke (Oklahoma State University, Biosystems & Agricultural Engineering)

Co-PI: Krushna Patil (Oklahoma State University, Biosystems and Agricultural Engineering)

Co-PI: Danielle Bellmer (Oklahoma State University, Biosystems and Agricultural Engineering)

Co-PI: Randy Lewis (Brigham Young University, Chemical Engineering)

End Date: 6/30/2010

Objectives:

Objective 1. Samples of biomass-syngas generated from switchgrass, corn gluten, wheat straw and coal mixed with switchgrass and wheat straw (co-firing) will be used to study the effects of the biomass-syngas on cell growth, CO/H₂ utilization, and ethanol/acetic acid formation. The gasification agent will be air. Coal alone will also be assessed to help determine the effects of coal on the co-firing. Chemical analysis of the biomass-syngas will be performed to identify a list of contaminants (i.e. types of tars, nitric oxide, SO₂, etc.) that may affect the fermentation process. A database of the generated contaminants and their associated compositions will be established. Objective 2. Four key enzymes (FDH, ADH, CODH, and Hydrogenase) will be assayed in the presence of biomass-syngas to assess the impact of the gas on enzyme activity. In addition, the most common biomass-syngas contaminants identified in Objective 1 will be added to “clean” syngas to quantify the impact of these contaminants on the enzyme function. Concentrations of these contaminants at least 2-fold greater than those observed in the biomass-syngas will be studied. Objective 3. Biomass-syngas will be obtained from differing gasifying agents including air, air/steam, and steam. Chemical analysis of the biomass-syngas, fermentation analysis, and enzyme analysis will be performed and compared with the results obtained in Objectives 1 and 2 using only air gasification. Additionally, the fluidized-bed gasifier at Oklahoma State University will be operated at different conditions for maximizing CO and H₂ concentrations and minimizing the tars, non-syngas gases, ash, nitric oxide and other possible contaminants. Initial gas clean-up issues will also be addressed, beginning with nitric oxide removal.

Methodology:

The gasification of biomass alone will be carried out at Oklahoma State University, using a 10-

inch diameter fluidized-bed gasifier. Corn gluten, wheat straw and switchgrass will be used as biomass to generate syngas. At Brigham Young University, a 4-inch diameter fluidized-bed gasifier will be used for co-firing of mixtures of biomass (switchgrass and wheat straw) and coal gasification.

The syngas resulting from gasification as well as co-firing will be bubbled through a bioreactor to study its effects on cell-growth, product distribution and enzyme activities of the microbial catalyst (denoted P11). Each of the syngas samples will be analyzed and the individual species will be quantified. These species will then be tested individually during the fermentation process.

Based on the above information, a gas clean-up system will be designed to remove any harmful species from the syngas. Cell-adaptability studies will be conducted to determine whether the cells can adapt to the syngas species upon prolonged exposure.

Expected Outcomes:

A summary of project deliverables includes:

1. A database of biomass-syngas constituents based on several feedstocks including switchgrass, wheat straw, and corn gluten as well as co-firing of switchgrass and wheat straw with coal.
2. An assessment of biomass-syngas constituents on the effects of cell growth, substrate consumption, ethanol production, and enzyme activity.
3. An assessment of the effects of gasification agents on generated biomass-syngas species and the fermentation process.
4. The development of a scrubbing system to reduce nitric oxide levels.
5. The development of gas clean-up systems to reduce or eliminate other biomass-syngas contaminants that are found to affect the fermentation process adversely.

The information obtained in the above mentioned deliverables would enable us to design a more efficient and economically viable gasification-fermentation process.

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

Principal Investigators and affiliations:

PI: Sergio Capareda (Texas Agricultural Experiment Station (Texas A&M University System), Biological and Agricultural Engineering)

Co-PI: Michael Mailander (Louisiana State University Agricultural Center, Biological & Agricultural Engineering)

Co-PI: Chandra Theegala (Louisiana State University, Biological and Agricultural Engineering)

Associate Investigators and affiliations:

Associate PI: Michael Farmer (Texas Tech University, Agricultural and Applied Economics Department)

End Date: 6/30/2010

Objectives:

The specific objectives of the project are as follows: a. investigate the technical feasibility of on-site thermal gasification systems with state-of-the art control systems for different unique biomass wastes in the region and evaluate the quality, composition, heat energy and power output (Capareda and Theegala) b. evaluate the economic feasibility of decentralized thermal gasification systems and develop economic models for different applications (Farmer) c. conduct systems analysis for the different applications within the region and lay-out strategies to reduce barriers for commercialization (Mailander) d. evaluate the environmental and air quality implications of the systems including permitting procedures (Capareda)

Methodology:

The overall methodology is divided into 4 general tasks as follows:

Task 1: Technical Feasibility. This task will prove the technical viability of the gasification systems for the different feed stocks identified

Task 2. Economic Feasibility. This task will provide a complete outlook of the economic feasibility of modular and decentralized applications.

Task 3. Systems Analysis. This task will evaluate strategies for on-site utilization and thresh out barriers to commercialization.

Task 4. Environmental Concerns. This task will evaluate environmental issues associated with the use of a gasification system including permitting processes required for adoption.

Expected Outcomes:

At the end of the study modular gasification systems shall be developed whose size is applicable to several biomass generating industries in the region. The technical feasibility of both the fixed bed and fluidized bed gasification systems should have been clearly evaluated by bench-scale and field scale demonstration systems. The field demonstrations should be able to prove the technical functionality of the systems and its impact on reducing the heat and power requirement of a facility particularly industries generating biomass wastes on site (i.e. cotton gins, animal farms, etc).

