MidSouth/Southeast BioEnergy Consortium
DE-FG3608GO88036
Final Report

Arkansas State University
University of Arkansas
University of Georgia

November 30, 2014

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EERE: Project Management Center Report Submission for:

DE-FG3608GO88036

Project Title: MidSouth/Southeast BioEnergy Consortium

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Reporting Period: September 1, 2009 to August 31, 2014
Date of Report: November 30, 2014

Executive Summary

GO88036 project was conducted at three universities: Arkansas State University, University of Arkansas and University of Georgia from 2009 to 2012, and University of Arkansas from 2012 to 2014. The funds were used at all three universities to build capacity: 1) infrastructure, such as purchase of laboratory equipment and laboratory set-up; and, 2) agronomic capabilities, including the establishment of field trials and acquisition of harvesting equipment. This infrastructure was critical to ramping bioenergy activities at all three universities. Thermochemical and biochemical conversion were investigated; algal, woody, annual and perennial herbaceous energy crops were established and monitored; educational and outreach events were organized; co-product production and extraction were investigated; and, the nutritional qualities of biorefinery co-products were evaluated. Funding from this project enabled 15 graduate students to submit PhD or MSc level theses; publication of one book and six book chapters; generation of 19 published abstracts; production of three lay press articles; and, dissemination of 44 peer-reviewed articles in good quality scientific journals.

University of Arkansas

Objective 1: To position the Mid-South bioenergy industry to expand from biodiesel and grain to ethanol to commercial production of cellulosic ethanol

Project 1 A.
Biochemical conversion platform of woody feedstocks

PIs: Danielle Julie Carrier and Mathew Pelkki

The overall goal of this research project was to investigate the saccharification of sweetgum (*Liquidambar styraciflua* L.), low density and high-density poplar (*Populus deltoides*), and pine (*Pinus taeda*) into fermentable sugar streams that can be converted to biofuels. We also worked on switchgrass as it presents an interesting hearbaceous model. Woody and herbaceous material used in this study were grown in Arkansas. Information obtained in this project was crucial for enhancing Arkansas' role as a biomass producer in the nascent US bioenergy industry. In an effort to accomplish this goal, pretreatment equipment, namely a fluidized sand bath and a 1 l Parr reactor, and a YSI glucose analyser were purchased with GO88036 project funds.

Poplar:

Poplar, bark and wood of low, non-irrigated, and high, irrigated, specific gravity clones were saccharified in unstirred batch stainless steel reactors (160 °C for 60 min in 1% (v/v) sulfuric acid) and washing the pretreated pellet with 20 volumes of water prior to enzymatic hydrolysis. Overall, the combined xylose and glucose recoveries for low and high specific gravity bark were 18.7 and 24.8 (g of sugar per 100 g of biomass), respectively, and 37.0 and 35.5 (g of sugar per 100 g of biomass) for low and high specific gravity wood, respectively. Total sugar yields of 66 and 60% were calculated for low and high specific gravity wood, while sugar yields of 50 and 73% were obtained for low and high specific gravity bark. The ratio of sugar-derived inhibitory byproducts to potential sugars in wood and bark were 0.34 and 0.64 (g per g), respectively. The low specific gravity clone displayed two advantages: 1) higher glucose recovery; and, 2) the ability to be cultivated under dry land conditions, which could be an important cultivation parameter where rainfall is the sole source of water.

Poplar biomass was pretreated in 0.98% (v/v) sulfuric acid at 140 °C for 40 min. Prior to enzymatic hydrolysis, pretreated biomass was either not washed, or washed with 1 ½, or 3 volumes of water, as compared to biomass. Rinsing the pretreated biomass with 1 ½ or 3 volumes of water resulted in glucose yields that were seven times greater than the non-wash treatment. Pretreatment hydrolyzates, wash waters and enzymatic hydrolysis hydrolyzates were analyzed for carbohydrate, aliphatic acid, aldehyde and phenolic content. An analysis of the wash waters showed the presence of gallic, vanillic, syringic, p-coumaric, ferrulic, trans-cinnamic and salicylic acids at concentrations below 0.07 mg per mL Washed and non-washed enzymatic hydrolyzates showed significant differences in gallic, vanillic, ferrulic, and salicylic acid concentrations, indicating that these compounds could be in part responsible for inhibiting enzymatic hydrolysis. Non-washed and washed enzymatic hydrolysates were fermented to ethanol with self-flocculating SPSC01 and non-flocculating ATCC4126 yeasts. While the biomass washed with 3 volumes of water produced the highest ethanol yields (up to 0.43 g g-1 glucose) and were significantly higher than those from the non-washed sample (≤ 0.28 g g-1 glucose), the ensuing differences between the 3 and 1 ½ wash samples were not significant. The SPSC01 strain generally outperformed the ATCC4126 strain in ethanol fermentation efficiency, in particular when the non-washed hydrolysates were used as feedstock.

Washing steps would be difficult to manage in commercial operations because of the unsustainable water consumption. The effects of formic acid and furfural on Accellerase ®1500 with cellulose powder and dilute acid-pretreated-poplar as substrates were investigated. Using cellulose powder as the substrate for enzymatic hydrolysis with the addition of 5 or 10 mg per mL formic acid, glucose recovery was reduced by 34% and 81%, respectively, in comparison to the control, consisting of cellulose powder and enzyme. The addition of furfural, at 2 or 5 mg per mL, to the enzymatic system reduced glucose recovery by 5% and 9%, respectfully. When 5 mg per mL of formic acid was combined with 5 mg per mL of furfural, glucose recovery in cellulose powder enzymatic system was reduced by 59%. Inhibition of sugar recovery was more pronounced when dilute acid-pretreated-poplar was used as a substrate for enzymatic hydrolysis. At 24 h incubation, recovery reductions were 94%, 97% and 93% in the presence of 5 or 10 mg per mL formic acid or of 5 the mg per mL combination.

Sweetgum:

The possibility of using sweetgum, growing as understory in southern pine dominated forests, as biobased refinery feedstock was investigated. Sweetgum wood and bark were pretreated with 0.98% (v/v) sulfuric acid at 140˚C for 30, 40, 50, 60 or 70 min and at 160˚C for 30, 40, 50 or 60 min. The water insoluble solid was washed with 30 volumes of water and hydrolyzed with Accellerase® 1500 enzyme cocktail. Maximum xylose and glucose yields from the wood were 82 and 86%, respectively. Similarly, the respective maximum yields of xylose and glucose from the bark were 93 and 24%. Acid based pretreatment also produced fermentation inhibitory compounds such as furfural, hydroxymethylfurfural (HMF), formic acid and acetic acid in concentrations ranging from 0.1 to 32.3 g per 100 g of raw dry biomass. Sweetgum bark was more recalcitrant to enzymatic hydrolysis than wood and also produced higher concentrations of formic acid. Sweetgum wood could be a good source of carbohydrates for a biobased refinery, but the removal of bark might be necessary to achieve better yields.

Due to their heterogeneous nature, forest residues may not be used to their full potential as biorefinery feedstocks. This work investigated the effect of mixing biomass species from the hardwood understory on xylose and glucose yields obtained from dilute acid pretreatment and enzymatic hydrolysis. The mixed biomass samples consisted of: 70% sweetgum wood + 30% sweetgum bark, 70% sweetgum wood + 30% oak bark, and 70% sweetgum wood + 30% oak wood. A feedstock of 100% sweetgum wood was used as the control. Samples were pretreated with 0.98% (v/v) sulfuric acid at 160 °C for 20 min, followed by hydrolysis with a cellulase enzyme cocktail. Resulting sugars from hydrolysis of the mixed samples were statistically compared to the sugar yield of the control; experimental and predicted sugar yields for the mixed samples were also compared to each other. Results showed that mixing biomass species did not negatively affect sugar yields. There were positive interactions between sweetgum wood and sweetgum bark during hydrolysis, as indicated by an increase of experimental xylose and glucose yields by 25% and 12%, respectively. However, the mixture with sweetgum bark increased formic acid concentration by 70% when compared to the control. In conclusion, the heterogeneous nature of forest residues would not prevent their use as a feedstock in biochemical biorefineries, as far as sugar yields are concerned; but, inclusion of bark biomass in forest residues could reduce the quality of the sugar stream, which could cause some processing challenges.

Switchgrass:

In order to establish the baseline quantities of key fermentable sugars, switchgrass (*Panicum virgatum* L., var. Alamo) stems and leaves were pretreated with dilute acid and the release of sugars was compared to that reported in the DOE Feedstock Database. Switchgrass was planted in the spring of 2008 and harvested in the spring of 2009. The feedstock was separated into leaves and stems and ground to particle sizes of 0.34 mm and 0.37 mm, respectively. Dilute acid pretreatment of the material, 130 °C in 0.98 % sulfuric acid for 25 min, yielded xylose recoveries of 76 and 61% for leaves and stems, respectively. Recoveries were calculated by dividing the recovered sugar by the values reported in the DOE Feedstock Database. Coupling a 2 h 85 °C hot water pre-soaking step to the 25 min 130 °C in 0.98% sulfuric acid pretreatment increased the xylose recovery by 23 and 5 % for leaves and stems, respectively. These results demonstrate that a pre-soaking step increases the carbohydrate concentration without escalating the severity of the pretreatment. Moreover, the 85 °C pre-soaking water contained the flavonoid, quercetin, which is a documented antioxidant. Pre-soaking water from leaves and stems displayed yields of 0.14 and 0.12 mg per g of dry switchgrass, respectively, of quercetin. Thus, the pre-soaking step not only increases carbohydrate recovery, but provides a slip stream that could be processed into a value-added revenue-generating stream for the biorefinery.

Outside storage as round bales is a likely mode of switchgrass storage; however, little is known of storage effects. The objective of this study was to determine the effects of baled storage method on saccharification, namely the recovery of glucose and xylose, and production of inhibitors after dilute acid pretreatment and enzymatic hydrolysis. Mature switchgrass (*Panicum virgatum* L. cv. Alamo) was harvested in Fayetteville, Arkansas and packaged in large round bales in October 2010. There were two baling times: one, soon after cutting when there was no rainfall and the other after a rainfall event. The bales that did not receive rain were stored either in an open barn or unprotected in the field. Bales made from rained-on switchgrass were only stored unprotected in the field. Samples were taken from the windrows right before baling, and after a maximum 65-day storage period. Field storage increased lignin content in biomass relative to barn storage, but carbohydrate constituents were not affected. Field storage decreased production of hydroxymethylfurfural (HMF) and increased production of furfural relative to barn storage. Results indicate that protected storage conditions for switchgrass biomass in round bales can lead to greater preservation of fermentable sugars and reduced production of the important inhibitor furfural.

Xylose oligomers are of interest to many fields of study and are intermediate reaction products of the hydrolysis of hemicelluloses. Protocols to generate xylose oligomers are reported, but these methods lead to the production of a pool of non-fractionated xylose oligomers with a wide range in degree of polymerization (DP). This work used switchgrass hemicelluloses as feedstock for production of purified xylose oligomers. Switchgrass hemicelluloses were autohydrolyzed at 160 °C. Yields of xylobiose, xylotriose, xylotetraose, xylopentose, and xylohexose were 24, 34, 23, 19, and 38 mg, respectively, per g of hemicelluloses. The crude xylose oligomer mixture was fractionated using centrifugal partition chromatography (CPC) with a butanol:methanol:water (5:1:4, V:V:V) solvent system. Xylose oligomers with a DP from two to six were successfully purified. Purities obtained by CPC separation, as calculated by mass of a given oligomer divided by the total mass of detected oligomers and degradation products and then reported on a percent basis, were 75 ± 7, 89 ± 1, 87 ± 2, 77 ± 6, and 69 ± 12 % for xylobiose, xylotriose, xylotetraose, xylopentose, and xylohexose, respectively. This work illustrates that a CPC-based process could be used to fractionate switchgrass xylose oligomers reference standards, which are currently not commercially available.

<u>Pine:</u>

Pine biomass was pretreated with 1% sulfuric acid at temperatures ranging from 140 to 160 °C. Pretreated biomass was subjected to enzymatic hydrolysis to release glucose. Pretreatment conditions consisting of 160 °C for 30 min were selected based on higher enzymatic hydrolysis achieved at this temperature, as compared to other tested temperature and time combinations. A portion of the treated solids was washed with 30 volumes water. Concentrations of glucose, formic acid, acetic acid, hydroxymethylfurfural (HMF) and furfural were determined in washates. Presence of glucose in hydrolysates and washates indicated that dissolution and degradation of cellulose during pretreatment step occurred, where approximately 10.5% glucan was lost in the pretreatment step.

Washed and unwashed solids were subjected to enzymatic hydrolysis in 100 mM or 10 mM citrate buffer. The final buffer strength in reaction mixture was 50 mM or 5 mM, respectively. The rationale for testing 10 mM conditions was to carry out enzymatic hydrolysis in a buffer that is similar to growth medium for fermenting yeast, as higher salt concentrations are not desirable because of their effect on yeast growth. There was no effect of buffer strength on hydrolysis efficiency when washed biomass solids were used; in both conditions, complete hydrolysis of cellulose present in biomass was achieved. Washing of biomass significantly removed the levels of organic acids, furfural and HMF, and in higher buffer strength, the effect of these inhibitors was alleviated to some extent. Using unwashed biomass, 65 % saccharification efficiency was achieved with 100 mM citrate buffer, while no saccharification was obtained with 10 mM buffer. It may be inferred from these results that reaction mixture buffers assist in countering the pH lowering effect of organic acids produced during pretreatment, maintaining favorable conditions necessary for enzyme integrity and action. At similar buffer strength, 65% saccharification of unwashed biomass was achieved, while complete saccharification of washed biomass was observed; it is possible that remaining levels of furfural and HMF in unwashed biomass could play a significant role at inhibiting the cellulase enzymes. It is important to note that all experiments were conducted at ~1% cellulose substrate loading and enzyme. It is more than likely that at higher cellulose loadings the saccharification efficiencies between buffer strength and biomass washing levels would be even more pronounced.

Funds from this project were critical to building the research capacity. For example, we had the high-pressure liquid chromatography equipment, but did not have the necessary pretreatment apparatus, which is essential to carry out the saccharification studies. Although, we had HPLC equipment, sugar analysis took 60 min per sample, as we ran our samples at flow rates of 0.2 mL per min such that the backpressure in our sugar columns did not build-up. Purchasing the YSI glucose analyzer was critical for our laboratory; we can now quickly analyze a suite of samples and then re-analyze by HPLC the samples that we need to further characterize. With pretreatment and analytical facilities, we were able to collaborate with our sister institution – Arkansas State University. We submitted two pre-proposals to the Biomass Research Development Initiative (BRDI), but were not selected for full proposals. We nonetheless obtained two SunGrant awards, that started in January 2013 and that received addition funding for a January 2015 to 2017. It is clear that without the GO88036 project funds we would not have been competitive for the SunGrant awards.

Under this project, we presented at least 16 posters or orals at scientific conferences, completed one MSc and one PhD, co-edited one book, published two book chapters, and published 11 manuscripts. One additional manuscript on pine saccharification is in preparation for submittal to a peer reviewed journal.

Publications:

<u>Theses</u>

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Lau, Chuan. 2012. Characterization and quantification of monomers, oligomers, and by-products from xylan during biomass pretreatment. PhD Thesis. College of Engineering, University of Arkansas, Fayetteville, AR.

<u>Books</u>

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<u>Book Chapters</u>

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<u>Peer-review Publications</u>

Bunnell K, Lau C, Lay JO, Gidden J and Carrier DJ. (2014). "Production and fractionation of xylose oligomers from switchgrass hemicelluloses using centrifugal partition chromatography." *Journal of Chromatography and Related Technologies* 38 (2015)

Frederick N, Zhang N, Ge X, Xu J, Pelkki M, Martin E and Carrier DJ. (2014). "Poplar (*Populus deltoides* L.): The effect of washing pretreated biomass on enzymatic hydrolysis and fermentation to ethanol." *Sustainable Chemistry and Engineering* 2: 1835–1842.