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.

Principal Investigators and affiliations:

PI: Dirk Hays (Texas A&M University, Soil and Crop Sciences)

Co-PI: Donghai Wang (Kansas State University, Biological and Agricultural Engineering)

End Date: 6/30/2010

Objectives:

Our goal is to develop a systems approach for designer sorghum cultivars to optimize the grain's endosperm matrix for bio-ethanol conversion and grain distiller's feed for low rain fed Texas environments. This approach will include protection of sorghum genetics with cultivars combining a high endosperm protein digestibility (HD) trait with a high amylopectin (waxy) starch trait. We have generated data that suggest that both traits individually improve the endosperm matrix for low energy input gelatinization, enzymatic hydrolysis, and total ethanol production. Together, these two traits will be ideal for bio-ethanol conversion. Bio-ethanol systems often sell the remaining grain by-products as a high protein feed supplement. Unfortunately, sorghum and corn grain are low in essential amino acids such as lysine. The HD trait also confers both a high protein bio-availability and high lysine content. As such, in the bio-ethanol-feed supplement system, the grain would also be optimized for feed. The proposal also explores the inclusion of vegetative tissue for lignocellulosic conversion into ethanol, best management practices to optimize the system for foliar and grain complex sugar retention, and a plan for future economic analysis of the competitiveness of this system with corn and sweet sorghum for the rain fed Texas environments.

Methodology:

We will use molecular markers in a marker-assisted breeding program and a near-infrared seed sorting technology to rapidly develop high yielding HD-waxy cultivars and determine the benefits derived from these modified endosperm sorghum genotypes for the development of new low energy input bio-ethanol methodologies.

Using existing waxy and HD endosperm sorghum lines, we will work as a team to develop an IP sorghum system using the following objectives:

1. Test existing high yielding HD, and waxy genotypes and early generation genotypes possessing both the HD and waxy traits to determine optimized low energy gelatinization and enzymatic hydrolysis procedures to liquefy and saccharify the starch into fermentable sugars.
2. Determine lysine content and feed potential of HD, waxy, and HD-waxy endosperm sorghums for potential use as improved grain distiller's feed material.
3. Determine soluble and lignocellulosic sugar content for potential bio-ethanol conversion of vegetative tissue in modified endosperm HD, waxy, and HD-waxy sorghum endosperm at different stages of grain maturity to maximize both sources of fermentable sugars.

Expected Outcomes:

This system could provide monetary benefit to Texas producers, TAES and the TAES sorghum royalty program. It is likely to provide an important source of market share growth for the bio-refineries and related distribution systems. The development of modified endosperm hybrids also has applications for use in celiac friendly beers, high value feed, and fast cook convenience foods.

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

Principal Investigators and affiliations:

PI: William Rooney (Texas Agricultural Experiment Station, Soil & Crop Science)
Co-PI: Jürg Blumenthal (Texas Cooperative Extension, Soil and Crop Sciences)
Co-PI: Brent Bean (Texas Cooperative Extension and Texas Agricultural Experiment Station, Soil and Crop Sciences)

Co-PI: Danielle Bellmer (Oklahoma State University, Biosystems and Agricultural Engineering)

Co-PI: Ray Huhnke (Oklahoma State University, Biosystems & Agricultural Engineering)

Co-PI: Donghai Wang (Kansas State University, Biological and Agricultural Engineering)

Co-PI: Mark Marsalis (New Mexico State University, Extension Plant Sciences)

Co-PI: Rick Kochenower (Oklahoma State University, Oklahoma Panhandle Research and Extension Center)

Co-PI: Scott Staggenborg (Kansas State University, Agronomy)

End Date: 6/30/2010

Objectives:

The specific objectives of this project are: (1) Develop and select sweet sorghum hybrids for use as a bioenergy feedstock; (2) Examine adaptability of high biomass sorghum and sweet sorghum to the South Central U.S., including the Coastal Plains, High Plains, and the Central Great Plains; (3) Develop production guidelines for sweet sorghums for these production regions. Agronomic emphasis will be on seeding rate, nitrogen management, and multiple harvest efficacy and water use efficiency; (4) Evaluate the effects of juice press operation and time of harvest on juice yield and sugar content of the expressed juice; (5) Determine the relative efficiency of ethanol conversion from sweet sorghum (lbs/sugar/gallon ethanol) and assess the relative role genotype, environment and genotype x environment interaction on ethanol production potential.

Methodology:

There are two research components. The first will focus on breeding, development and release of sweet sorghum hybrids for commercial production and the second will focus on production, processing and conversion issues of sweet sorghum cultivars and hybrids. Given that the cooperators are strategically located through a diverse set of production environments, we have divided the South Central area into three regions; (i) the Coastal Plain, from the Rio Grande through Louisiana, (ii) the High Plains region, including eastern New Mexico to Western Kansas, (iii) the South Central Great Plains region, including Central Kansas and Oklahoma.. Within each region, specific research objectives will be addressed based on the expertise and research interests of the faculty in that region. A common theme of all research is that it will focus on the sweet sorghum hybrids so that production systems are optimized for their release, adaptation and utilization.

Preliminary sweet sorghum hybrid evaluations in 2007 will be used to identify 3-5 specific hybrids for advanced testing. Parental lines for selected hybrids will be grown in the TAMU winter nursery near Guayanilla, Puerto Rico and bulk quantities of hybrid seed will be produced. Experimental hybrid seed will be distributed amongst the agronomists and they will plant these

hybrids (along with checks appropriate for their region) in replicated tests in locations throughout the region.

These trials will also be used to develop agronomic “best management practices” for the three production regions. At each location, specific agronomic and/or engineering objectives will be addressed based on the interest of each co-PI. In South Texas, emphasis will be placed on multiple harvests, on the High Plains, population density will be studied and in the Great Plains, nitrogen utilization will be studied. Juice extraction studies will be conducted in Stillwater while ethanol conversion will be completed in Manhattan.

Expected Outcomes:

From this project we will release inbred lines necessary to produce a sweet sorghum hybrid specifically for bioenergy production. These lines will be distributed based on licensing agreements negotiated by the Office of Technology Commercialization at Texas A&M University. Our expectation is that these materials will be available for distribution in 2010 and possibly if needed as early as 2009. At the time of distribution, and on a regional basis, extension bulletins and production management guides will be made available on hybrid adaptation, expected sugar yield, and optimum seeding rate under both dryland and irrigated conditions.

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

Principal Investigators and affiliations:

PI: Stephen Searcy (Texas Agricultural Experiment Station (Texas A&M University System), Biological and Agricultural Engineering)

Co-PI: Michael Popp (University of Arkansas, Agricultural Economics and Agribusiness)

End Date: 6/30/2010

Objectives:

1. Evaluate the energy, labor and capital requirements for converting standing switchgrass to chopped material suitable as feedstock for a bio-fuels refinery using conventional round bale and large modules as the storage forms. 2. Determine the optimum chopped switchgrass characteristics for formation and long-term stability of modules. 3. Determine the storage losses and protection requirements for switchgrass modules to minimize field to biorefinery losses.

Methodology:

Project activities will address engineering and economic aspects of using modular packages for switchgrass. The study has an underlying assumption that large-scale collection of energy crops will be based on a custom harvest and transport business model. That system would include in-field chopping of the crop and immediate storage in modules. This approach can only be successful if trade-offs related to particle size, moisture content, equipment capacity, harvest weather and seasonal yield are evaluated. The system anticipated is analog to the cotton module

system. There are several differences between cotton and energy crops that will require modification of the system. The length of time the material would be stored in the package will be greater, and the moisture content likely will be higher.

The impact of switchgrass moisture content and particle size on the visco-elastic and internal friction properties will be evaluated. These properties are critical to the formation of a viable module. As the engineering properties of the harvested switchgrass are documented, mini-modules of switchgrass will be made using an existing ¼ scale module builder. Following successful module formation on the reduced scale, field scale harvesting and module formation will be evaluated.

Activities in Arkansas will be focused on evaluation and optimization of the harvest, storage and transport system within the constraints of historical weather and potentially other farming operations. The eventual model will be evaluated for weather conditions at four locations, two each in Texas and Arkansas. Dr. Popp will modify and evaluate the Integrated Biomass Supply Analysis and Logistics Model (IBSAL), which tracks economic cost and energy requirements of equipment necessary to harvest, transport and store biomass from the field to the biorefinery subject to daily weather data.

Expected Outcomes:

In addition to regular progress reports, the deliverables from this grant funding and the anticipated dates of completion will be:

- a. knowledge of the visco-elastic properties of chopped switchgrass material as a function of moisture and particle size (July 2009)
- b. information regarding trade offs between harvest moisture content and length of chop for purposes of module stability, handling and transport weight limitations (December 2009)
- c. a preliminary economic model that can perform sensitivity analysis on energy, labor and capital cost associated with the two proposed harvest, storage and transport systems (July 2008)
- d. a breakeven analysis regarding tradeoffs related to more extensive equipment utilization vs. likely switchgrass yield losses associated with extending the harvest window (December 2008)
- e. estimation of annual harvest capacity per harvest unit given typical weather data and optimal harvest window identified above (December 2009)
- f. detailed cost of production budgets from field to biorefinery for round baling and module building (July 2010)
- g. report on switchgrass production and harvest activity impact on other farm operation and processing activities (July 2010)

Project Title: Evaluation of the nutritional and feeding value of ethanol by-products for animal production.

Principal Investigators and affiliations:

PI: Travis Whitney (TX A&M Agricultural Experiment Station, Experiment Station)

Co-PI: James Muir (Stephenville Agricultural Experiment Station, Stephenville)

Co-PI: Barry Lambert (Tarleton State Univ., Animal Science)

Co-PI: Chris Lupton (Texas A&M University Agricultural Research and Extension Center, Animal Science)

Co-PI: Mike Salisbury (Angelo State University, Animal Sciences)

Co-PI: Kirk Braden (Angelo State Univ., Animal Science)

End Date: 6/30/2009

Objectives:

Objectives of this project are to determine how animal performance, metabolism, digestibility, and wool and carcass characteristics of growing lambs and kids are affected by replacing protein (cottonseed meal) and energy (milo) feeds with DDG. Trials 1 and 2 (Period 1; Spring and Summer, 2008) will evaluate use of DDG on lamb and kid performance and wool and carcass characteristics. Trials 3 and 4 (Period 2; Fall, 2008) will evaluate the use of DDG on feed digestibility and nutrient metabolism in small ruminants.

Methodology:

Two feeding trials (Trials 1 and 2) will be used to evaluate effects of substituting cottonseed meal (CSM) and milo with distiller's dried grains (DDG) on lamb growth performance, metabolism, health, and wool and carcass characteristics. In Trial 1, lambs will be fed four different feedlot rations. These rations will differ in the amount of distiller's dried grains (DDG), which replaces a portion of cottonseed meal (CSM) in the diet. In Trial 2, lambs will be fed four different rations differing in the amount of DDG, which replaces a portion of CSM and milo in the ration.