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Djioleu A, Martin E, Pelkki M and Carrier DJ. (2014). "Mixtures of sweetgum wood with sweetgum bark, oak wood and oak bark: Effects on xylose and glucose yields from dilute acid pretreatment and enzymatic hydrolysis. " *Transactions of* ASABE 57: 1175-1185.

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Djioleu A, Arora A, Martin E, Smith JA, Pelkki M and Carrier DJ. (2012). "Sugar recovery from high and low specific gravity poplar clones post dilute acid pretreatment/enzymatic hydrolysis." *Agricultural and Analytical Bacterial Chemistry* 2:121-131.

Djioleu A, Martin E, Pelkki M and Carrier DJ. (2012). "Xylose and glucose yields from dilute acid pretreatment and enzymatic hydrolysis of sweetgum (*Liquidambar styraciflua* L.)." *Agricultural and Analytical Bacterial Chemistry* 2: 175-186.

Martin E, Cousins S, Talley S, West C, Clausen E and Carrier DJ. (2011)."The effect of pre-soaking coupled to pretreatment on the extraction of hemicellulosic sugars and flavonoids from switchgrass (*Panicum virgatum*, var. Alamo) leaves and stems." *Transactions of ASABE* 54: 1953-1958.

Martin E, Bunnell K , Lau C**,** Pelkki M, Patterson D, Clausen E, Smith J and Carrier DJ. (2011). "Hot water and dilute acid pretreatment of high and low specific gravity *Populus deltoids* clones." *Journal of Industrial Microbiology* 38:355-361.

Project 1 B.
Biochemical conversion platform of herbaceous feedstocks

PIs: Jerry King and Ya-Jane Wang

GO88036 funding was utilized to investigate the following areas, using environmentally-benign approaches and chemicals:

1.) Development of measurement techniques and correlation of solubility and mass transport data on solutes and reaction products in aqueous media. Also included was the development of multiple chromatographic techniques to measure the efficacy of hydrolysis when pretreating several diverse herbaceous feedstocks.

2.) Development of both static and dynamic treatment methods using compressed fluids (carbon dioxide and water) to offer an alternative for the reactive pretreatment of renewable feedstocks such corn stover, switchgrass and rice substrates. The effect of these pretreatment methods was evaluated after aqueous pretreatment conditions as well their subsequent effect on the application of carbohydrase cocktails after pretreatment.

3.) The integration of these hydrothermal-based reactive conditions into a biorefinery concept for the pretreatment of mixed carbohydrate-lipid-protein substrates as well as production of specific chemicals.

Using both our own experimentally determined and literature solubility data, useful correlations were developed for predicting the solubility of solutes in hot compressed water that is used as both a hydrolysis and extraction medium. A continuous flow method was developed and applied to measure the solubility of sugars derived from cellulosic and hemicellulosic feedstocks, and a similar dynamic method used to evaluate the diffusion coefficients of solutes as a function of temperature using a chromatographic band-broadening technique. Several methods were applied to measure the extent of reactive hydrolysis achieved by employing subcritical water, both with and without high-pressure carbonation to accelerate conversion. These included the use of an accelerated solvent extraction module (ASE), a static hydrolysis technique using a modified supercritical fluid extractor to deliver compressed CO_2 to an aqueous slurry of biomass held under pressure, and the development of a continuous flow reactor, with and without the introduction of compressed CO_2 for facilitating hydrolysis with environmentally benign media.

Pretreatment of corn stover, switchgrass and rice substrates is critical in their use for generating monomeric sugars for conversion to transport fuels, such as bioethanol. High pressure reactive pretreatment and hydrolysis of cellulose- and hemicellulose-containing substrates, principally corn stover and switchgrass, were conducted over the temperature range of 150–190 °C utilizing carbon dioxide pressures ranging from 150 to 450 bar. Experiments were designed employing an orthogonal design criteria consisting of the variables of temperature, CO_2 pressure, the time of pretreatment-hydrolysis, and substrate particle size. Initially, high-pressure carbonation using water-biomass mixtures were conducted in a batch reactor and the resultant hydrolyzate mixtures analyzed for sugar content using both SEC and HPLC-RI detection. The resultant hydrolyzates - after either hot water or carbonated water pretreatment - were further hydrolyzed using commercial enzymes to saccharify the remaining oligomeric sugars to xylose and glucose.

These results were than compared to those obtained using dilute mineral acid pretreatment. High pressure carbonated water pretreatment yielded 9–13% less sugars than found in the sulfuric acid-derived hydrolyzate and only 6–10% less sugars upon further treatment of these hydrolyzates with carbohydrase enzymes. Interestingly it was determined that carbonated water pretreated switchgrass hydrolyzates required 33% less enzyme for post pretreatment saccharification relative to that required using dilute sulfuric acid pretreatment. There were no enzyme savings in using carbonated water to pretreat corn stover compared to sulfuric acid pretreatment, the opposite of which was found for switch grass pretreated with carbonated water. However dissolving supercritical carbon dioxide in water providing an environmentally benign pretreatment method for depolymerizing the sugar oligomers present in biomass substrates, as well as facilitating their saccharification without the need of base to neutralize the hydrolyzate mixture prior to application of a carbohydrase enzyme (Depol 692L).

The carbonated water pretreatment was also investigated using a dynamic flow, semi-continuous mode. Both water and compressed CO_2 were delivered by three high-pressure precision syringe pumps, using a tubular vessel held under pressure to reactively pretreat the biomass substrates. Aqueous-based hydrolyses of switchgrass using both neat subcritical water as well as carbonated water were conducted at temperatures between 150 - 190 °C. Using a 3×3 factorial experimental design varying temperature and flow rate, the generation of cellobiose, glucose, xylose, acetic and formic acids, as well as furfural and 5-hydroymethylfurfural (HMF) could be assessed. The composition of untreated Alamo switchgrass obtained from Dr. Chuck West, which was analyzed using NREL procedures by the Microbac Laboratories. Using both hydrolytic approaches, the yield of xylose increased with increasing reaction temperature; 3 to 8.5 fold depending on the water flow rate between 150 C to 190 °C. The maximum xylose could be produced at a temperature of 190 °C with the flow rate of 0.3 ml/min at a system pressure of 1000 psig. Slightly less xylose was produced during the carbonated water hydrolysis compared to hot water hydrolysis at flow rates of 0.3 ml/min and 0.5 ml/min.

Hydrolysis of switchgrass was further conducted at a higher pressure to study the effect of this variable on sugar production. The yield of xylose was slightly decreased when the higher system pressure was applied to the subcritical water hydrolysis. The accumulative concentration (w/w) of glucose and xylose decreased by 10 percent when the system pressure was adjusted to 2000 psig, while the cellobiose yield increased by 20 percent. The xylose yield increased 1.5 fold at this higher pressure for the carbonated water hydrolysis; while the production of cellobiose and glucose increased about 35 and 23 percent, respectively. This demonstrates the benefit of employing a carbonated water hydrolysis in the flow mode at 2000 psig, since the carbonated water acts as a autocatalytic reaction medium.

Utilizing this principle, we focused on optimizing reaction conditions using carbonated water under pressure for the production of carbochemicals from switchgrass, *i.e*, a biorefinery for converting biorenewable resources into value-added chemicals and products. Traditionally, carbochemicals, such as furfural and HMF, are produced using inorganic acids such as H_2SO_4, HCl, and H_3PO_4. Again, a three-syringe pump system was employed to feed water and high pressure CO_2 into the reactor simultaneously. The carbochemicals formed were detected in the aqueous reaction mixtures using a Waters HPLC employing a Bio-Rad Aminex HPX-87H column with a PDA (photo diode array) detector scanning at wavelengths from 210 to 280nm. Oligosaccharides were detected using Dionex HPLC employing Bio-Rad Aminex HPX-87P column using a Shodex RI-101 refractive index detector. The kinetics of the formation and degradation of HMF and furfural were modeled using the solver function in Microsoft Excel. Carbonated water clearly showed catalytic activity resulting in increased yields of HMF and furfural at temperatures of 220, 250 and 280°C. The highest catalytic activity was observed for HMF formation in carbonated water with a nine-fold increase in yield over that using just neat subcritical water.

Similarly with a European partner subcritical water was successfully used as a solvent for the reactive extraction of compounds obtained from hydrolysis vegetable oils and hemicellulose-containing biomass. To demonstrate the versatility of subcritical water medium, hydrolysis was performed on both rice bran oil (lipids) and rice bran (carbohydrates) as model substrates. The results from batch and continuous hydrolysis systems indicated that carbohydrate polymers and triacylglycerols (lipid oils) were efficiently hydrolyzed within 60 minutes. A multi-factorial experimental design was used to optimize both the batch and continuous flow subcritical water mediated hydrolysis of the rice bran and rice bran oil. Such an experimental design protocol allowed an evaluation of the overall efficacy of the subcritical water hydrolysis. Our results showed that both carbohydrate polymers and triacylglycerol moieties were efficiently hydrolyzed within 1 hr. extraction-reaction time, using conditions between 270 – 330 °C at pressures between 100 – 200 bar. This approach allowed not only the assessment of critical fluids for biomass -substrate pretreatment, but the reactivity and downstream transformation of hydrolysis products such as free fatty acids. Using a continuous flow of supercritical carbon dioxide, the free fatty acids were bio-catalytically transformed to ethyl esters under continuous flow in SC-CO_2. All of the above reaction sequences were conducted and optimized to give over 90% of the theoretical yields for the expected hydrolysis products.

This new research capacity established at University of Arkansas has served to build new collaborative relationships. Scholarly outcomes from the project have been communicated through nine presentations, four application notes, five published proceedings, wo thesis, and five peer-reviewed publications.

Publications:

<u>Theses</u>

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Project 1 C.

Thermochemical conversion platform

PI: Samy Sadaka

Arkansas is a rich state in its biorenewable resources. According to Arkansas Energy Office, the available biorenewable resources including woody residues, agricultural wastes, animal manure and solid wastes is about 22.4 million dry ton per year. These vast amounts of bioresources contain energy sufficient to generate 150% of our state electricity needs.

Bioresources are scattered all over the state. Long distance transportation of these types of bulky biomass will affect the cost of electricity generation significantly, which discourage the use of these bioresources. Therefore, three systems were developed with GO88036 project funds.

1) Fluidized bed gasifier

A mobile fluidized bed gasifier / pyrolyzer system (125 kW thermal) was designed, fabricated and tested to gasify or pyrolyze not only woody biomass, but also low-density feedstock. The system is unique in its flexibility to run on the farm, which allows saving transportation cost of low-density biomass. The system was tested and proven to convert various types of feedstock to bioenergy, which can provide cheap energy to our farms all year around. The system can be used by our farmers without complications.

2) Auger gasification system

An externally-heated auger gasifier/pyrolyzer unit was designed, fabricated and tested in the Rice Research and Extension Center at U of A. The unit consists of the following subunits: (a) biomass-feeding unit, (b) air supply unit, (c) auger reactor, heating element and temperature controller unit, (d) char collection unit, (e) tar and water collection unit, (f) gas yield and analysis unit and (g) data acquisition unit. The system was used to gasify and prolyze woody biomass, dried manure, algal biomass and cotton gin waste, which is biomass available in Arkansas.

3) Biodiesel production unit

A biodiesel unit was manufactured to provide firsthand information to Arkansas farmers and youth on biodiesel production from soybean oil and animal fat. The unit had been utilized to make several batches of biodiesel. A standard operating procedure describing how to make biodiesel was established and demonstrated to several county agents as well as faculty members. This unit encouraged other research centers to establish biodiesel units. The unit was used to demonstrate how to make biodiesel to high school students. This unit works as a model to the public to show them how they can create their own jobs in the biofuel industry.

4) Biochar

Biochar is a low cost soil amendment and is a by-product resulting from thermochemical biomass processing for bio-oil production. Biochar has properties, such as high surface area and cation exchange capacity, and can remove heavy metals from aqueous solutions to reduce their bioavailabilty. Moreover, biochar is negatively charged and can adsorb positively charged components; steam activated biochar has increased anion-cation exchange capacity. Arkansas is in the southeast US and 22 % of its 18.0 million acres of timberland is dominated by loblolly pine. Pine biochar was produced with the pyrolysis set-up located at the Rice Research and Extension Center in Stuttgart, in a batch system at 550 °C for 1 h under steady state conditions in the absence of oxygen and activated in a steam environment.

This new research capacity established at University of Arkansas has served to build new collaborative relationships. Scholarly outcomes from the project have been communicated through 21 energy “e-Tips”, two contributions to national web sites, three peer-reviewed publications, one thesis, and eight fact sheets. In addition, more than 50 demonstrations, workshops, poster presentations and farm visits were provided to faculty members, graduate students, high school students, farmers and county extension agents.

Publications:

Theses

Mahmoud Sharara. 2010. MSc. Biodrying-gasification of dairy manure-wheat straw mixture. University of Arkansas.

Peer-review Publications

Sadaka S and Negi S. Improvements of biomass physical and thermochemical characteristics via torrefaction process. 2009. Environmental Progress & Sustainable Energy. Vol. 28 (3): 427–434.

Sharara M, Sadaka S, Costello T and Van Devender K. Influence of Aeration Rate on the Physio-Chemical Characteristics of Biodried Dairy Manure - Wheat Straw Mixture. 2012. Applied Engineering in Agriculture. Vol. 28(3): 407-415.

Davis J, Johnson D, Edgar D, Wardlow G and Sadaka S. NOx Emissions and Performance of a Single-Cylinder Diesel Engine with Emulsified and Non-Emulsified Fuels. 2012. Applied Engineering in Agriculture. Vol. 28(2): 179-186.

Ashworth, A, Sadaka, S, Allen F, Sharara M, Keyser P. 2014. Influence of pyrolysis temperature and production conditions on switchgrass biochar for use as a soil amendment. BioResource. 9: 7622-7635.

Sharara, M, Holeman N, Sadaka S, Costello T. 2014. Pyrolysis kinetics of algal consortia grown using swine manure wastewater. Bioresource Technology. 169: 658-666.

Sharara, M, Sadaka S. 2014. Thermogravimetric analysis of swine manure solids obtained from farrowing, and growing-finishing farms. Journal for Sustainable Bioenergy Systems. 4: 75-86.

Sadaka, S, Sharara M, Ubhi G. 2014. Performance assessment of an allothermal auger gasification system for on farm grain drying. Journal for Sustainable Bioenergy Systems. 4: 19-32.

Sadaka, S, Sharara M, Ashworth A, Keysser P, Allen F, Wright A. 2014. Characterization of biochar from switchgrass carbonization. Energies. 7: 548-567.

Objective 2: To develop economic and environmentally viable systems to produce, harvest and process relevant feedstocks for advanced generation biofuels, and match available feedstocks to specific conversion technologies

Project 2.A:
Algal biomass production

PIs: Tom Costello and Marty Matlock

Feasibility of algal feedstock production in a temperate ecoregion through four seasons was evaluated. GO88036 funding was utilized to design, install, operate and evaluate a test bed algal turf scrubber (ATS) in Springdale, AR, a 50,000-inhabitant town located in Northwest Arkansas. The ATS was operating with Springdale treated wastewater. The pilot scale algal growth bed was monitored for growth during the period from March 2011-December, 2012. After August 2012, there was minimal growth--not enough growth even in the sample collection areas for a dry weight analysis. The lack of algae growth illustrated the important dependency between wastewater qualities and the ATS. The wastewater quality varied and the algae stopped growing.

Nonetheless, this work enabled our team to obtain a NIFA grant to examine the use of algae to mitigate swine waste. An algal production system was constructed at the University of Arkansas Swine Research Center near Savoy, AR (Northwest corner of the state). The system was designed to use swine wastewater as input to four parallel flow ways that are each 5 ft wide X 200 ft long on a 2% slope. The base of the flow way area was constructed using compacted, structural fill with a cap layer of precision-graded crushed limestone (SB-2). A felt pad was used between the crushed limestone and a 45 mil rubber liner. An upper layer of commercial grade indoor-outdoor carpet provided a growth medium to support attachment and growth of periphytic algae in an open system. Wastewater from a swine growout facility is circulated in each flow way to provide water and nutrients for the algae. Water from the ponds can run through the flow way and back to the pond; or, the wastewater can recirculate within a single flow way or plumbed in sequence to simulate 200, 400, 600 or 800 ft long pathways.