Ruminally fistulated goats will be used in Trial 3 to evaluate the use of DDG on feed digestibility. The goats will be fed the same diets as described for Trials 1 and 2, and small bags of forages will be placed into their stomachs. After incubating for various periods of time, bags will be removed and digestibility will be calculated from change in bag weight. For Trial 4, total fecal and urine output will be collected from four goats fed the same diets as previously discussed, to determine effects of DDG on nutrient metabolism.

Expected Outcomes:

Results from these trials will assist nutritionists in establishing a level of DDG that can be safely and efficiently fed to lambs and goats in growing rations. This knowledge should greatly increase the use of DDG since this study will quantify positive and negative effects associated with feeding DDG, which are high in phosphorus, sulfur, and fat. If DDG can effectively replace a portion of a protein or energy feed source, then it will become a more viable feed alternative when formulating feed rations. We believe that DDG will enhance animal performance in this study, which will increase utilization and value of this by-product.

Project Title: Breeding and testing of new switchgrass cultivars for increased biomass production in Oklahoma, Arkansas, Texas and Kansas

Principal Investigators and affiliations:

PI: Yanqi Wu (Oklahoma State University, Plant and Soil Sciences)

Co-PI: Charles West (University of Arkansas, Crop, Soil & Environmental Sciences)

Co-PI: Charles Taliaferro (Oklahoma State University, Plant and Soil Sciences Department)

Co-PI: John Fritz (Kansas State University, Agronomy)

Co-PI: James Muir (Stephenville Agricultural Experiment Station, Stephenville)

End Date: 6/30/2010

Objectives:

1. Conduct a breeding program to develop switchgrass cultivars with increased biomass yield and wide adaptation. 2. Establish a testing network in the south-central United States

Methodology:

Population improvement techniques will be used to develop improved cultivars. Inbreeding and specific combining ability will be studied. Field plots will be established using randomized complete block design at five locations.

Expected Outcomes:

The deliverables from the project from July 1 2007 to June 30 2010 will include: a) four breeding populations advanced from generation C2 to C3, and from C0 to C1, respectively in 2009; b) 6 to 8 new synthetics developed in 2009; c) data on stand density and biomass yield of new synthetics and major commercial cultivars in the establishment year at five locations in 2010; d) data on inbreeding and development of hybrid cultivars in 2010.

SEED GRANTS

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

Principal Investigators and affiliations:

PI: Kalyan Annamalai (Texas A&M University, Mechanical Engineering)

Co-PI: John M. Sweeten (Texas Agricultural Experiment Station, Texas A&M Univ. Agric. Research & Extension Cntr.)

End Date: 6/30/2009

Objectives:

2.1 Goal: 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 and Hg.

2.2 Objectives/Tasks: The proposed work falls into four broad objectives/tasks: (1) CB Fuel and Coal Characteristics, (2) Facility and Experiments, (3) Modeling, and (4) Economics. The tasks are selected so as to supplement the ongoing DOE project in developing new technology and providing an additional market for CB as fuel in coal fired plants. Task 1: CB Fuel and Coal

Characteristics. Obtain fuel characteristics for the fuels to be tested. Coal, DB and FB Sub-task 2.1: Collection, Composting, and grinding Sub-task 2.2: Obtain all relevant fuel properties for the fuels to be tested Sub-task 2.3: Thermogravimetric and Differential Scanning Calorimetry (TGA/DSC) Task 2: Facility and Experiments. Sub-task 2.1: Modify the Low NO_x burner for firing CB with Coal Sub-task 2.2: Study the effects of operating parameters and various fuels Task 3: Conduct Zero D modeling Task 4: Conduct economic analysis on fuel collection, transportation, and processing. Task 1: CB Fuel and Coal Characteristics. Obtain fuel characteristics for the fuels to be tested. Coal, DB and FB Sub-task 2.1: Collection, Composting, and grinding Sub-task 2.2: Obtain all relevant fuel properties for the fuels to be tested Sub-task 2.3: Thermogravimetric and Differential Scanning Calorimetry (TGA/DSC) Task 2: Facility and Experiments. Sub-task 2.1: Modify the Low NO_x burner for firing CB with Coal Sub-task 2.2: Study the effects of operating parameters and various fuels Task 3: Conduct Zero D modeling Task 4: Conduct economic analysis on fuel collection, transportation, and processing.

Methodology:

The proposed experiments will yield a first set of data on NO_x and Hg when CB is co-fired with coal. The following methodology will be adopted:

- 1) Select Wyoming Powder River Basin Coal as the base fuel and blends of coal and FB for experimental runs. Combustion tests will involve coal as a standard fuel. Cattle biomass and a blend of CB and Coal will be tested in order to confirm preliminary data obtained in the conventional burner.
- 2) Collect bottom ash and fly ash and analyze the samples to determine combustion efficiency and LOI.
- 3) Measure CO, CO₂, O₂, NO_x, and elemental Hg. Since the Hg instrument is limited to measure elemental mercury alone, and to present more exhaustive results on mercury capture, it is required to report both elemental and oxidized concentrations of mercury in flue gas. For this purpose a wet chemistry based flue gas conditioning system is used based on modified Ontario. Limited Hg measurements will include both elemental and oxidized Hg due to time consuming procedure for wet chemistry method in determining oxidized Hg.
- 4) Determine lowest amount of CB needed in the blend to achieve highest NO_x and Hg reduction.

Expected Outcomes:

Demonstrate the use of CB as a co-fired fuel in Low NO_x burners. Once the proposed technology has been demonstrated, there is the possibility of significant emissions reductions from stored FB and the generation of additional revenue to both farmers and power companies. Moreover, there is the prospect of improving the environment in terms of improved air quality and the sustainability of clean streams and watersheds in rural America. Manure-based biomass can add a significant portion to the list of new, renewable fuels available to the country's power production facilities from a growing, industrialized agricultural sector.

Project Title: Advanced technologies for biodiesel production

Principal Investigators and affiliations:

PI: Dorin Boldor (Louisiana State University Agricultural Center, Biological and Agricultural Engineering)

Co-PI: Vadim Kochergin (Louisiana State University Agricultural Center, Audubon Sugar Institute)

Co-PI: Cristina Sabliov (Louisiana State University, Biological and Ag. Eng. Dept.)

Co-PI: Marybeth Lima (LSU, Biological & Agricultural Engineering)

End Date: 6/30/2009

Objectives:

The objectives of the proposed research are to use batch and continuous microwave technology to extract oil from traditional (soybeans) and alternative (rice bran, Chinese tallow tree seeds) feedstocks, to convert these oils into biodiesel, and to estimate the feasibility and economic viability of the process.

Methodology:

To achieve these objectives, the feedstock will be subjected to: pre-processing, oil extraction, separation, trans-esterification, and separation of trans-esterification products (biodiesel and glycerol). The results will be assessed using universally accepted analytical methods.

Expected Outcomes:

The outcomes of the proposed research project will (a) further develop and strengthen the bioenergy-targeted bioprocessing programs at LSU AgCenter; (b) develop new technologies for post-harvest processing of biodiesel feedstock, and (c) accelerate the technology transfer process from research to commercialization using existing agreements with industry partners.

Project Title: *Vibrio furnissii*: a biotechnology platform for biomass bioconversion

Principal Investigators and affiliations:

PI: Paul de Figueiredo (Texas A&M University, Plant Pathology and Microbiology)

End Date: 6/30/2009

Objectives:

To further our long-term aim of developing an efficient and economical platform for the direct bioconversion of biomass into kerosene and other long-chain alkanes, we propose to pursue the following objectives: 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. 3. To characterize the genetic lesions in *V. furnissii* strains harboring altered hydrocarbon biosynthesis and accumulation phenotypes.

Methodology:

Objective 1. We shall employ chemical and molecular genetic methods to induce mutations in *V. furnissii* cells. We shall then screen mutants for strains with altered hydrocarbons biosynthesis and accumulation phenotypes. These strains will be useful for elucidating the genetic pathways mediating hydrocarbon metabolism in this organism. In addition, strains with enhanced

hydrocarbon biosynthesis and accumulation properties are expected to hold significant promise as tools for the industrial-scale bioconversion of biomass into kerosene and other light oils. Two general strategies will be used to generate the desired mutants. First, we will employ transposon mutagenesis to induce marked mutations in genes that regulate hydrocarbon biosynthesis. Alternatively, ethyl methane sulfonate (EMS) or N-ethyl-N-nitrosourea (ENU) mediated chemical mutagenesis will be employed to generate the desired mutations.

Objective 2. We will employ high perform thin layer chromatography (HPTLC) and gas chromatography-mass spectrometry (GC-MS) to analyze hydrocarbon biosynthesis and accumulation in the most promising strains identified in our screen. We shall, however, be particularly interested in strains that accumulate high levels of long-chain hydrocarbons. These strains will hold significant promise as biofuel biosynthesis platforms, and therefore, shall be further examined using genetic methods (see below).

Objective 3. We shall clone the genes that are responsible for the altered hydrocarbon accumulation phenotypes in mutant strains. Information about these genes will prove useful in efforts that seek either to further enhance hydrocarbon biosynthesis and accumulation in *V. furnissii*, or to engineer corresponding capacities into genetically tractable model microbes (e.g., *E. coli*).

Expected Outcomes:

1. Overview. Results derived from the proposed experiments will benefit the bioenergy research and development communities by providing new insights into alternative routes for light oil synthesis. In addition, the proposed technology platform will facilitate efforts to transform biomass into fuel oils that are compatible with existing petrochemical transportation and refining infrastructures. This technology will therefore help satisfy our nation's bioenergy needs. 2. Economic analysis. World energy demand is projected to increase by more than 70% in the next 25 years. Biofuels derived from biomass hold great promise as a source of renewable energy that can help to meet this demand. However, cost-efficient methods for the industrial conversion of biomass into desirable fuels are not yet available. Current goals set by the US Secretary of Energy state that 30% of transportation fuels are to be derived from biomass by the year 2030. If this ambitious goal is to be achieved, then the proposed bioconversion platform may play an important role. Assuming that the proposed (or a similar) platform produces (a modest) 0.1% of the transportation-compatible biofuels by 2030, then the proposed approach would provide an annual domestic contribution of more than 300 million gallons (with a current retail value of ~\$US 600M). 4. Project deliverables. The proposed work will provide the following deliverables: (a) *V. furnissii* strains with enhanced hydrocarbon biosynthesis ability; (b) Intellectual property associated with the hydrocarbon biosynthesis profiles of selected strains generated in this research; (c) Information about *V. furnissii* genes responsible for altered biofuel production and accumulation; (d) Enhanced awareness of bioenergy technology development among undergraduate, graduate student, and postdoctoral trainees will constitute an important deliverable associated with this research effort.