Issues related to water quality and its impact on algal growth is currently being explored. Eventually, production experience will allow life cycle analyses of system energy transformations, economic feasibility and impacts on whole farm carbon footprint. These issues will be critical in assessing the feasibility of using livestock wastewater with commercial algae production. The basic system design can be scaled to a much larger size; hence, the operating experience with the system will provide insight into feedstock characteristics and operating/fixed costs for envisioned commercial production.

Publications:

Peer-review Publications

Sandefur, H., M. Matlock, and T. Costello. 2011. Seasonal productivity of a periphytic algal community for biofuel feedstock generation and nutrient treatment. Ecological Engineering, 37(10): 1476-1480.

Project 2.B:

Evaluation of short-rotation woody tree feedstock production

PI: Jamie Shuler

The project consisted greenhouse and field trials to determine the potentials for producing lignocellulosic feedstocks from short-rotation woody species. The species were willow (*Salix* spp.), cottonwood (*Populus* spp.), and American sycamore (*Platanus occidentalis*), all lowland types native to the Mississippi Alluvial Valley, where the field studies took place. The greenhouse trials also included several clones and hybrids of cottonwood. The overall aim was to examine the interaction of environmental factors and tree physiology to develop a better understanding of short-rotation woody tree production. More specifically, the trials were designed to identify species differences in productivity and allocation patterns for different levels of fertility, determine root and shoot carbon and nitrogen dynamics through the rotation, monitor fine-root and leaf tissue production and turnover in relation to resource acquisition, and finally to describe canopy development as it relates to resource use.

Planting occurred in March 2009 and consisted of approximately 5000 seedlings/cuttings. In 2009-2011, measurements were done on tree growth, foliar nutrient concentrations, fine root densities, soil carbon content, weed control and herbicide tolerance in black willow. Cottonwood and sycamore responded more to fertilization than did black willow as measured by height (Figure 1). Faster growth of sycamore corresponded to greater uptake of nitrogen and other elements than in the other tree species. The low native fertility of soils used in these trials indicated that fertilization would be recommended for rapid biomass production in short-rotation systems.

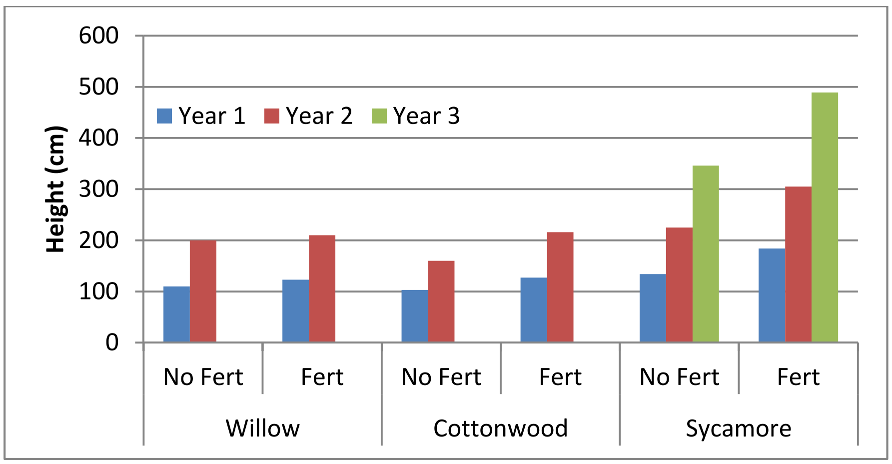


Figure 1. Height development for cottonwood, black willow and sycamore grown with or ithout fertilization at the Pine Tree location.

Weed control trials indicated that weeds interfered with black willow growth (height) during the establishment year (Figure 2). The herbicide Oust was most effective in reducing the effect of weeds on willow growth, but incomplete weed control still resulted in a reduction in willow growth. The untreated control and all weed control treatments resulted in lower tree survival rates than the weed-free check.

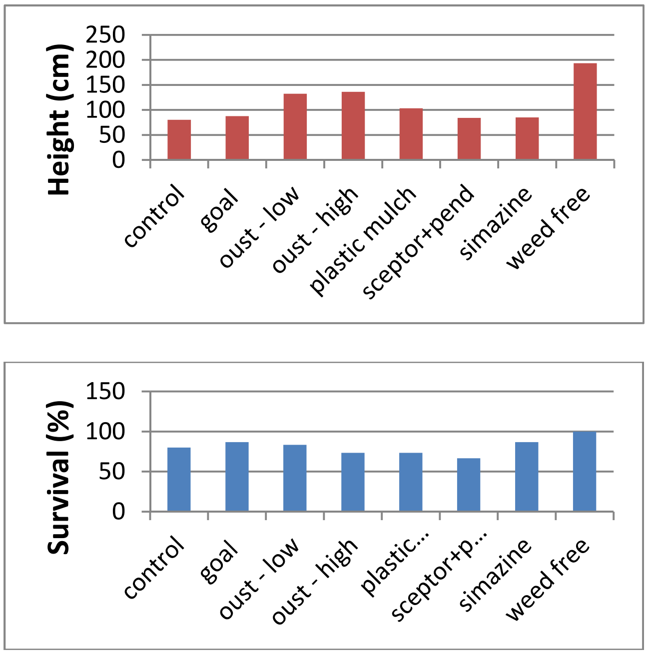


Figure 2. Assessment of various weed control treatments and pre-emergent herbicides used to establish black willow cuttings on a clayey soil.

Publications:

Published Proceedings

Schuler, J.L. 2011. Comparing second year growth of American sycamore, black willow, and eastern cottonwood with and without fertilization. 16th Biennial Southern Silvicultural Research Conference. Feb. 15-17, 2011, Charleston, SC. Extended Abstract.

Schuler, J.L. 2011. Initial results of a biomass production system using black willow, eastern cottonwood and American sycamore grown with and without fertilization. 16th Biennial Southern Silvicultural Research Conference. Feb. 15-17, 2011, Charleston, SC.

Project 2.C:
Herbaceous feedstock production and environmental sustainability

PIs: Bert Bluhm, Nilda Burgos, Charles West, Rick Oliver Dirk Philipp, Michael Popp Dharmendra Saraswat and David TeBeest

a) Subproject Sweet sorghum weed control and productivity: (PI Nilda Burgos)

1) Screening of herbicides for weed control in sweet sorghum at the Southeast Research and Extension Center (SEREC), Rohwer, Arkansas.

Herbicides or herbicide programs that provided good weed control and high biomass yield include (Table 1):

a. Saflufenacil
b. Pyroxasulfone
c. Sulfentrazone + S-metolachlor
d. Atrazine + S-metolachlor followed by dicamba
e. Mesotrione + S-metolachlor followed by dicamba

2) Efficacy and crop safety of herbicides, Northwest Arkansas Research and Extension Center, Fayetteville.

Tests were conducted with preplant and preemergence treatments at different rates of mesotrione, S-metolachlor, saflufenacil and sulfentrazone. The treatments resulting in highest biomass production included: mesotrione (0.21 kg /ha) + S-metolachlor (1.39 kg/ha); S-metolachlor alone (1.39 kg/ha); saflufenacil (0.075 – 0.15 kg/ha) (data not shown).

3) Preplant herbicide option and planting depth, Northwest Arkansas Research and Extension Center, Fayetteville.

Spartan Charge® + glyphosate is an excellent weed control option for sweet sorghum production either preplant or at planting burndown treatment regardless of planting depth, 0.5 in or 1.5 in (Table 2). This treatment produced about 90 to 120 Mg/ha fresh biomass. Spartan Charge is a premix of sulfentrazone and carfentrazone; the former provides residual weed control.

4) Planting date, variety, and herbicide effects on biomass production, SEREC, 2009 – 2011.

Sweet sorghum biomass production fluctuated greatly across years. In 2009, the highest yield (>100 Mg/ha) was obtained when planted in the first week of June, averaged across varieties (Dale, M-81, Topper), either with S-metolachlor alone or with mesotrione + S-metolachlor (Table 3). There was no consistent advantage of the tank mixture over S-metolachlor alone with respect to biomass yield, but the tank mixture generally resulted in more consistent overall weed control than S-metolachlor alone (data not shown). Biomass production in 2010 was generally lower than that in 2009. The tank mixture resulted in about 50 Mg/ha yield with Topper when planted in late April to the first week of June. Planting earlier or later than this window, using the tank mixture of S-metolachlor and mesotrione, resulted in lesser yield. Dale produced the lowest yield of the three varieties tested when planted in late June while M-81 produced the lowest yield when planted early (mid-April). In 2011, three late planting dates were tested, which showed M-81 to the highest yielder (91 Mg/ha) when planted in the 3rd week of June and the second week of July (78 Mg/ha), but no difference between varieties (62-67 Mg/ha) when planted in the 3rd week of July. Overall, a wide range of average yields, 22 – 74 Mg/ha, could be obtained when planting in April and 26 – 67 Mg/ha when planting in late June to the 3rd week of July.

5) Effect of maturation period and irrigation on sugar content, Northwest Arkansas Research and Extension Center, Fayetteville.

Juice from sweet sorghum grown under irrigation contained higher total sugar concentration than that of sweet sorghum produced without irrigation (Figure 1). It is also better to harvest sweet sorghum biomass at the hard dough stage (114 d after emergence for Dale in Fayetteville, AR) (Figure 2).

6) Effect of planting date and cultivar on sugar concentration in sweet sorghum juice, SEREC. Sugar analysis was conducted for the 2009 planting season with three varieties of different maturation dates. In general, sugar concentration tended to increase from late April to early June planting, then reverted to the April levels in mid-June planting. When planted on April 22, Dale headed 50% at 100 days after planting (DAP), M81-E and Topper at 121 DAP. Days to heading progressively shortened with later plantings, with Dale heading at 80 DAP when planted in mid-June. Averaged across planting dates and herbicide treatments, Topper had the highest sucrose concentration. The optimum harvest timings for the varieties tested were: Dale = 50% heading; M81-E = 20 days after 50% heading; and Topper = 10-20 days after 50% heading.

Table 1. Response of sweet sorghum to herbicide treatments, Southeast Research and Extension Center, Rohwer, Arkansas, 2011. PRE = preemergence; POST = postemergence.

Treatment description	Product rate (oz/ac)	Timing	Plant response to herbicide treatment			
			Stand count	Plant injury (%)	Plants/ha	Biomass (Mg/ha)
Untreated	n/a	n/a	31	0	155556	72
Callisto 4 EC (mesotrione)	6	PRE	29	0	180556	98
Callisto 4 EC (mesotrione)	12	PRE	21	14	143056	91
Dual Magnum 7.62 EC (*S*-metolachlor)	20.8	PRE	27	0	150000	87
Warrant 3 EC (acetochlor, encapsulated)	42	PRE	24	0	166667	90
Warrant 3 EC (acetochlor, encapsulated) Warrant 3 EC (acetochlor, encapsulated)	42 42	PRE fb POST	28	0	158333	89
Zidua (pyroxasulfone)	2	PRE	12	43	155556	105
Dual Magnum (7.62 lb ai/gal) fb Paramount 75 DF (quinclorac)	20.8 8	PRE fb POST	23	0	169444	94
Atrazine 4L	32	PRE	27	0	145834	86
Spartan 4F (sulfentrazone)	2	PRE	26	0	161111	92
Spartan 4F (sulfentrazone)	4	PRE	22	10	123611	84
Sharpen (saflufenacil, 2.85 lb ai/gal)	3	PRE	26	0	168056	118
Sharpen (saflufenacil, 2.85 lb ai/gal)	6	PRE	21	0	140278	89
Callisto + Dual Magnum	6 + 20.8	PRE	27	0	163889	91
Callisto + Dual Magnum	12 + 20.8	PRE	20	26	158333	91
Atrazine + Dual Magnum	32 + 20.8	PRE	22	0	168056	95
Spartan + Dual Magnum	2 + 20.8	PRE	21	6	166667	101
Spartan + Dual Magnum	4 + 20.8	PRE	19	13	172222	102
Dual Magnum fb Peak 57 WDG (prosulfuron)	20.8 0.75	PRE fb POST	25	0	184722	96
Callisto + Dual Magnum fb Clarity (dicamba)	6 + 20.8 8	PRE fb POST	26	0	173611	93
Callisto + Dual Magnum fb Clarity 4L (dicamba)	12 + 20.8 8	PRE fb POST	21	20	162500	99
Atrazine + Dual Magnum fb Clarity (dicamba)	32 + 20.8 8	PRE fb POST	24	0	170833	101
LSD $_{0.05}$			7	13	29392	20

Table 2. Sweet sorghum 'Dale' response to Spartan® Charge application, Arkansas Agricultural Research and Extension Center, Fayetteville, 2010.

Depth	Herbicide[a]			Stand[b]	Flower[c]	Plants harvested[d]		Height[e]	Injury[f]	
	Timing	Treatments	Rate			Number	Weight		21 DAP	42 DAP
in	--	---	kg oz/ha	no./m	days	no./ha	mt/ha	cm	-------- % -----------	
0.5	--	Nontreated	--	2	97	11	1	91	0	0
	PPL	Spartan® Charge	0.076	5	97	13	3	162	85	40
		Spartan® Charge + glyphosate	0.076 + 0.56	14	82	11	107	253	0	0
	PLT	Spartan® Charge	0.076	2	97	18	4	174	80	90
		Spartan® Charge + glyphosate	0.076 + 0.56	14	89	85	87	238	40	33
1.5	--	Nontreated	--	2	97	11	1	91	0	0
	PPL	Spartan® Charge	0.076	2	98	0	0	91	95	0
		Spartan® Charge + glyphosate	0.076 + 0.56	16	78	85	94	238	18	0
	PLT	Spartan® Charge	0.076	2	92	15	2	104	78	90
		Spartan® Charge + glyphosate	0.076 + 0.56	12	85	107	119	259	37	17
$LSD_{0.05}$[g]		Depth (A)		NS	2	NS	NS	NS	NS	NS
		Timing (B)		2	2	15	NS	NS	NS	16
		Herbicide (C)		2	3	27	43	61	23	16
		B x C (1)		NS	4	NS	NS	NS	NS	30
		B x C (2)		NS	4	NS	NS	NS	NS	21

[a]PPL (Preplant) = 7 d before planting; PLT = at planting; + = tank mixed; Spartan® Charge = carfentrazone + sulfentrazone
[b]Stand count from 1-m row, 21 DAP
[c]Days from planting to 50% flowering
[d]Plants sampled from 2-m row; total weight of above-ground plants sampled; number = no./ha x1000.
[e]Average of 5 plants
[f]Injury at 21 d after planting (DAP) = stand reduction; 42 DAP = stunting, leaf necrosis
[g]LSD values (A), (B) and (C) may be used to compare the main effects of depth, timing or herbicide; B x C (1) to compare application timings at the same or different levels of herbicide treatment; B x C (2) to compare herbicide treatments at the same level of application timing; other interaction effects were NS.

Table 3. Response of the three sweet sorghum cultivars on different dates of planting and herbicide treatments within years, Southeast Research and Extension Center, Rohwer, AR., 2012.