Project Title: Optimizing a new downdraft gasification system for synthesis gas production from low bulk density biomass materials

Principal Investigators and affiliations:

PI: Krushna Patil (Oklahoma State University, Biosystems and Agricultural Engineering)

Co-PI: Ray Huhnke (Oklahoma State University, Biosystems & Agricultural Engineering)

Co-PI: Danielle Bellmer (Oklahoma State University, Biosystems and Agricultural Engineering)

End Date: 6/30/2009

Objectives:

A unique downdraft gasifier design has been evolved at OSU to generate synthesis gas (syngas) consisting of CO, H₂ and methane as the major combustible elements. The project aims at optimizing the new downdraft system for selected low bulk density biomass materials to generate synthesis gas high in carbon monoxide and hydrogen concentrations and low in tar and particulate contents, and to demonstrate its readiness for commercial deployment for distributed energy applications in Oklahoma and South Central region. The specific objectives are as follows: 1) to fabricate a unique downdraft gasifier system capable of gasifying low bulk density biomass materials and develop a test set-up; 2) to test and evaluate the gasifier system developed under the objective 1 for chopped switchgrass, wheat straw, wood shavings, saw dust, and corn fermentation byproducts to generate synthesis gas high in carbon monoxide and hydrogen concentrations and low in tar and particulate contents and incorporate modifications as needed; 3) to evaluate mass and energy balance and synthesis gas generation cost details for each of the biomass material tested under the objective 2; and 4) demonstrate the developed gasifier technology to selected industries of Oklahoma and South Central region.

Methodology:

OSU has well equipped gasification laboratory and workshop facilities necessary to carryout the proposed work. PI and CO-PI's have extensive experience in biomass gasification research. To accomplish the objectives outlined in the proposal, initially fabrication details will be developed for the new gasifier system and it will be constructed in the workshop of BAE department. The system will be installed and instrumented at the gasification laboratory of BAE department. Instrumentation will include: mass air flow meter, type-K thermocouples for reactor temperatures, and differential pressure transmitters for pressure drop measurement, gas chromatograph for syngas analysis, an electric air heater for preheating the gasifier input air, a tar and particulate measurement system developed in-house to quantify the tar and particulate content in the syngas, a GC-MS system for product distribution analysis of tars. Gasifier performance will be evaluated using chopped switchgrass, wheat straw, wood shavings, saw dust, and corn fermentation byproducts as the feed stalks. ASTM standard methods will be adopted for Proximate and elemental composition of the selected biomass materials. Effects of specific gasification rates, air-fuel ratios, biomass particle size and its moisture content, and the temperature of the input gasifying air on gasifier performance will be studied for all the selected biomass materials. Necessary modifications will be incorporated as identified. Mass and energy balance and synthesis gas generation cost details would be evaluated for each of the biomass material tested. System economic evaluation report will be developed. Lastly, selected industries in Oklahoma and South Central region will be identified and demonstrations would be organized to create awareness about the gasifier technology.

Expected Outcomes:

1. An exploratory test facility for a unique downdraft gasifier system to generate synthesis gas from low density biomass material.
 2. An optimized engineering design of a unique downdraft gasifier system capable accepting low bulk density biomass material as the feed stalks to generate synthesis gas high in carbon monoxide and hydrogen concentrations and low in tar and particulate content.
 3. System testing and evaluation report for each of the selected biomass materials.
 4. A report on economic evaluation of the gasifier system.
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Project Title: Saline extractive distillation for ethanol separation

Principal Investigators and affiliations:

PI: Peter Pfromm (Kansas State University, Chemical Engineering)

End Date: 6/30/2009

Objectives:

The objective is to reduce capital and operating costs (energy demand) of the current multi stage separation process for recovery of fuel-grade ethanol from fermentation broth. A single distillation unit will suffice to obtain fuel-grade ethanol. The principle is the addition of salt to the distillation column and the subsequent recovery and recycle of the salt by electro dialysis. The recycling of salt to the distillation column by electro dialysis is the enabling process for saline extractive distillation.

Methodology:

1. Electro dialysis experiments
2. Computer simulation, mass and energy balances
3. Cost estimate

Published computer simulations for saline extractive distillation of fermentation broth will be the foundation. The novelty of this work is the use of electro dialysis for salt recovery from the bottoms of the saline extractive distillation column. Small pilot scale electro dialysis experiments with simulated and actual fermentation broth will give the data to computer-model saline extractive distillation with electro dialytic salt recovery. A steady state computer simulation will be completed to obtain mass and energy balances. Cost estimates will be done according to accepted chemical engineering principles.

Expected Outcomes:

Experiment-based design data, computer simulation, and cost estimates for saline extractive distillation of ethanol from fermentation broth. Publication in peer reviewed journals.

Project Title: Breaking the cost barrier for bio-ethanol: reactive adsorption of fermentation broth

Principal Investigators and affiliations:

PI: Mary Rezac (Kansas State University, Chemical Engineering)

End Date: 6/30/2009

Objectives:

The long-term goal of the proposed work is to develop reactive adsorption technology for the efficient technical-scale recovery of ethanol from fermentation broths. Unlike conventional ethanol recovery systems, where separation of ethanol from water relies upon differences in the boiling points of the components, in the proposed system, separation will be achieved 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 revolutionary approach for recovering ethanol from fermentation broth has the potential for reducing production costs by 40% [Vane, 2005]. Literature data demonstrates that moieties exist which will selectively and reversibly react with ethanol to form stable products. An increase in temperature is all that is needed to reverse the reaction. Our challenge is to identify a reactive moiety that has very high specificity for ethanol in the presence of water and for which the moiety-ethanol product can be converted back into the individual molecules with only moderate energy input.

Project Objectives The main objectives of this seed grant project are to:

- Evaluate the feasibility of recovering ethanol from fermentation broth via solid-phase reactive adsorption.
- Evaluate the capacity of the adsorbent to be regenerated and reused.
- Prepare a preliminary process design and determine energy requirements for the proposed system.