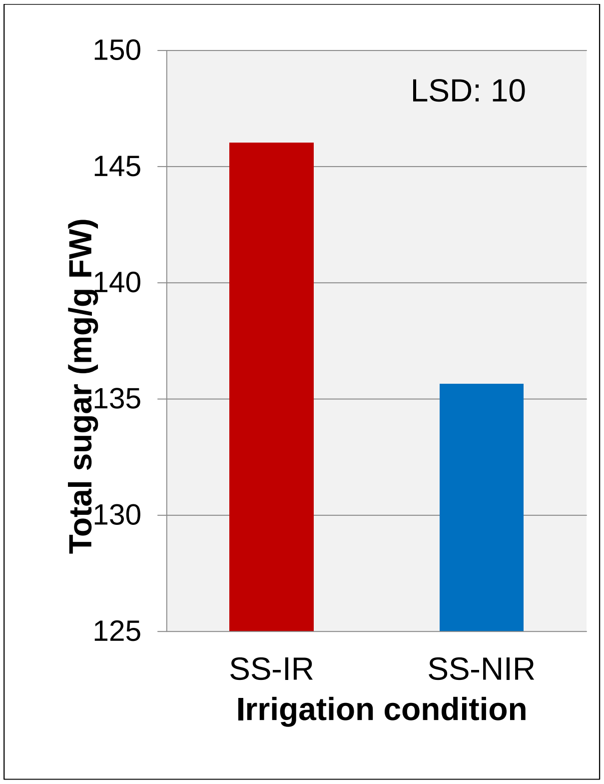
		2009[c]				2010[c]				2011[c]					
Planting	Weed	Injury 56 DAP			Biomass	Injury	Biomass (mt/ha)			Injury 56 DAP			Fresh biomass (mt/ha)		
date[a]	control[b]	Dale	M-81	Topper	(mt/ha)	56 DAP	Dale	M-81	Topper	Dale	M-81	Topper	Dale	M-81	Topper
PD1	Weedy check	--	--	--	57.0	--	9.1	11.3	9.0	--	--	--	--	--	--
	Dual magnum	0	0	0	72.7	0	34.8	35.0	39.7	--	--	--	--	--	--
	Dual + Callisto	0	0	0	73.7	0	42.3	22.0	31.0	--	--	--	--	--	--
PD2	Weedy check	--	--	--	53.0	--	23.7	7.5	5.3	--	--	--	--	--	--
	Dual magnum	0	0	8	93.6	0	45.0	46.5	65.7	--	--	--	--	--	--
	Dual + Callisto	0	5	9	77.9	0	62.2	54.3	50.3	--	--	--	--	--	--
PD3	Weedy check	--	--	--	57.8	--	5.5	0	9.5	--	--	--	--	--	--
	Dual magnum	5	0	12	115.0	0	25.0	24.2	22.7	--	--	--	--	--	--
	Dual + Callisto	12	5	12	138.8	3	24.2	32.7	52.0	--	--	--	--	--	--
PD4	Weedy check	--	--	--	50.6	--	18.0	26.3	37.8	--	--	--			
	Dual magnum	0	0	0	65.0	0	19.6	34.5	54.4	0	0	0			
	Dual + Callisto	25	0	5	94.1	0	27.8	49.0	52.2	0	0	0	66.2	91.0	77.0
PD5	Weedy check	--	--	--	--	--	8.0	4.4	13.7	--	--	--			
	Dual magnum	32	25	22	--	0	50.0	37.8	47.0	5	0	5			
	Dual + Callisto	42	32	38	--	0	57.5	47.0	48.0	8	5	12	67.0	78.0	72.0
PD6	Weedy check	--	--	--	--	--	2.4	6.8	24.0	--	--	--			
	Dual magnum	0	8	6	--	0	24.4	40.7	35.2	0	0	0			
	Dual + Callisto	12	12	12	--	0	26.3	45.8	39.0	5	5	0	66.6	63.4	62.1
$LSD_{0.05}$	Plant date (A)												A(9.0) x B(8.2)		
	Cultivar (B)				A(9)	A(2)									
	Herbicide (C)	A(6) x B(6) x C(6)			x C(28)	x C(2)	A(12.3) x B(12.3) x C(12.6)			A(2) x B(2) x C(2)					

[a]Date of planting in 2009: PD1 on 22 April, PD2 on 20 May, PD3 on 03 June, PD4 on 17 June, PD5 on 07 July, and PD6 on 17 July; 2010: PD1 was planted on 15 April, PD2 on 29 April, PD3 on 13 May, PD4 on 26 May, PD5 on 09 June, and PD6 on 24 June; 2011: PD4 was planted on 20 June, PD5 on 11 July, and PD6 on 18 July.

[b]Dual magnum (S-metolachlor) at 1.12 kg ai/ha and Callisto (mesotrione) at 0.21 kg ai/ha were applied 0 – 1 DAP.

[c]DAP = d after planting; above ground fresh biomass at 21 d after 50% heading.

[f]LSD values A x B, (A) to compare dates of planting at the same or different cultivar, (B) to compare cultivar means at the same date of planting; A x C, (A) to compare planting dates at the same or different herbicide treatment, (C) to compare herbicide treatments at the same planting date; A x B x C, (A) to compare planting dates at the same combination of cultivar and herbicide treatment, (B) to compare cultivars at the same planting date and herbicide treatment, (C) to compare herbicide treatments at the same combination of planting date and cultivar.



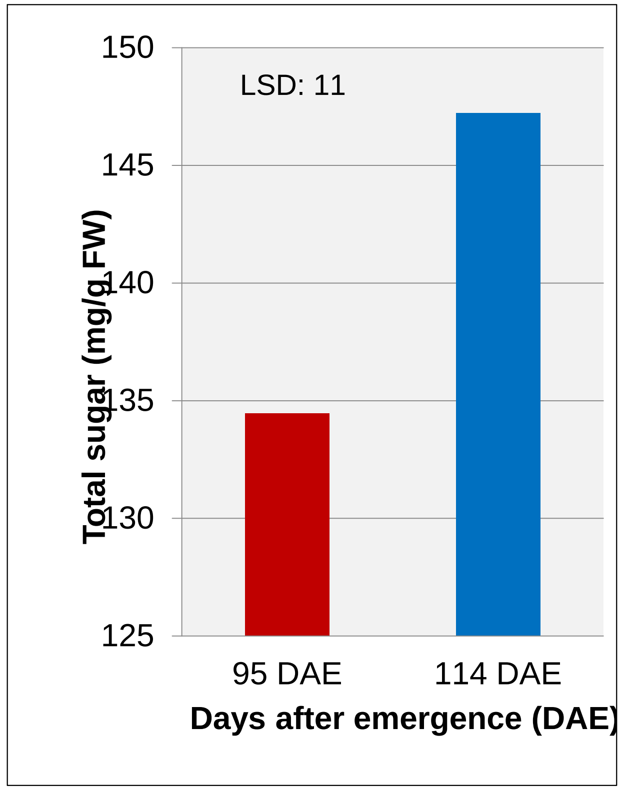


Figure 1. Effect of irrigation on total sugar.

Figure 2. Effect of plant age on total sugar.

Publications:

<u>Published Proceedings</u>

Alcober, E.A., N.R. Burgos, V.K. Shivrain, D. Motes, T.M. Tseng, and L.E. Estorninos. 2009. Residual effects of herbicides in summer crops to spinach and canola. Abstr. Arkansas Crop Protection Assoc. Res. Conf. 13:8.

Burgos, N.R., V.K. Shivrain, E.A. Alcober, D. Motes, S. Eaton, T.M. Tseng, L. Martin. 2009. Canola response to residual effects of summer-applied herbicides. WSSA Abstr. Vol. 50. Access at https://srm.conference-services.net/reports/.

Sapkota, P., N.R. Burgos, L. E. Estorninos, Jr., E.A. L. Alcober, and T.M. Tseng. 2009. Tolerance of sweet sorghum to metolachlor and mesotrione herbicides in non-irrigated conditions. Abstr. Arkansas Crop Prot. Asso. Res. Conf. 13:12.

Tseng, T.M., N.R. Burgos, L.E. Estorninos, and E.A.L. Alcober. 2010. Efficacy and crop safety of preplant and preemergence herbicides for sweet sorghum. Abstr. Arkansas Crop Prot. Asso. Res. Conf. 14:9. Accessible at http://acpanews.com/conference/abstracts/

<u>*b) Subproject: Switchgrass weed control, disease observations, and response to poultry litter; (PIs Charles West, Bert Bluhm, David TeBeest, Rick Oliver, Michael Popp)*</u>

Switchgrass was no-till seeded into sod-killed, old pasture in spring 2009, and 18 herbicide treatments were applied to test for effective weed control and switchgrass stand establishment. By fall of 2009, ground cover of switchgrass ranged from 18-48%, with 4 treatments attaining >40% cover, which would be considered rapid and full establishment. By the end of 2010, stand cover ranged from 74-94%, and dry biomass yields from 2.5-3.7 tons/acre. The treatment giving the combination of highest yield and ground cover was with the herbicide Certainty (sulfosulfuron), while the treatments with lowest cover and yield were the untreated check and Facet pre-emergence. By the end of 2011, there was very little difference among the treatments for cover and yield. Greenhouse trials were conducted simultaneously, which showed that switchgrass must attain at least the 6-leaf stage to avoid crop injury by post-emergence herbicides used to control johnsongrass. Pursuit+Induce was the most effective herbicide at controlling johnsongrass.

A rust pathogen of switchgrass was defined based on a range of morphological and molecular parameters. This was the first known report of rust on switchgrass in Arkansas, and highlights the need for resistance and management strategies to be developed against this potentially devastating disease.

Poultry litter applications of 0, 1.2, and 2.4 tons/acre generally had slight or not significant effects on switchgrass biomass yields, but significantly increased biomass yield of a daylength-sensitive hybrid sorghum. The sorghum took up and removed substantially more N, P, and K nutrients than did switchgrass. In conclusion, switchgrass grown for biomass would not be an effective crop on which to apply poultry litter as a means of disposing and removing excess nutrients such as P, whereas a high biomass-producing sorghum hybrid would be a more appropriate crop.

Publication:

Peer-review Publications

Li, Y.H. and D.O. TeBeest. 2009. Temporal and spatial development of sorghum anthracnose in Arkansas. Plant Disease 93:287.

Hirsch, R.L., D.O. Tebeest, C.P. West, B.H. Bluhm. 2010. First report of rust caused by *Puccinia emaculata* on switchgrass in Arkansas. Plant Disease 94:381.

Published Proceedings

Ashworth, A.J., C.P. West, and K.R. Brye. 2009. Seasonal trends in biomass accumulation and nutrient removal of switchgrass. In Annual meetings abstracts [CD-ROM]. ASA, CSSA, and SSSA, Madison, WI.

Slaton, N.A., R.E. DeLong, R.K. Bacon, and J. Kelly. 2010. Canola response to nitrogen, sulfur, phosphorus, and potassium fertilization in Arkansas. p.30-35. In N.A. Slaton (ed.) Wayne E. Sabbe Arkansas Soil Fertility Studies – 2009. Univ. Arkansas Agric. Exp. Stn. Res. Ser. 578.

West, C.P., B.C. Grigg, C.A. Guerber, R. Farris, and K.R. Brye. 2011. Poultry litter effects on switchgrass and sorghum biomass yield and macronutrient removal. In Annual meetings abstracts [CD-ROM]. ASA, CSSA, and SSSA, Madison, WI.

c) Subproject: Vetch nitrogen benefit; (*PI Dirk Philipp)*

Winter-annual legumes were no-till planted into bermudagrass in the fall and compared to bermudagrass without legumes for growth and nitrogen uptake during the subsequent spring and summer. Hairy vetch demonstrated the greatest ability to economically replace nitrogen fertilizer in a subsequent bermudagrass crop in 3 sequential years. This demonstrates the ability of hairy vetch, a winter annual legume, to be interseeded into existing stands of perennial warm-season grasses to supply low-cost nitrogen in a biomass production system.

d) Subproject: Development of a watershed-scale nutrient balance model using SWAT; (PI Dharmendra Saraswat)

This series of studies evaluated the potential for the SWAT model to develop a spatial analysis of Arkansas to delineate where switchgrass and hybrid miscanthus could be produced, and simulate their impacts on water flow and quality, and soil erosion. A thorough collection of spatially referenced digital data describing elevation, land use/land cover, soil type and attributes, stream network location, weather data, stream discharge, water quality/point pollution sources and crop management practices data was made for the L'Anguille watershed in eastern Arkansas. A total of 1048 hydrologic response units were created. Fifty-six percent of the watershed land area was determined to be appropriate for switchgrass, suboptimal for the principal row crops of east Arkansas (soybean, cotton, rice, corn), and lacking in soil erosion risk. A detailed map of those soil sites was produced. Simulation of Alamo switchgrass on marginal land resulted in a 2% decrease in sediment, 1% decrease in nitrate-nitrogen, and 27% decrease in total phosphorus loading at the watershed outlet. However, when Alamo switchgrass was simulated on non-marginal cropped land, it resulted in 1% decrease in sediment, 17% in nitrate-nitrogen, and 23% in total phosphorus loading.

Publications:

Published Proceedings

Singh, G., D. Saraswat, and N. Pai. 2012. Water quality impacts of biofuel crop production on marginal lands. July 31, 2012, ASABE Annual International Meeting, Dallas.

Objective 3: Conduct educational programs to deliver information on feedstock production, harvesting, and processing with farm and industry audiences

Project 3.A:
Conversion technology education

PIs: Don Johnson, Samy Sadaka, Don Edgar

This project took a multifaceted approach to developing and delivering educational materials about bioenergy and its utilization in farm engines. We acquired and developed digital video resources related to engine performance and emissions of biofueled engines. Portable engine-dynamometer-emissions measurement unit was developed for demonstrating comparative engine performance (torque and power), fuel efficiency (kg/kW-hr) and exhaust emissions (ppm or kg/kW-hr) for bio-fueled engines. We obtained a two-wheeled trailer for transport of demonstration unit. We obtained compression-ignition and spark-ignition engines for demonstration unit. Each demonstration engine was equipped with auxiliary fuel tanks and weigh scales for determining mass fuel consumption. We modified exhaust systems as necessary to facilitate emissions measurement. Appropriate safety guards and safety equipment (hazardous materials spill kit, fire extinguisher, etc.) were developed and installed. We developed appropriate signage for portable demonstration unit to include Division and project/sponsor logos. We developed an operator's manual for the demonstration unit to facilitate use by trained individuals.

We completed the project web site as an on-line repository of instructional materials, research articles and reports, and other project materials. An outline and identified key resource was developled for instructional materials targeting Grades 10-14 and adults. We conducted educational programs for county agents, schools, community colleges, civic, consumer, farm, and industry audiences on biofuel production and utilization. We completed basic educational materials for high school and adult audiences in biofueled engine education. An auger gasification system was developed and tested to convert woody biomass and poultry litter to usable heat that can be used for heating poultry houses. Educational materials were developed to educate public about manure to energy utilization.

We continued educational programs with adult, public school and 4-H groups. Project methods and results were disseminated through conference presentations and journal manuscripts. Three educational on-line short courses were developed and added to the Extension website. These courses focused on biodiesel production, biomass gasification and energy conservation and were available to the public.

We examined the use of alternative energy for assisting in pumping water for rice irrigation, as AR is the US state with the highest rice production. In rice flooding production systems, conventional tail water recovery systems utilize a large storage and canals to return tail water back to the field for reuse in flood and furrow irrigation systems. The implementation of a tail water recovery system can improve irrigation efficiency from 50-60% to 70-90%. According to the 2013 US Census, about 76% of farmland is surface irrigated, either flood or furrow. Half of the pumps in Arkansas are diesel, with the other half being electric with a seasonal average of about 1000 hours per year in the 20 to 200 hp range. Therefore there is considerable amounts of fossil fuels that are consumed due to poor irrigation efficiencies in the region. Improving irrigation efficiencies in the region are critical to reduce unnecessary energy demand and reduce greenhouse gas emissions for irrigation. This project studied the feasibility of improving irrigation by using a variable-flow solar powered tail water recovery system. The concept is feasible and a prototype is being constructed as a result of this project. This approach takes advantage of industrial automation and reduced solar panel costs and technology advancement to develop a new approach to improving irrigation efficiency. A new low-cost pump was developed, intake and support structure, solar array, and controls were designed. The project designed a new kind of tail water pump that can operate autonomously and is much more efficient than current practices. It also has potential to improve profitability of surface irrigated farmers while reducing groundwater overdrafts. It has a high potential for adoption because land that can be used to grow crops will not be taken out of production. The prototype is under construction and will be tested in 2015 cropping season.

Publications:

Peer-review Publications

Hudson, G.T., Sallee, C.W., Johnson, D.M., Wardlow, G.W., Edgar, D.W., and Davis, J.A. 2010. A mobile unit for demonstrating performance, efficiency, and emissions of biofueled engines. Applied Engineering in Agriculture, 26: 521-525.

Sallee, C.W., J.A. Davis, D.M. Johnson, D.W. Edgar, and G.W. Wardlow. 2010. Effectiveness of a biodiesel education program for secondary students. Applied Engineering in Agriculture 26:937-941.

Hunt, C.L., Johnson, D.M., & Edgar, D.W. (2013). NOX emissions and performance of a compact diesel tractor fueled with emulsified and non-emulsified biodiesel. Journal of Agricultural Systems, Technology, and Management, 24, 12-22.

Johnson, D.M., D.W. Edgar, L.D. Edgar, M.L. Pate, and R.W. Steffen. Biodiesel: Awareness, use, and perceptions of students at four U.S. universities. NACTA Journal 59.1: 54-62 (2015)

Published Proceedings

Hunt, C.L., D.M. Johnson, D.W. Edgar, and G.W. Wardlow. 2012. Performance and emissions of a compact diesel tractor fueled with emulsified biodiesel. Annual International Meeting of the American Society of Agricultural and Biological Engineers. Dallas, TX.

Kulkarni, S., D.M. Johnson, and J.A. Davis. 2011. Irrigation power unit performance, efficiency, and NOX emissions with petroleum diesel, biodiesel, and biodiesel blends. Annual International Meeting of the American Society of Agricultural and Biological Engineers, Louisville, KY. (ASABE Paper #1110969).

Davis, J.A., D.M. Johnson, D.W. Edgar, G.W. Wardlow, and S. Sadaka. 2011. NOx emissions and performance of a single-cylinder diesel engine fueled with emulsified biodiesel blends. Annual International Meeting of the American Society of Agricultural and Biological Engineers, Louisville, KY. (ASABE Paper #1111084).

Johnson, D.M., D.W. Edgar, L.E. Edgar, and M.L. Pate. 2012. Awareness, use and perceptions of biodiesel: A comparison of agriculture and non-agriculture majors. Annual Conference National Association of Colleges and Teachers of Agriculture (NACTA). Abstract published in the NACTA Journal 56 Supplement 1:67.

Arkansas State University

Objective 1:
To develop economic and environmental viable systems to produce, harvest and process relevant feedstocks for biodiesel and ethanol operations, matching feedstock availability to specific conversion technologies.

Project 1.A:
Soil and water sustainability in dedicated energy crop production

PIs: Steven Green and Jennifer Bouldin

Bioenergy production is receiving unprecedented attention due to society's need to reduce dependence on fossil fuels, increase use of renewable energy, and mitigate climate change. The overall goal of this research project was to evaluate alternative bioenergy cropping systems to determine their effect on soil and water quality and production potential in the Delta Region of Arkansas in order to develop sustainable cropping systems. A major emphasis was placed on cellulosic biomass crops, though other alternative crops were also evaluated on a minor scale.

In an effort to accomplish this goal, an 8-acre field at the Arkansas State University Farm Complex was precision leveled and converted into a biomass crop research site. Five crop treatments (switchgrass, eastern gamagrass, switchgrass/big bluestem mix, high biomass sorghum, and high biomass sorghum rotated with soybean) were established, each with four nitrogen source treatments (urea fertilizer, poultry litter, municipal biosolids, no nitrogen control), replicated four times in a randomized complete block arrangement. This resulted in 80 experimental units. The plots are 24 ft wide by 90 ft long; these large plots enable us to install additional treatment variations within the plots in order to answer additional research questions in the future. Specific objectives included determining soil quality impacts of bioenergy crop management, nitrogen use efficiency of bioenergy crop management, determining yield potentials representative for the region, and determining suitability of alternative energy crops for growth in the region.

Over the course of this project, we collected data on the impact of alternative nitrogen sources on earthworm preference and water quality of leachate from a column study. We evaluated soil quality parameters, including soil carbon, aggregate stability, organic matter, active soil carbon, and soil enzyme activity, under the various biomass cropping system treatments. We determined nitrogen-use efficiency effects from different biomass cropping system treatments as well as effects of cropping system on biomass yields. We collected preliminary data for a camelina research program to evaluate this bioenergy oil seed crop as a potential winter rotational crop for the region. Current variety trials for camelina include 16 entries, which will increase with new funding pending from USDA-NIFA.

The research capacity built with funds from this project have been showcased to other entities in the bioenergy sector and have resulted in collaborations and interest in placing trials at our location. We leveraged resources to be able to conduct variety trials for rapeseed and sweet sorghum, region adaptability studies for energy beets, and collaborations have been established with bioenergy industry practitioners and promoters. This list includes Biodimensions, Delta Biorenewables (sweet sorghum and rapeseed variety trials), Betaseed (energy beet trials), Montana State University (camelina), MFA Oil Biomass (miscanthus), USDA-NRCS Plant Materials Center, Booneville, AR (switchgrass and eastern gamagrass), and Consulting (rural development). Additionally, our increased research capacity has helped us to secure other competitive funding. We were recently awarded a USDA-NIFA, Capacity Building Grant for Non Land Grant Colleges of Agriculture with a focus on camelina production in the AR Delta region. Preliminary data gathered under the GO88036 project was instrumental in securing the USDA-NIFA award.

Under this project, we have given seventeen presentations at scientific conferences, completed four M.S. graduate theses, published two book chapters, and published one manuscript in a scientific journal. Four additional manuscripts are in preparation for submittal to peer reviewed journals. Over the course of this project, five graduate students were trained, four of whom completed theses; four undergraduate students were trained, two of whom have gone on to pursue graduate education in agricultural sciences at land grant universities with assistantships. We have also produced fact sheets on biomass-grass species in our research trials and posted them to our outreach website.

Through this project, we have been able to secure needed research equipment, establish a long-term bioenergy crop management study, collect necessary data for designing more sustainable bioenergy cropping systems, establish critical collaborations, enhance our visibility in the bioenergy crop management research area, produce outreach materials on biomass crops, train graduate and undergraduate students, and produce scientific publications.

Publications:

<u>MS Theses</u>

Acosta Gamboa, Lucía, 2012. Nitrogen dynamics of switchgrass, eastern gamagrass and high biomass sorghum from different sources of chemical and organic nitrogen fertilizers. M.S. Thesis. College of Agriculture and Technology. Arkansas State University-Jonesboro.

Awale, Rakesh, 2010. Performance of high biomass sorghum and nitrogen dynamics by the application of urea, pelleted poultry litter, and class A municipal biosolids. M.S. Thesis. College of Agriculture and Technology. Arkansas State University-Jonesboro.

Khatenje, Jane Achando, 2010. Soil organic matter dynamics in bioenergy cropping systems. M.S. Thesis. College of Agriculture and Technology. Arkansas State University-Jonesboro.

Klasky, John Wesley Phillip. 2010. Comparison of water and soil quality from locally-available soil amendments to biomass sorghum (*Sorghum bicolor* (L.) Moench). M.S. Thesis. Department of Biological Sciences. Arkansas State University-Jonesboro.

Book Chapters

Green VS, 2011. Soil Sustainability Issues in Energy Crop Production, in: E. Hood, et al. (Eds.), Plant Biomass Conversion, Wiley and Sons, Inc., Ames, Iowa. pp. 143-155.

Bouldin, JL, 2011. Soil Sustainability Issues in Energy Crop Production, in: E. Hood, et al. (Eds.), Water Sustainability in Bioenergy Cropping Systems, Wiley and Sons, Inc., Ames, Iowa. pp. 143-155.

Peer-review Publications

Ge X, Green VS, Zhang N, Sivakumar G, Xu J. (2012) Eastern gamagrass as an alternative cellulosic feedstock for bioethanol production. Process Biochemistry 47:335-339. DOI: 10.1016/j.procbio.2011.11.008.

Project 1B:

On-farm biofuel production and deployment to small non-road engines

PI: Kevin Humphrey

The project addressed farm-scale biodiesel production and local deployment for small, non-road engines used in farm operations. We established at Arkansas State University College of Agriculture and Technology's (ASU COAT) research farm an integrated operational farm-scale research system that includes a seed-crush process with biodiesel conversion capabilities having flexibility for processing of multiple oil seed crops to extract crude vegetable oils. Our research demonstrated the oil seed crops canola and camelina are candidates for economical and sustainable production of high quality biodiesel for on-farm operating use in the Arkansas Delta region. This farm-scale system has the capacity to annually produce 32,000 gallons of biodiesel, which has generation considerable interest by regional producers for adapting similar technologies to their operations.

Research completed at the ASU COAT research farm and farm-scale facility included establishing yields from test plots for specialty oil seed production (canola and camelina), determining the quality and quantity of oils extracted from harvested seeds (as well as extracted from regionally-produced soybean and cottonseed seeds), and determining yield and quality of biodiesel converted from these oils. Finally, the biodiesel produced in our operation was deployed for evaluating use as a fuel in small, non-road compression (diesel) engines, with assessment of emissions and performance. Data indicated favorable results up to a 20 percent biodiesel/fossil fuel diesel blends.

This project also addressed use of by-products from farm-scale oil-seed biodiesel production. Crude glycerin is considered a hazardous material that must be effectively processed for disposal. The project addressed this by incorporating into the on-farm system a process to recapturing methanol, which enable effective recycling in the biodiesel process, as well as rendering a refined non-biohazard glycerin co-product for use in further refinement into higher value products. The outcome of this was to demonstrate an improved environment and economical profiles for farm-scale biodiesel production. Seed meal generated during the crushing phase was also investigated for conversion to value-added products. Our research facility has generated over 5 tons of meal since February of 2012. We have initiated evaluation for quality for use as livestock feed. However, continued research is needed to integrate new uses for this material from our on-farm system.

This ASU project leveraged university funds used for upgrading an existing storage space to 3,444 square feet of heated and cooled laboratory and classroom space to thus establish a research and demonstration facility at the Farm complex that enabled use of DOE funds for on-farm renewable biofuels production and evaluating their use in small non-road engines. This new research capacity established at ASU has served to build new collaborative relationships with community farmers and producers, state and regional constituency groups and associations, which is being leveraged for new continuing research funding. Scholarly outcomes from the project have been communicated through 11 presentations at scientific conferences and 5 in the popular press and local mass media. A collateral benefit has been to promote understanding of biorenewable fuels through undergraduate classes and engaging graduate students in research at ASU. A new web site was developed at ASU to further stimulate research cooperation and public understanding of specialty oil seed crops and regional biofuels production. Other public outreach outcomes from this project include over eleven formal informational tours and demonstrations of the on-farm systems lab. These have included local, state and regional stakeholders, students and teachers from regional schools, as well as notable visitors to the University including, USDA Undersecretary Catherine Woteki and U.S. Congressman Rick Crawford (AR1) with his Washington and Jonesboro Arkansas office personnel. Both were impressed with the capability of the on-farm system and its potential for rural economic development. This farm-scale facility is a showcase for the ASU COAT that is promoting cross-discipline interactions between faculty and regional cooperators for research and educational activities for undergraduate and graduate students with outreach benefits to the general public.

Publications:

<u>Popular Press Communications</u>

Biodiesel Magazine (March 2011 Issue, p. 14), Springing towards applied skills: a new biodiesel process is installed at Arkansas State University.

Jonesboro Sun (Vol. 108, No. 180, Wednesday, June 29, 2011), “Educator sees pressing need for soybeans”.

Arkansas State University Herald (October 25, 2012), Agriculture professor converts cooking oil into fuel.

Project 1.C:
Algal oil and biofuels production from Arkansas agricultural biomass

PI: Jianfeng Xu **CoPIs:** Pamela J. Weathers**,** Ganapathy Sivakumar

Specific research objectives of this project were to: 1) develop a sustainable integrated biorefinery process for recycling agricultural wastes with algal biodiesel production; and to 2) establish sustainable and economic platform for biochemical conversion of cellulosic biomass to advanced biofuels at Arkansas State University (ASU). In order to accomplish this goal, multiple sub-projects were conducted over the course of the project.

For Objective 1, we collaborated with a commercial partner, Green Wisdom Inc. (Cotton Plant, AR) to construct a complete algae cultivation facility for a pilot-scale (1000 L) photo-bioreactor station at the ASU farm. We also designed and built two additional smaller-scale (100 L) photobioreactors for cost-effective screening of experimental conditions culturing algae under natural sunlight and ambient temperature conditions. Anaerobic digestion methodology was investigated for rapid recycling and conversion of regional-specific agricultural waste residues (catfish processing, soybean meal, and cotton gin fines) to low cost nutrients for algae cultivation and for generating biogas for capture and use. The oil-rich alga (*Ettlia oleoabundans*) was effective cultured on anaerobic digestion effluents and determined to accumulate high contents of recoverable oil (up to 50% of cell dry weigh). In addition, a new algae species *Stichococcus bacillaris* was isolated and identified. This latter alga accumulated extraordinary high levels of triacylglycerols (TAGs) with fatty acids profiles suitable for converting to high quality liquid fuels in range for use as jet fuel. Molecular biology approaches (e.g., creation of a cDNA library) were employed to identify key genes responsible for TAG production.

For Objective 2, we demonstrated integrated methods for processing and biochemical conversion of agricultural biomass to ethanol. This includes the technology for biomass pretreatment, enzymatic saccharification, and fermentation. We introduced a novel self-flocculating yeast strain to improve fermentation rates for more cost-effective ethanol conversion. Lignocellulosic biomass crops produced in Arkansas (gamagrass, miscanthus, switchgrass, elephant grass) that was used for studies was provided by regional research cooperators (USDA-ARS in Booneville and ASU COAT). We also investigated an alternative biomass source, duckweed (*Lemna minor*) as a feedstock for ethanol production. Duckweed showed benefits for use in phytoremediation of agricultural wastewater and, due to lack of lignin, does not require any thermal-chemical pretreatment before enzymatic saccharification. These benefits promote further investigation as an economic and environmentally sustainable feedstock. During these studies, energy beet production in Arkansas emerged as an alternative biomass crop for producing low-cost and high-quality sugars for fermentation. We demonstrated that either beet juice or liquefied whole roots (enzymatic-saccharification of ground beet roots with juice) were effective feedstocks for generating very high ethanol yields (up to 0.49 g/g sugar), particularly using the self-flocculating yeast.

This project established the first research capacity in biochemical engineering for agricultural biomass-to-biofuel conversion at ASU. We established new collaborations with industrial, academic, and federal research entities, including Green Wisdom Inc. (photobioreactor, anaerobic digestion), Missing Link Technologies LLC (algal oil extraction), University of Arkansas (algal oil chemical analysis), Worcester Polytechnic Institute (algal biotechnology), Virginia tech (pretreatment processing), Cornell University (cellulase engineering), and USDA-ARS (fermentation, energy crops). The funded project also generated sufficient preliminary data that enable us to prepare proposals for seeking new competitive funding. In the final year of the project, two NSF proposals and one Sun Grant proposal related to bioenergy research were submitted.

We established a new capability in biochemical engineering to support research, training, and outreach program at ASU. We communicated sixteen presentations at national and regional scientific conferences and published eight peer-reviewed research papers (two additional manuscripts are in preparation). Training of next-generation researchers was enabled through research participation by one postdoc, one full-time research associate, three graduate students, and eight undergraduate students were trained. Of them, one graduate student (Ying Yang) completed her PhD dissertation; another MS graduate student (Ningning Zhang) completed her internship by working on bioethanol production from both cellulosic biomass and energy beet, and chose to continue working in biomass research for her PhD degree in the Dr. Xu lab. New research capabilities in the Bioprocess Engineering Lab (Dr. Xu) now supports on-going bioenergy research through various bioreactor systems, algal culture and oil extraction facilities developed during the course of the project. We can now more effectively promote use of Arkansas' varied agriculture biomass resources as feedstock for biofuels production, which will support sustainable associated rural economic development in the region.

Publications:

Theses and Dissertations

Yang, Ying, 2010. Algal culture on anaerobic digester effluents. PhD dissertation, Worscester Polytechnical Institute, Worscester, MA.