Methodology:

We will determine the effectiveness of reactive moieties tethered to solids for the recovery of ethanol from water. The planned activities include:

- Using combinatorial chemistry approaches, prepare a library of potential surface bound reactive groups with a diverse range of moieties incorporated.
- Measure the thermodynamic equilibrium of a model fermentation broth with the adsorbents prepared.
- Measure the thermal transitions to determine adsorbent regeneration schemes.
- Measure the degree of separation in the expected system configurations.

Equipment necessary for these evaluations is available.

Expected Outcomes:

An ethanol recovery system that eliminates the highly energy intensive distillation process provides a route to making substantial reductions in the cost of ethanol production. Energy costs are expected to be reduced by 40%. Capital costs should also be reduced due to reduced complexity. This breakthrough technology will enable the entire concept of a biorefinery to be more rapidly advanced. Replacing the current petrochemical feedstocks with renewable biomass for transportation fuel production will lead to economic opportunities for rural America, more stable prices for transportation fuels, and a reduced dependence on foreign oil to power the American economy. Furthermore, adsorption systems can easily be scaled down providing a route to the construction of smaller-scale biorefineries which are likely to promote rural economic development.

Project Title: Nanoparticle systems for delivery of biological antimicrobial compounds to limit microbial contamination in industrial yeast fermentation

Principal Investigators and affiliations:

PI: Steven Ricke (University of Arkansas, Department of Food Science)

Co-PI: Michael Johnson (University of Arkansas, Food Science Department, IFSE)

Co-PI: Jin-Woo Kim (University of Arkansas, Department of Biological and Agricultural Engineering)

End Date: 6/30/2009

Objectives:

Our long term goal is to find feasible antimicrobial intervention method (s) that can be routinely integrated with economical delivery systems in large scale industrial yeast fermentation systems. Specific objectives are: 1) Evaluate *B. bifidum* NCFB 1454 fermented corn grain extract against potential contaminants of yeast fermentation in a model system and screen for effective bacteriocins; 2) Evaluate the effective concentrations of polylysine or *B. bifidum* NCFB 1454 fermented corn grain extract bacteriocins or their combinations against potential contaminants in yeast fermentation system. 3) Synthesize and characterize chitosan nanoparticles containing polylysine peptide dispersed in organic acids and evaluate the effectiveness of the antimicrobial nanoparticles containing poly-lysine peptide incorporated organic acid spray treatment in yeast fermentation system.

Methodology:

Materials: Corn meal or corn starch will be obtained from commercial sources. *S. cerevisiae* (Yeast) and commercial strain *Bifidobacterium bifidum* NCFB 1454 is maintained at Center for Food Safety and Microbiology -IFSE, Dept. of Food Science, Univ. of Arkansas, Fayetteville, AR.

Enzymatic Pre-treatment for corn grain to facilitate *Bifidobacterium bifidum* NCFB 1454 fermentation: Corn grain obtained from a commercial source will be milled and passed through a 60 mesh screen. Corn grain will be homogenized by mixing with water (20g: 200ml Corn grain: Water) followed by sonication (Virtishear Tempest, The Virtis Co., NJ) (10min, at temperature 30°C). Amylase (0.4g) will be added to the sonicated homogenate and the pH will be as adjusted to 6.5 (optimum pH of amylase), incubated at 50°C in a shaker (200rpm, 4.5h, Microenviron shaker, Melrose Park, Illinois). The extracts will be separated by centrifugation at 153 x g for 20 minutes and enzyme in the supernatant will be inactivated at 90°C for 10 min. This extract will be freeze dried and kept until use.

Objective 2: Evaluate the effective concentrations of polylysine or *B. bifidum* NCFB 1454 fermented extract or their combinations against potential contaminants in yeast fermentation. *S. cerevisiae* will be cultured in PDB at 30°C aerobically with shaking at 200 rpm. Early stationary phase *S. cerevisiae* cells at 8 h will be harvested from 100 ml of PDB culture medium. The raw material (corn grain / corn starch) will be prepared according the standard procedures used by the fermentation industry for ethanol production.

Objective 3: Synthesize and characterize chitosan nanoparticles containing polylysine peptide dispersed in organic acids and evaluate the effectiveness of the antimicrobial nanoparticles containing poly-lysine peptide in combination with most effective fermented extract. Chitosan-

nanoparticles will be prepared by methods described by Alonso-Sande et al. (2006) with appropriate modifications.

Expected Outcomes:

Microbial contamination is the one of the major problems in the industrial yeast fermentation creating great economic losses to the fermentation industries during processing and requiring control in the initial processing steps of yeast fermentation. Failure to do so ensure an eventual shutdown of the fermenter and loss of production time until the system can be purged of contaminants and re-inoculated with the yeast strains normally used for the fermentation process. Therefore it becomes critical to develop broad spectrum antimicrobial additives to prevent potential bacterial contamination “blooms” prior to irreversible contamination and shut down of the fermenter. The objective of the proposed research will be to evaluate economically feasible biological compounds for their broad spectrum capability to limit and/or inhibit microbial contaminants in yeast fermentations. Because yeast fermentations are used for the production of the majority of ethanol, this study will be an important step to increase the quality of the fermentation process and therefore provide the Arkansas biofuel industry with better interventions for maintaining the quality of the fermentation process. Finally, the ethanol fermentation industry is one of the fastest growing industries with great potential economic benefit in Arkansas for development of biofuel from agricultural byproducts. Successful completion of this project should provide a useful intervention strategy utilizing polymer-nano-composite system incorporating chitosan nanoparticles containing polylysine peptide dispersed in fermented extract biologicals to effectively control contaminants in yeast fermentation systems.