Zhao, Zhiqiang, 2013. Hydroxyproline-O-glycosylation patterns in green algae cell walls. M.S. in Molecular Biosciences Program, Jonesboro, AR.

Zhang, Ningning, 2013. Biofuels and biobased products generated from Arkansas energy beets. Ph.D. in Molecular Biosciences Program. College of Science and Mathamatics. Arkansas State., Jonesboro, AR. (Co-advisee with B. Savary.)

Peer-review Publications

Ge, X, Zhang, N, Phillips, G. and Xu, J. (2012). Growing *Lemna minor* in agricultural wastewater and converting the duckweed biomass to ethanol. *Bioresource Technology* 124:485-488.

Sivakumar, G, Xu, J, Thompson, RT, Yang, Y, Randol-Smith, P and Weathers, PJ (2012). Integrated green algal technology for bioremediation and biofuel. *Bioresource Technology* 107:1-9.
Ge, X, Green, VS, Zhang, N, Sivakumar, G and Xu, J (2012). Eastern gamagrass as a promising cellulosic feedstock for bioethanol production. *Process Biochemistry* 47:335-339.
Yang, Y, Xu, J, Vail DR, and Weathers, PJ (2011). *Ettlia oleoabundans* growth and oil production on agricultural anaerobic waste effluents. *Bioresource Technology* 102:5076-5082.
Ge, X, Burner, DM, Xu, J, Phillips, G, Sivakumar, G. (2011). Bioethanol production from dedicated energy crops and residues in Arkansas. *Biotechnology Journal* 6:66-73.
Sivakumar, G, Vail, DR, Xu, J., Burner, DM, Lay, JO, Ge, X and Weathers, PJ (2010). Bioethanol and biodiesel: Clean energy for future generations. *Engineering in Life Science* 10:8-18.
Weathers, PJ, Towler, MJ and Xu, J (2010). Bench to batch: advances in plant cell cultures for producing useful products. *Applied Microbiology and Biotechnology* 85:1339-1351.
Xu, J, Weathers, PJ, Xiong, XR and Liu, CZ (2009). Microalgal bioreactors: challenges and opportunities. *Engineering in Life Sciences* 9:178-189.

Objective 2: To develop alternative uses for by-products and create new co-products that generates revenue streams to complement biofuel production.

Project 2. A:
Biochemical technologies for generating valuable co-products

PI: Brett J. Savary

The goal of this project was to develop biochemical technologies useful for processing agricultural biomass generated in the Arkansas Delta region to create value-added co-products such as functionally improved food and feed products and new industrial bio-based materials. Efficient biomass processing and generation of co-products are critical to the overall economic and environmental sustainability for producers and processors. This project targeted conversion of conventional crop processing residues, such as those from rice, as well as new bioenergy crops such as energy beets. Polysaccharides in rice straw and hulls can be converted to cellulosic ethanol, whereas sucrose from energy beets may be directly fermented to liquid biofuels. Objectives of this project were to 1) establish advanced bioanalytical technologies for protein and polysaccharide materials, 2) produce recombinant (monocomponent) enzymes useful for biomass (polysaccharide) modification, and 3) evaluate enzyme systems for efficient processing and conversion of biomass to value-added products.

We developed routine analytical methodologies for a new Waters MALDI-TOF mass spectrometer that had recently been acquired in the ASU Arkansas Biosciences Institute (ABI) through a separate NSF EPSCoR research program using project funds. We also evaluated application of other ABI bioanalytical instrumentation (e.g., Smiths Detection system FTIR spectrometer with ATF and microprobe) to identify and quantify polysaccharide components of plant cell walls. These tools provided new capabilities for unequivocal identification of recombinant proteins (such as those produced under objective 2), for defining action patterns of enzymes on polysaccharide substrates, and for structural characterization of polysaccharide-based products created from enzyme treatments. These tools have been used in training research staff and students in bioanalytical methods through a formal graduate-level techniques course and workshops. These new research capabilities have been demonstrated in numerous presentations and publications by ASU students, postdocs, and scientists.

Bioproduction of reagent-grade cell wall hydrolases (i.e., recombinant enzymes) using the *Pichia* yeast-cell expression system represents a critical new research capability at ASU and the ABI. Recombinant expression provides a stable, reproducible source for monocomponent enzymes, which we continue to define biochemical and action patterns on polysaccharide substrates. We have produced over twenty enzymes acting on the key linkages in all major classes of plant cell wall structural polysaccharides. This includes both endo-glycanases and accessory enzymes for pectin homogalacturonan, arabinan, and galactan and for hemicellulose glucuronoarabinoxylan. Bioproduction technology of reagent enzymes has also been incorporated into student training and research participation through new lab courses and independent studies. The various enzymes produced are being characterized in detail for use in conversion studies of rice and energy beet biomass. We are investigating how to use these enzymes for efficient production of putative bioactive oligosaccharides. This includes a new state-wide multidisciplinary research initiative between ASU COAT faculty with faculty at UAF's Department of Food Science and research scientists at the USDA-ARS National Rice Research Center to generate oligosaccharides from rice biomass and establish their structural and functional property relationships.

We have targeted bioproduction of a novel plant thermally-tolerant plant pectin methylesterase for biomass processing. This enzyme identified from previous research for which we recently obtained a US patent. Continuing research is addressing bioproduction and alternative application of this enzyme to efficiently reduce water-holding capacity in pectin-rich biomass such as beet pulp. An enzyme technology developed for this can significantly reduce energy-inputs and associated costs during biomass drying, while potentially improving nutritional and functional properties as animal feed. The beet sugar industry has provided some seed grant money to continue this research at ASU. Other enzymes produced from the GO88036 project will be investigated further utility in generating new value-added polysaccharide-derived products for beet pulp generated from energy beet processing.

This project leveraged GO88036 project funds with various resources at ASU and its Arkansas Biosciences Institute and College of Agriculture to establish new research capacities targeting carbohydrate-based products from plant biomass applying state-of-art biochemical and bioanalytical technologies. During the course of this project, GO88036 project funds provided partial funding support for 2 Ph.D.-level scientists, two support scientists, three graduate students, and three undergraduate students. There were 34 total oral and poster presentations at international, national, and regional conferences by project staff (including 7 invited talks and seminars by the PI) to disseminate results generated during the course of our research. Four student research projects were directly supported and four publications were generated (3 additional ones are in preparation). Finally, a network of collaborators was established in AR for addressing continuing bioenergy and biomass research in the AR Delta region.

Publications:

Theses and Dissertations

Miller, James W, 2010. Determination of the substrate action pattern of an *Aspergillus nidulans* pectin methylesterase. Honors' Senior Research Thesis, Chemistry and Physics, College of Science and Mathamatics, Arkansas State University, Jonesboro, AR.

Zhang, Ningning. Cellulosic ethanol production from energy crops in Arkansas. M.S. in Biotechnology. College of Science and Mathematics. Arkansas State University, Jonesboro, AR. (Co-advisee with J. Xu.)

Tovar, Jose T. Bioproduction and evaluation of a thermostable pectin methylesterase for biomass processing. Ph.D. Dissertation, Molecular Biosciences Program, College of Science and Mathematics, Arkansas State University, Jonesboro, AR.

Book Chapters

Savary, BJ, and P Vasu (2011). Chapter 2: Routine identity confirmation of recombinant proteins by MALDI-TOF mass spectrometry. IN: Recombinant Gene Expression: Reviews and Protocols, 3rd ed.; Lorence, Argelia, editor (Humana Press/Springer: New York).

Vasu, P, Bauer, S, and Savary, BJ (2011). Chapter 12: Cloning and expression of hemicellulases from *Aspergillus nidulans* in Pichia pastoris. IN: Recombinant Gene Expression: Reviews and Protocols, 3rd ed.; Lorence, Argelia, editor (Humana Press/Springer: New York).

Peer-review Publications

Cameron, RG, Luzio, GA, Vasu, P, and Savary, BJ (2011). Enzymatic modification of a model homogalacturonan with the thermally tolerant pectin methylesterase from Citrus: I. Nanostructural characterization, enzyme mode of action and effect of pH. J Agric Food Chem 59:2717-2724.

Savary, BJ, Vasu, P, Nuñez, A, and Cameron, RG (2010). Identification of thermolabile pectin methylesterases isolated from sweet orange fruit by peptide mass fingerprinting. J Agric Food Chem 58:12462-12468.

University of Georgia

Project 1:
Bioenergy crop production systems

PI: Dewey Lee

Farm owners and growers desire to help increase our country's energy independence while capturing additional on-farm value in an environmentally conscientious manner. One of the greatest challenges is providing a consistent supply of quality feedstocks for conversion to some form of energy without negatively impacting the food, feed, or fiber industry or harming the environment. Previous work conducted by several investigators has proven the southeastern area of the U.S. to be an excellent choice for producing various annual and perennial species of grasses and oilseeds for either food, feed or fiber.

The main goal of this project was to develop a better understanding of annual and perennial cropping systems using multiple plant species as potential bioenergy feedstocks; and determine the impact of nutrient uptake and water use on production efficiency of the perennial species.

The expected outcomes of this project were to demonstrate that the Southeast U.S. is ideally suited for production of perennial grasses and that they can be used to produce cellulosic ethanol. It was also expected that some annual species could possibly be suited as a substitute or supplemental feedstock in addition to the perennial grasses.

Several annual and perennial species were assessed and evaluated for their potential to produce dry matter and energy under irrigated and dryland conditions. Replicated plots of annuals, including sweet sorghum, forage sorghum, tropical corn, non-food sweet potatoes and camelina and perennial feedstocks, such as bermudagrass, napiergrass, energycane, miscanthus X giganteus, and switchgrass, were established primarily at the Southeast Research and Extension Center near Midville, GA. Other sites were used to develop some of the data sets.

Dry matter yields (15-20 +Mg/ha) of annual species, such as sweet sorghum and forage sorghums (*Sorghum bicolor* L.), compared favorably to bermudagrass, switchgrass or miscanthus but not to energycane or napeirgrass grasses with similar inputs. Other annuals, such as true tropical corn hybrids (*Zea Mays* L.) did not have the agronomically acceptable characteristics or yield to be competitive. Non-food sweet potatoes (*Ipomoea batatas*) demonstrated better potential for dry matter yields than current varieties grown for food in the southern U.S. Fall production of camelina (*Camelina sativa*) produced yields similar to yields obtained in the northernwestern U.S. (1500-1700 kg ha) however spring production was not sustainable due to significantly reduced yields (< 50%).

Dry matter yield and ethanol yield of perennial grasses species such as energycane (*Saccharum* sp.), napiergrass (*Pennisetum purpureum* Shumach), bermudagrass (*Cynodon dactylon* L.), switchgrass (*Panicum virgatum* L.), and Miscanthus X giganteus (*Miscanthus x giganteus* Greef et Deu) were compared under irrigation and dryland conditions. In addition, the five species were evaluated under three harvest systems to determine the effects on both yield and ethanol production. One oilseed perennial, jatropha (*Jatropha curcas* L), was evaluated and dropped from the study due to the lack of tolerance to low temperatures experienced in Georgia.

Overall, energycane and napiergrass provided more dry matter (20-40 Mg/ha) than bermudagrass, switchgrass, and miscanthus (4-20 Mg/ha) under similar conditions, yielding best under irrigation when rainfed conditions were significantly less. Surprisingly, the percent of ethanol, produced via conversion, in general ranged from 1.2 to 1.5 % for all grass species, though it was slightly affected by species and harvest system.

Throughout the project, problems were encountered with establishment of various species, weed control and /or herbicide injuries, and lodging. While some of these may prove too difficult or expensive to overcome in large commercial productions, most of the plant species were eventually established and provide some useful data. The problems though cause many variances in the studies. Additional studies in 2011 were added to evaluate the effects of nitrogen and potassium on energycane, napiergrass and miscanthus biomass. An energycane variety and miscanthus x giganteus clonal study was planted to evaluate differences in biomass and energy yield. Hopefully these additional studies will expand our knowledge on incorporating these species into a perennial feedstock cropping system.

a) Subproject: Identify, establish and compare non-food annual oilseed and starch feedstock

The expected outcomes were that non-food type annual crops could be used as potential bio-feedstocks and provide a source of biomass for conversion to some form of bioenergy in a cropping system in the southeastern U.S. Replicated studies were established during 2009-2011 to demonstrate the productivity of annual crops, such as sweet sorghum, forage sorghum, tropical corn, non-food sweet potato and camelina on sandy loam soils in Midville and Tifton, GA. In general plots were 3.7 m X 9.1 m. Row widths were 91.4 cm for sorghum *sp*, corn, and sweet potato. Plant populations varied according to common Cooperative Extension recommendations. Plots were established from April to June depending on the site and cropping system. Camelina plots were established either in Nov./early Dec. and April/May depending on the study.

Approximately, 4.5 kg ha of seed were drilled per plot with a Brillon ® Seeder. Atrazine (2.3 L ha) was applied to control weeds in sorghum and tropical corn. No herbicides were used on the camelina plots. Only metolachlor (1.6 L ha) was used in the sweet potato production. Nitrogen (112-168 kg N/ha) was applied to sorghum and corn studies depending on year. Phosphorus and potassium was applied during the length of the studies according to standard soil test. Soil test values for the annual crop production were determined by the University soil laboratory. No applications of phosphorus or potassium were made on soils testing medium high or higher. Fertilizer applications of 48 kg/ha of N, P, and K were made to the sweet potato plots. Camelina trials received 112 kg N/ha only. Sorghum plots were harvested and weighed with a tractor driven silage chopper with a weighing apparatus carried by the tractor. Sweet potatoes were dug by a tractor driven potato digger and weighted by hand. Camelina was harvested by a small self propelled combine. Seed was collected and weighed by hand.

In most cases, only sweet and forage sorghum compared favorably to the perennial grass species at similar N application rates. True tropical corn hybrids (140+ day maturity) proved too difficult to produce successfully due to very poor lodging resistance and, therefore were abandoned as a potential annual crop. Data from 2009 was discarded due to the large effects of lodging across multiple plots and replication. In addition, one study was abandoned due to extreme droughty conditions and herbicide injury.

Table 1. 2008 dry matter production (Mg ha^{-1}) of sorghum sp.		
AT (forage sorghum)	31% dry matter*	17.6
Dale (sweet sorghum)	27 % dry matter	21.7
Topper 76 (sweet sorghum)	27% dry matter	22.8

Planted April, 2008 under irrigation in 91.4 cm rows.
Applied 168 kg ha^{-1} nitrogen, no P or K.
* Dry matter % at harvest, Oct.

Table 2. 2010 dry matter production (Mg ha^{-1}) of sorghum.	
ES 5201 (forage)	16.1
Topper 76 (sweet)	20.6
ES 5200 (forage)	16.5
M 81-E (sweet)	14.3

Planted in June, 2010 under irrigation in 91.4 cm rows
Applied 168 kg ha nitrogen, no P or K.
Harvested Oct. 10^{th}.

Under irrigation, these forage sp. performed as expected with moderate to low inputs. These yields are similar to some sorghum varieties found in the University statewide variety trials comparing various forage species (http://www.swvt.uga.edu).

Sorghums have shown that under irrigation, competitive yields can be produced when planted April to June under moderate levels of nitrogen, phosphorus and potassium. These yields cannot be expected under rainfed conditions on soils common to the coastal plains of the southeastern U.S.

In 2010, 2011, and 2012, a non-food sweet potato (CSX-1 variety, CARethanol LLC) was compared to Hernandez and Beauregard, popular food sweet potatoes. In previous studies in South Carolina, CSX-1 produced dry matter yields in excess of 40 Mg ha^{-1} however yields were lower than those reported earlier. While the yields of the food type sweet potatoes ranged from13 to 18 Mg ha, the yield of CSX-1 ranged above 25 Mg ha. Though slightly lower than reported in S. Carolina and California, yields were competitive or superior to other annuals.

Varieties of camelina were compared to determine whether winter or spring production was most suitable for the southern U.S. In a pilot production prior to 2008 at two sites in Georgia, one variety, Calena produced approximately 2200 kg ha^{-1} when planted in December. Camelina is known for its high oil content (> 32%), however, its production capabilities were not known on the sandy loam soils of the southeastern U.S. Winter production of camelina planted in November ranged from 900 to 1600 kg ha^{-1} depending on varieties. Spring production (April planted) was significantly reduced.

Yields were less than 50% on average of the fall plantings. These levels are unsustainable in any cropping system. Camelina did not response to nitrogen or phosphorus rates above 112 kg ha^{-1}. Surprisingly, camelina did not respond to any applied rate of potassium (56, 112, or 168 kg ha^{-1}). Most soil test values exceeded 100 kg ha of available potassium per hectare.

In 2010, camelina planted in late Oct. flowered December 27th. Pollination during December was undamaged by freezing temperatures however during Jan.and Feb., it was apparent during seed development that freezing temperatures significantly reduced development and yields. Yields of 16 varieties ranged from 280 to 420 kg ha^{-1}. All other tests were abandoned.

b) Subproject: Establish and compare crop yield and energy yields of potential perennial feedstocks.

The advantages of perennial grasses are that they do not need to be replanted every year as with annuals thus cost should be reduced over the life of the crop. In addition, potential soil losses would be lower due to significantly reduced tillage. They also would ultimately have a deeper root system (thus better water use efficiency), better drought tolerance and higher potential for C sequestration. In addition, it was expected that yields would be higher and pest problems potentially lower. The five perennial grasses (switchgrass, miscanthus, bermudagrass, napiergrass, and energycane) chosen for this project were divided into senescent grasses and subtropical grasses. Bermudagrass was added because it is a popular perennial forage grass in the southeastern U.S.

Switchgrass and miscanthus x giganteus are senescent grasses as they flower mid to late summer, then senesce prior to the onset of winter. Their stems are thinner and easily dry when harvested. Napiergrass and energycane are sub-tropical grasses that flower very late, and may suffer significant potential losses in freezing temperatures. Stems are generally thick and dry little during the fall and winter prior to the onset of spring growth the following year.

The expected outcomes of this objective were that the five perennial grasses could possibly be used as a bio-energy feedstock for some undetermined bio-energy conversion process. In addition, this project may, by comparing the five different species, show enough differences to demonstrate each perennial grass may be better suited for some type particularly conversion based on yield, growth, life cycle, and harvest capability.

Five different perennial grass species were planted during the fall 2008 to the spring 2009. Plots were 14.6 m wide x 9.1 m in length. Energycane cultivar, L79-1002 and napiergrass cultivar, Merkeron was planted in the fall 2008 from stem cuttings, 1m in length. Cuttings were planted 12 cm deep in rows 1.8 m's apart. Miscanthus rhizomes were planted in the greenhouse in small cones then removed and used for planting in the spring 2009. Each treatment was three 45.7 cm rows, 45.7 cm apart. Switchgrass cultivar, EG 1101 (Blade Seed Co.) was seeded in the greenhouse in small pots and planted similarly to miscanthus at the same time. Bermudagrass cultivar, Tifton 85 stolons were dug and planted by broadcasting and disking into the soil in March, 2009.

Weed control was a preemergence application of 2.3 L ha^{-1} of atrazine and 1.75 L ha^{-1} of oryzalin on napiergrass and energycane plots. Atrazine alone was used on the switchgrass, miscanthus and bermudagrass plots. Energycane, napiergrass, switchgrass, and miscanthus were harvested by a tractor driven silage chopper with a weighing apparatus attached. Bermudagrass was harvested by a self-propelled forage harvester (Carter Mfg.). Samples of all grass plots were collected, weighed fresh, dried to completion in an oven and weighted again to determine dry matter concentration. Samples were ground for nutrient and ethanol determination.

Biomass samples for ethanol production were ground in a Wiley mill to pass a 2 mm screen. Samples were subjected to pretreatment with dilute H_2SO_4 at 121 degree C for 1 hr in an autoclave. After cooling the pH was adjusted to 5.0 with $Ca(OH)_2$ and citric acid. Total dry matter in the reaction was 10% w/v. Saccharifying enzymes were Celluclsat 1.5 L cellulase and Novo 188 cellobiase (both from Novozymes Corp.). The yeast (*Saccharomyces cerevisiae*) was a xylose-fermenting strain YRH400, provided by Ronald Hector, USDA-ARS. Simultaneous saccharification and fermentation was carried out for 72 hr at 30 degrees C in a shaking incubator. Samples were taken every 24 hr, were filtered and then frozen at -20 degrees C until analysis. Total reducing sugars were quantified by the dinitorsalicylic acid (DNS) method, and ethanol was quantified by gas chromatography.

Miscanthus plots in the original study, which included the four other grass plots were severely damaged due to herbicide injuries from an overspray of imazapic. Switchgrass productivity was severely limited by the lack of weed control. Due to these variances, an additional study was added to meet the overall objectives of the study. Similar material and methods were used.

Dry matter yields of the senescent grasses are much lower than the subtropical grasses as shown in tables 4 and 5. Surprisingly, miscanthus yielded significantly less than switchgrass. However, it must be noted that while miscanthus x giganteus and switchgrass emerged from dormancy in early March (soil temperatures 50+ degrees F), MxG flowered much earlier than switchgrass thus reducing the potential biomass yield. In addition, it must be noted that delayed harvesting had more impact on miscanthus yields than switchgrass (Tables 4 & 5).

Table 4. 2010 Production Season, Mean Dry Matter (Mg/ha), Two Harvest

Grass Species/ Irrigated only	Jan., 2011	Feb., 2011
Miscanthus	3.79	3.47
Switchgrass	10.67	10.67

Table 5. 2011 Production Season, Mean Dry Matter (Mg/ha), Three Harvest Periods

Species	Dec., 2011	Jan. 2012	Feb. 2012
Miscanthus	6.40	6.13	5.35
Switchgrass	21.60	17.56	19.02

Preliminary studies (data not shown) suggest that switchgrass and miscanthus are less fermentable than the other grasses used in the overall study. However, they may be more N efficient (data not shown) and certainly are drier at harvest. Harvest moisture was generally 12 to 14%. It is expected that these grasses would be much better suited due to the lower moisture at harvest for combustion processing into direct heat energy.

Tifton 85 is a popular bermudagrass grown for hay and forage and it was hypothesized that this variety could fill a gap in biomass production during summer months. A harvest scheme was designed to demonstrate the potential production of biomass and ethanol that could be maximized in a current cropping system. Table 6 shows the harvest dates for 2010 and 2011. They were designed so that bermudagrass could be compared with delayed harvest of the other species. Plots were either harvested once, twice or three times.

Table 6. Harvest dates of Tifton 85 bermudagrass

Year	Harvests	June	Mid-summer	Fall
2010	3	18-Jun	30-July	8-Sept
	2	-	23-July	8-Sept
	1	-	-	27-Sept
2011	3	21-Jun	3-Aug	27-Sept
	2	-	6-July	8-Sept
	1	-	-	3-Sept

Overall, there was little change in ethanol conversion efficiency over the harvest dates (Fig.1). Under irrigation, a two to three cut system maximized yields. However, the three cut system removed much more N that the two cut system (data not shown). Ethanol yields of 3500 L/ha are possible with bermudagrass. Under the dryland production a single harvest would be the most efficient with respect to N usage. This single harvest could be done at any point throughout the summer to provide supplemental feedstocks in a cellulosic conversion system. About 2000 L ha^{-1} could be produced under this system.

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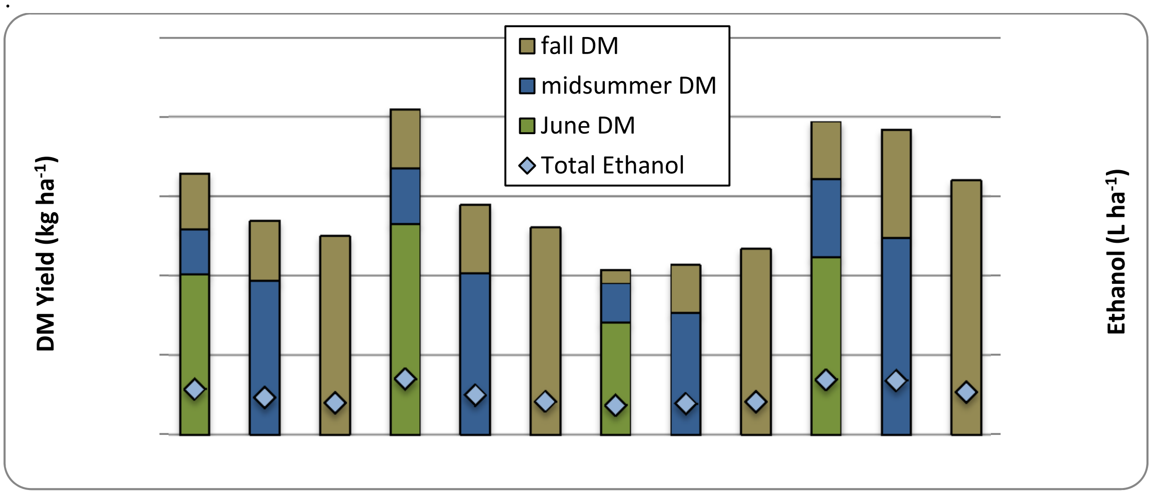

Figure 1. Dry matter yield and ethanol production of bermudagrass under dryland and Irrigated conditions

Energycane and napiergrass produced significantly more biomass than any of the other perennial grasses. In the fall 2009, both species were only harvested once in early February, 2010. There were no differences between irrigated and dryland plots. However, napiergrass was more productive under both conditions than energycane.

Table 7. 2009 Energycane & Napiergrass Production Season		
Treatment	Grass	Dry Matter (Mg ha^{-1})
Irrigated	Napiergrass	20.18
	Energycane	16.91
Dryland	Napiergrass	2125
	Energycane	17.94

Planted Oct.30, 2008; Applied 100 lbs N & no P or K
Harvested Mar. 4, 2010
P>=0.05;

After a period of establishment, energycane was more productive in the following years than napiergrass under irrigation(Fig. 2 and 3). In 2010-2011, irrigation had no effect on yield (Fig.2) in a year with normal rainfall (rainfall data not shown). Delaying harvest from Dec. to Feb. resulted in significant reduction in dry matter yield due to a colder winter. Reduction in yield ranged 25 to 30% for energycane and 23% for napiergrass.

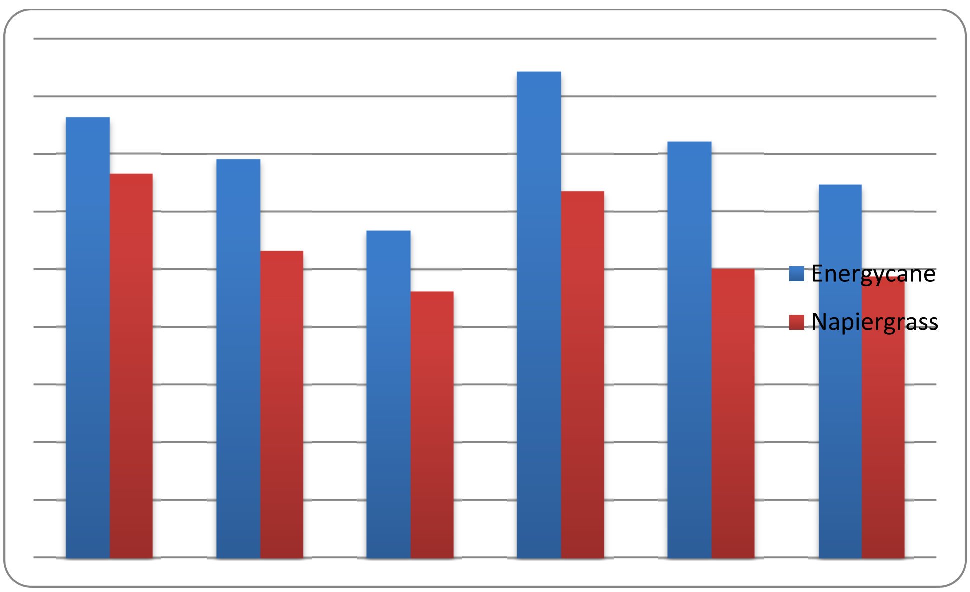

Figure 2. Dry matter yields (Mg/ha) of napiergrass and energycane (2010-2011)

In 2011-2012, dry matter yields greatly increased with irrigation for energycane only (53%) (Fig. 3). Irrigation only increased napiergrass dry matter by 18%. Rainfall during 2011 was significantly below average (data not shown). Delayed harvest resulted in significant reduction in dry matter yields of napiergrass primarily to leaf loss. Energycane maintained a larger portion of leaf tissue and maintained stalk integrity due to much warmer temperatures during the winter months (data not shown). Under rainfed conditions, it appears that napiergrass tends to be more drought tolerant and less responsive to irrigation.

Table 8. Ethanol yield (%) of napiergrass and energycane in 2010-2011

Species	Harvest	0 hr	24 hr	48 hr	72 hr
Energycane	Dec.	0.032	1.453	1.512	1.578
	Jan.	0.018	1.189	1.304	1.343
	Feb.	0.017	1.004	1.105	1.151
Napiergrass	Dec.	0.028	1.141	1.263	1.321
	Jan.	0.026	1.140	1.247	1.288
	Feb.	0.027	1.132	1.260	1.271

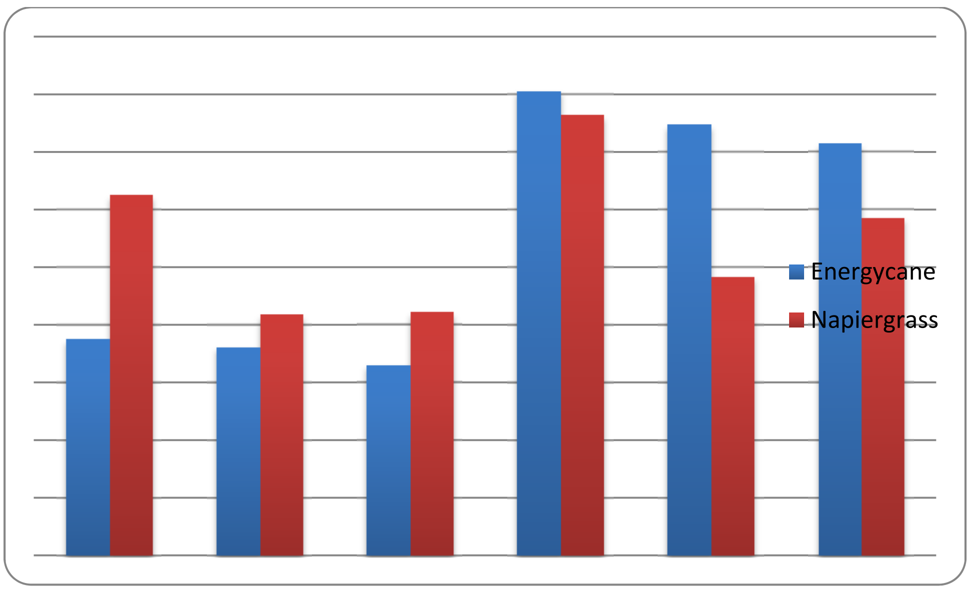

Figure 3. Dry matter yields (Mg/ha) of napiergrass and energycane (2011-2012)

Energycane appears to produce a slightly higher percentage of ethanol (Table 8 and Fig. 4) as expected due to slightly higher fermentable sugars. In the colder winter of 2010-2011, delayed harvest resulted in a decrease of conversion to ethanol (loss of free sugars). In the warmer winters (2011-2012), this effect was much less (Fig. 5).