Project Title: Biodiesel Feedstock Development for the Southern Great Plains

Principal Investigators and affiliations:

PI: Michael Stamm (Kansas State University, Agronomy)

Co-PI: Gary Pierzynski (Kansas State University, Agronomy)

End Date: 6/30/2009

Objectives:

1) Evaluate germplasm with high monounsaturated fat content (oleic acid), reduced levels of polyunsaturated fat (linolenic and linoleic acid), and low amounts of saturated fatty acids for general adaptability to the southern Great Plains. 2) Develop adapted winter canola cultivars with superior oil quality for production of a high quality feedstock to produce biodiesel.

Methodology:

Superior oil quality germplasm will be acquired from winter canola breeding programs of public universities. Germplasm will be exchanged between universities according to germplasm use agreements. Some breeding lines may be facultative winter-types and generally not adapted to the southern Great Plains. Therefore, lines will be bred to adapted winter canola cultivars through conventional breeding methods such as backcrossing. Two backcross generations can be

obtained per year in the greenhouse to expedite development. Single and double cross populations will also be initiated. Populations will be grown in isolated field trials for evaluation of agronomic acceptability, winter survival, oil quality, and yield potential. Multiple generations of single plant and bulk plot selecting will continue until a desirable level of uniformity is reached within populations. Yield trials will begin in approximately the F4 or F5 generations. The best performing lines will be tested in the Great Plains Germplasm Evaluation System operated by the K-State and Oklahoma State University joint canola breeding program.

Expected Outcomes:

Specialty canola cultivars will be the primary deliverable from this research project. Specialty canola cultivars will possess high oleic acid content in addition to low-linoleic, low-linolenic acid content, and lower levels of saturated fatty acids. High-oleic cultivars are defined as having approximately 75% oleic acid, with reduced amounts of polyunsaturated fatty acid. Specialty canola cultivars will be available approximately 8 to 10 years following creation of breeding populations. A reputable biofuel industry will likely establish in the region over this same time period. Oil produced from these specialty cultivars will provide a high quality feedstock with improved stability for the production of biodiesel. Superior oil quality will strengthen the value-added farm economy of the southern Great Plains, providing farmers an incentive to grow the cultivars.

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

Principal Investigators and affiliations:

PI: Donghai Wang (Kansas State University, Biological and Agricultural Engineering)

Co-PI: Ronald Madl (Kansas State University, Grain Science & Industry)

Co-PI: Scott Staggenborg (Kansas State University, Agronomy)

Co-PI: Michael Woolverton (Kansas State University, Agricultural Economics)

End Date: 6/30/2009

Objectives:

The goal of this proposed research is to develop comprehensive understanding and utilization of regular sorghum stover and bmr sorghum (sorghum biomass) for ethanol production. The specific objectives are: 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; 3) to increase the ethanol yields by identifying and reducing the effects of potential inhibitors formed during pretreatment of sorghum biomass; and 4) to investigate energy inputs and outputs associated with bioprocessing sorghum biomass.

Methodology:

High yield regular sorghum lines and bmr sorghum will be selected, grown under selected environment conditions. Plant samples will be collected at various stages of crop development

and at maturity from each genotype. All samples will be analyzed for moisture content, cellulose, hemicellulose, lignin, structural protein, acid insoluble residue content, etc. Crystallinity, morphology, and surface area accessible for cellulase binding will be analyzed using XRD, SEM, and confocal laser scanning microscopy. The chemical and physical properties will be linked to the fermentable sugars and ethanol yields. Optimized pretreatment and enzymatic hydrolysis procedures will be developed to increase fermentable sugars yield from sorghum biomass. The compounds derived from the pretreatment and hydrolysis of cellulosic biomass will be determined by HPLC and LC-MS/MS methods. Economic analysis on energy efficiency associated with bioprocessing sorghum biomass will be conducted.

Expected Outcomes:

The main results will be the finding of the impacts of chemical composition, microstructure, physical properties, pretreatment methods, and degradation products on bioprocessing of sorghum biomass for ethanol production. This will lead toward a study of the relationship among the “composition-structure-pretreatment-bioconversion sequence”. The chemical composition and physical properties will be correlated to genotype and production environment. Input/output flows and estimated processing cost will be used to conduct economic analysis. Outcomes will be communicated to the processing industry for use in implementation. The outcomes will include baselines of chemical composition of regular sorghum stover and bmr forage sorghum, and optimized pretreatment and enzymatic hydrolysis procedures for high sugar yields. At least one peer reviewed publications is expected from this research.

Project Title: A multifunctional frequency-response permittivity sensor for biodiesel concentration measurement and impurity detection

Principal Investigators and affiliations:

PI: Naiqian Zhang (Kansas State University, Biological and Agricultural Engineering)

Co-PI: Wenqiao Yuan (Kansas State University, Biological and Agricultural

End Date: 6/30/2009

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 fuel-injection adjustment, and 3) Prove the accuracy, reliability, and durability of the sensors through a well-designed experiment.

Methodology:

The sensor uses the “frequency response” concept to measure the dielectric and conductive behaviors of biodiesel blends and accurately estimate the blend ratio and impurity concentrations from the “frequency response” data. Preliminary tests on biodiesel-diesel and ethanol-gasoline blend ratio measurements have achieved accuracies of 0.88% and 0.70%, respectively.

Expected Outcomes:

1) A portable sensor for quick measurement of blend ratio and impurities concentrations for biodiesel, 2)an embedded sensor for use in diesel engines, 3) peer-reviewed publications.