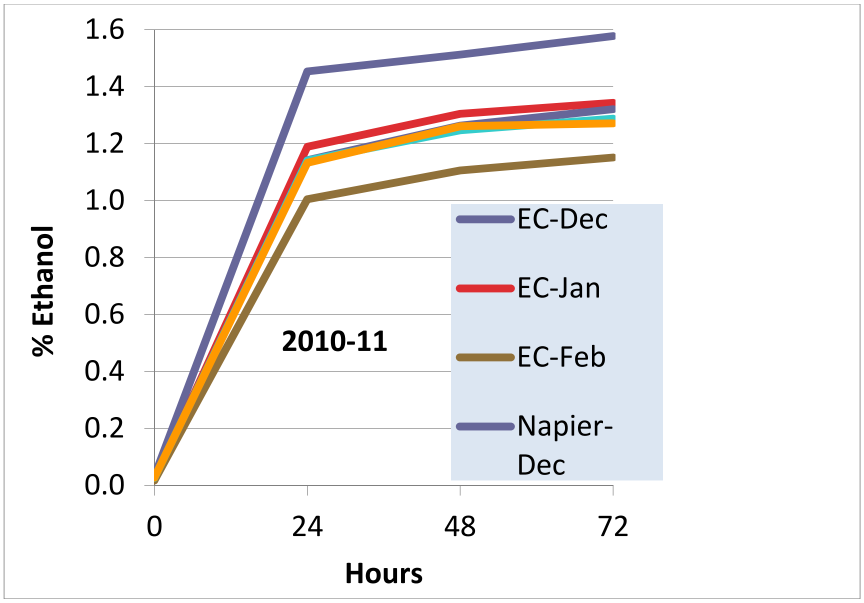

Figure 4. Ethanol yield % of energycane and napiergrass in 2010-11.

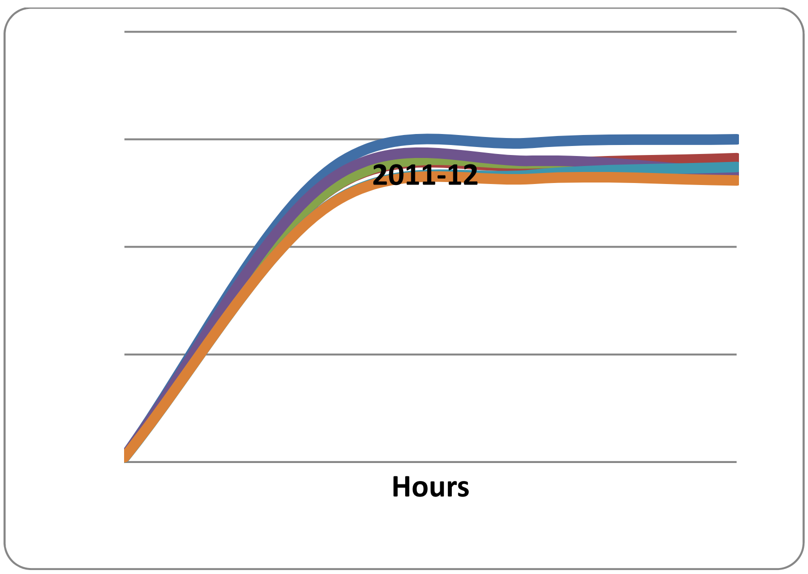

Figure 5. Ethanol yield % of energycane and napiergrass, 2011-12

Napiergrass appears to be more drought tolerant than energycane (2010 data, Fig.3). From observations, leaf drop may have caused some harvestable loss in biomass over the winter in napiergrass. It appears that leaf drop and the freezing and thawing of the energycane stalks may also have caused loss or degradation of fermentable free sugars. Fermentability of napiergrass was unaffected by harvest date.

In summary, under the conditions of this study, napiergrass can produce between 3000-4700 L ha^{-1} of ethanol (325-500 gal per acre) while energycane could produce between 3900-6200 L ha^{-1} of ethanol (420-700 gal per acre).

c) Subproject: Determine the effect of crop residue removal on soil quality and nutrient availability of the perennial feedstocks.

It is important that in a cropping system designed for energy production, the environmental effects such as nutrient removal and carbon sequestration be determined as to fully account for the cost of the energy produced. Though samples were collected and most analyzed, datasets were still incomplete to draw any conclusions in time for a final report. Manuscripts for publication will be prepared that will include this data.

d) Subproject: Establish deliverable Extension programs via county meetings, website and electronic media, and on-site demonstration and training programs.

During the time period of 2009-2012, these plots were viewed by over 600 producers, students, agents, local, state and national legislatures, university personnel and agri-businessmen, and media. Data collected from these studies were shared in local and regional extension meeting plus five regional, national and international professional meetings. In addition, the results of these studies will continue to be analyzed in order to develop and modify current crop protection and fertilizer recommendations for use by growers when conversion technologies can successfully utilize the potential of these various feedstocks.

Additional studies underway that have been initiated from this project are:

- The effects of four rates of N & K on miscanthus, napiergrass, and energycane.
- The evaluation of weed control methods on miscanthus production.
- A variety comparison of energycane varieties.
- A planting rate and method study on establishing miscanthus
- The potential productivity of seeded types of miscanthus sp.
- The conversion of five perennial grasses to bio-oil through fast pyrolysis.

The preliminary results of this study appear to demonstrate that both annual and perennial grasses and herbaceous species used in this project can be incorporated into some type of cropping system that can be used as a feedstock for bioenergy. Further studies will be necessary though to determine the economic sustainability of these feedstocks as conversion technologies are developed that can convert these materials to a form of energy on a commercial level.

Final note: The principal investigator thanks the contributions of the following:
USDA-ARS: Dr. Bill Anderson, Freddy Cheek, Tony Howell
University of Georgia: Robert Pippin, Anthony Black, Jacob Hall, John Ware
East Georgia College: Joe Knoll
Auburn University: Jennifer Johnson

Project 2:
Nutritional quality of by-products from bioenergy production

PI: Amy Batal, Mike Lacy

Prior to detailing the accomplishments of the project, some general context in regard to why this project was important may be useful. During the past decade it has become increasingly evident that alternatives must be developed to reduce dependence on fossil fuels. The recognition of this need is hardly new, nor is it limited to the United States. Wind, tide, and geothermal sources of energy have long been employed in different countries to varying degrees. The generation of ethanol for use in motor vehicles has been common for some time in Brazil, with sugarcane being the source of substrate. By contrast, in the United States the abundance of corn has generated considerable interest in its fermentation so as to obtain ethanol for motor fuels. Similarly, the use of fats and oils to generate bio-diesel has attracted attention. While still in the experimental stage, the fermentation of cellulose to obtain ethanol is being actively explored.

While the primary objective of fermentation has been to obtain ethanol, it has not escaped the attention of producers that one or more of the products of these processes may have value as animal feed ingredients. Obtaining value from co-products is clearly of benefit both to the biofuel industry and to those directly involved in poultry and animal production. In order to professionally contemplate the use of such ingredients their nutritive value must be reliably determined. By doing so nutritionists will be able to assign an economic value to such products, which can then be taken into account by those assessing the economic feasibility of establishing a fermentation facility. It has been the objective of this project to study the nutritional attributes of a number of co-products of biofuel generation and thus address the needs of both the biofuels industry and animal producers.

While a number of biofuel co-products have been evaluated in this study, the major focus has been on those derived from the fermentation of corn. In 2011 it was estimated that approximately 40% of the United States corn crop was employed for ethanol generation. As is evident from the various quarterly progress reports submitted as part of this project, a number of different aspects of the nutritional quality of such co-products have been examined so as to provide both producers and users the opportunity to objectively and professionally assess the feeding value of corn based DDGS. Initially, and in order to provide a baseline of data, a number of commercially prepared samples of corn DDGS were evaluated for their proximate composition and energy. Proximate proposition consists of protein, fat, fiber, ash, moisture, and by calculation, carbohydrate. A variety of samples from different locations were employed in this overview, as it is recognized that considerable variation exists in corn cultivars, growing conditions and manufacturing processes (Table 1.).

In addition to the basic nutrient components, it was necessary to determine the amino acid composition of the many samples, in addition to their energy bearing components. It is well recognized that excessive heat during the drying process can damage amino acids, particularly lysine, as has been documented for many decades in the soybean industry. The vitamin composition of DDGS as found in the standard tables of nutrient composition was in need of revision, as many of these values were determined for beverage grade DDGS. Samples of both standard and low-oil DDGS were evaluated for a range of vitamins (Table 3.), fulfilling a need of the animal feed industry. Similarly, the mineral composition of DDGS was determined on 20 samples (Table 2.), in addition to obtaining background mineral compositions on both corn and soybean meal. Once again, this research was essential as beverage grade DDGS may not be typical of today's DDGS in terms of mineral content. Of particular interest is the phosphorus component of DDGS. Phosphorus is of course is needed for proper skeletal development. However excess phosphorus can lead to environmental problems. Thus the amount of phosphorus in DDGS, and its relative digestibility, were two concerns. As documented in quarterly reports, the phosphorus in DDGS is much more available for the animal than that in the parent corn, presumably due to the microbial action during fermentation. This finding has been well received by the poultry industry.

Until recently DDGS contained approximately 10% oil. Some concern had been expressed as to the stability (i.e. possible rancidity) of this ingredient. A number of samples were checked for rancidity. Happily, it was determined that using the OSI technique (which has been generally accepted by the commercial sector) the fat in DDGS is remarkably stable. The particle size of DDGS is of concern, as this ingredient is occasionally associated with poor pelleting quality. As pelleting is an essential feature of feed preparation for poultry, the determination of particle size has led to a better appreciation of the pelleting process when employing this ingredient (Table 4.). Similarly, the effect of DDGS on poultry pigmentation (skin and yolk), was of concern especially those markets where pigmented product is of higher value. This was investigated as well.

While not envisioned at the time this project was planned, the decrease in oil in DDGS has been received with considerable concern by poultry producers. Originally, a "low-oil" DDGS might contain 7.5 - 8% oil. Currently, using centrifugation technology, this can be reduced to as low as 4%. We felt this project would not be complete without at least an initial evaluation of the energy content of the very low-oil samples. Using two approaches it was determined that the very low-oil (5%) DDGS contains approximately 15% less energy than the initial product. This may well preclude its use in high energy broiler diets but may still prove valuable in lower energy pullet developing rations.

The research staff was pleased to have the opportunity to evaluate the nutrient composition of several other co-products of the bio-fuels industry. Glycerin, obtained from the preparation of bio-diesel, was found to have a reasonable energy level and can be used in feeds. DDGS from barley, as opposed to corn, had a somewhat higher fiber value. Single cell protein from yeast, (obtained in the fermentation process) was evaluated, as was algae meal. This product is also made available as a residue of obtaining oil for bio-diesel. *Camilina sativa* meal was originally promoted as a source of omega-3 fatty acids for poultry. However it soon became apparent in those regions producing camelina (Montana and other northern states) that the oil might be more valuable as bio-diesel. Much of the oil is now being removed from these seeds, with the remaining camelina meal available for poultry feeds. It has been especially attractive in areas close to the actual sites of production, principally Oregon and California. Duck weed residues have also been evaluated but found to be relatively high in fiber.

Correlation among nutrient compositions in traditional DDGS samples was established. The completed table of values is now available for poultry nutritionists to utilize. In addition, the prediction equation for nitrogen corrected apparent metabolizable energy (AME_n) in traditional distillers dried grain with solubles (DDGS) samples has been determined. Data indicate that there are negative correlations between AME_n and lignin, but positive correlations exist between crude protein (CP) and lignin or acid detergent fiber (ADF). The average particle size distribution, geometric mean diameter (d_{gw}), was positively correlated with crude fat but negatively correlated with crude fiber.

In summary, this project has allowed us to determine:

- Proximate composition of Distillers Dried Grains with Solubles (DDGS) from a variety of sources
- Amino acid compositions of DDGS from a variety of sources
- First time evaluation of vitamin composition of DDGS
- Mineral composition of DDGS
- The effect of particle size and feed pelleting in regard to DDGS quality
- The stability of fats in DDGS (rancidity)
- The effect of DDGS on pigmentation of skin and egg yolks
- Energy content of newer low-oil DDGS
- Correlations between nutrient composition and DDGS fat content (traditional and low-fat)
- Development of prediction equations for poultry nutritionists to use in the estimation of the metabolizable energy and amino acid values of traditional and low-fat DDGS
- The nutrient composition of several other co-products of the bio-fuels industry - glycerin, DDGS from barley (as opposed to corn), single cell protein from yeast, algae meal and duck weed residues were evaluated.

Table 1. Proximate composition (%) of 13 low-fat corn DDGS samples from different ethanol plants, as-fed basis

	Moisture	CP	NPN[2]	Crude fat	Ash	Starch	Crude fiber	Lignin	ADF[2]	NDF[2]
Mean	10.5	27.3	0.3	8.1	3.9	5.37	7.2	3.8	11.3	24.6
Range	8.0-12.8	25.0-32.2	0.1-1.3	6.6-10.2	1.9-4.6	3.19-7.37	6.4-8.8	1.6-8.3	8.4-15.6	21.2-33.7
Standard deviation	1.03	1.98	0.31	1.05	0.72	1.30	0.79	1.79	1.81	3.87

[1] Distillers dried grain with solubles (DDGS) samples was obtained from different ethanol plants throughout the United States.

[2]NPN=Non-protein nitrogen; ADF = Acid detergent fiber; NDF = Neutral detergent fiber.

Table 2. Mineral composition of 13 low-fat corn DDGS samples from different ethanol plants, as-fed basis

	Macro minerals, %							Micro minerals, ppm					
	Calcium	Phosphorus	Phytate phosphorus	Sodium	Potassium	Sulfur	Magnesium	Choline chloride	Copper	Zinc	Manganese	Iron	Selenium
Mean	0.02	0.75	0.17	0.14	0.96	0.67	0.26	541	5.0	56	12	94	0.28
Range	0.01 - 0.04	0.45 - 0.90	0.12 - 0.21	0.05 - 0.23	0.42 - 1.17	0.33 - 1.16	0.13 - 0.31	332 - 754	4.0 - 6.2	38 - 74	8 - 16	44 - 144	0.12 - 0.44
Standard deviation	0.01	0.12	0.03	0.06	0.20	0.24	0.05	128.72	0.68	9.53	2.20	26.08	0.11

Table 3. Vitamin composition of traditional cDDGS[1] and low-oil cDDGS samples

	Tradition cDDGS				low-oil cDDGS				NRC (1994)
	1	2	3	Mean	1	2	3	Mean	
Obtained state	IL	WI	NE		WI	IA	NE		
Vitamin A, IU/kg	ND[2]	ND	ND	ND	ND	ND	ND	ND	Not listed
Vitamin D, IU/kg	ND	ND	ND	ND	ND	ND	ND	ND	Not listed
Vitamin E, mg/kg	9.0	8.5	6	7.8	4.5	6	7	5.8	40
Biotin, µg/kg	ND	ND	ND	ND	ND	ND	ND	ND	Not listed
Thiamin, mg/kg	8.6	10.9	7.6	9	7.4	6.9	4.5	6.3	2.9
Riboflavin, mg/kg	1.9	3.8	2.1	2.6	2.2	2.7	1.1	2.0	8.6
Pyridoxine, mg/kg	2.4	9.6	1.5	4.5	3.8	2.3	1.3	2.5	2.2
Pantothenic acid, mg/kg	6.5	16.3	8.4	10.4	17.4	13.4	3.3	11.4	11.0

[1]cDDGS = corn distillers dried grain with solubles.

[2]ND: not detectable

Table 4. Particle size, gross energy, and color of 13 low-fat corn distillers dried grain with solubles (cDDGS) samples from different ethanol plants[1]

	Particle size (d_{gw}[1])	Gross energy, kcal/kg	Color[2]		
			L*	a*	b*
Mean	765	4,520	53.8	7.55	35.2
Range	509 – 1,090	4,358 - 4,695	42.0 - 61.9	5.34 - 11.53	21.1 - 15.8
Standard deviation	184.4	90.1	5.77	1.40	6.21

[1]d_{gw}: geometric mean diameter.

[2]Measured using a Minolta colorimeter. Higher values for a* and b* indicate a greater degrees of redness and yellowness, respectively; L* = lightness of DDGS; where 0 = black to 100 = white.

Publications:

Peer-review Publications

Jung, B., and A.B. Batal. 2011. Nutritional and feeding value of crude glycerin for poultry. 1. Nutritional value of crude glycerin. J. Appl. Poultry Res. 20:162-167.