

Agro-ecosystem Approach to Sustainable Biofuels Production via the Pyrolysis-Biochar Platform

**April 2016** 



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#### **LEGAL NOTICE**

This report was prepared by Iowa State University and CenUSA Bioenergy research colleagues from Purdue University, United States Department of Agriculture-Agricultural Research Service, University of Illinois, University of Minnesota, University of Nebraska, Lincoln, University of Vermont, and the University of Wisconsin in the course of performing academic research supported by Agriculture and Food Research Initiative Competitive Grant No. 2011-68005-30411 from the United States Department of Agriculture National Institute of Food and Agriculture ("USDA-NIFA").

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# Agro-ecosystem Approach to Sustainable Biofuels Production via the Pyrolysis-Biochar Platform (AFRI-CAP 2010-05073)

Second Quarter Report: February 1, 2016 – April 30, 2016

# **Project Administration, Project Organization and Governance**

Ken Moore (Professor, Iowa State University) continues as the CenUSA Bioenergy Project Director with Anne Kinzel as the Chief Operating Officer. Rebecca Staedtler (ISU Bioeconomy Institute) provides assistance with project financial matters.

# CenUSA Bioenergy Advisory Board

We continue to integrate our Advisory Board into our project activities. This quarter our primary communication has been through informal project updates and sharing of information. We have invited the Board to participate in our April 2016 Co-project director monthly meeting (virtual) where our Health and Safety Objective Co-project director Chuck Schwab will be presenting on the totality of his CenUSA work to date.

# Executive Team Meetings

The Co-Project directors representing each of the ten project objectives continue to meet monthly with Ken Moore and Anne Kinzel via online meetings held in CenUSA's dedicated Adobe Connect meeting room. The virtual meeting room allows documents to be viewed by all participants, enhancing communications and dialogue among participants. Tom Binder, the Advisory Board chair also attends these meetings on behalf of the Advisory Board.

The CenUSA co-project directors will be attending a leadership team 2016 annual meeting in Ames, Iowa, June 22-23, 2016.

## Financial Matters

The Administrative Team continues to monitor all project budgets and subcontracts to ensure adherence to all sponsor budgeting rules and requirements. Our no-cost extension request has been approved and we are currently updating our contractual arrangements with our partner institutions.

# **Germplasm to Harvest**

# **Objective 1. Feedstock Development**

Feedstock Development focuses on developing perennial grass cultivars and hybrids that can be



used on marginal cropland in the Central United States for the production of biomass for energy.

# 1. Significant Accomplishments Summary

• Plant Pathology and Entomology. This research provides important information on the arthropods associated with bioenergy grasses and valuable information on the host suitability of switchgrass and other bioenergy grasses to four aphids within a system that has been largely overlooked, indicating that there are genetic differences among switchgrass populations for resistance. The ultimate goal of this project is to develop effective and sustainable management strategies for the key arthropod pests affecting switchgrass.

#### 2. Planned Activities

- Breeding and Genetics ARS-Lincoln, Nebraska and Madison, Wisconsin (Mike Casler and Rob Mitchell)
  - ✓ Prepare for the 2016 field season.
  - ✓ Fertilize and apply herbicides, as needed, to all field plots.
- Feedstock Quality Analysis (Bruce Dien ARS Peoria and Akwasi Boateng ARS Wyndmoor)
  - ✓ Continue analyzing data generated from NIR models and write manuscript(s).
  - ✓ Add mineral content, biomass composition, and pyrolysis product yield from the 88 grass samples (indiangrass and big bluestem) to the NIR models. These samples need to be analyzed by the NIR.
  - ✓ Complete processing the FY 2015 sample set for enzymatic sugar release following hot-water pretreatment.
  - ✓ Begin to evaluate hydrolysates received from Renmatix for lipid production.
- Plant Pathology and Entomology University Nebraska-Lincoln (Tiffany Heng-Moss and Gary Yuen)
  - ✓ Finalize feeding monitoring for yellow sugarcane aphid.
  - ✓ Begin writing arthropod survey publication.
  - ✓ Prepare publication on results from CenUSA varietal trials focusing on resistance to rust.



#### 3. Actual Accomplishments

# • Breeding and Genetics – Lincoln, Nebraska and Madison, Wisconsin (Mike Casler and Rob Mitchell)

- ✓ Completed grinding of 2015 samples.
- ✓ Completed data compilation from 2015.
- ✓ Completed early-season fieldwork on CenUSA field plots (fertilization, herbicides, staking, and flagging).
- ✓ Established new trials to document the most recent advances in breeding for increased biomass yield in switchgrass and big bluestem, including evaluation of 12 new populations, not previously tested.

# • Feedstock Quality Analysis (Bruce Dien and Akwasi Boateng)

- ✓ Completed processing FY 2015 sample set for enzymatic sugar release following hotwater pretreatment and entire biomass measurement set shared with collaborators.
- ✓ Demonstrated feasibility of converting Renmatix sugar stream into microbial lipids with good lipid titer. Results distributed to Renmatix for comment.
- ✓ All data generated from NIR models has been analyzed. Results from enzymatic hydrolysis and py-GCMS are being incorporated into two manuscripts examining the effects of harvest time on conversion.

# • Pathology and Entomology - University Nebraska-Lincoln (Tiffany Heng-Moss and Gary Yuen)

- ✓ The analyses for the arthropod survey sample from 2015 are complete.
- ✓ The analyses of the electronic feeding monitoring for greenbugs are complete.
- ✓ Progressing toward completion of the arthropod survey publication.

## 4. Explanation of Variances

None noted.

#### 5. Plans for Next Quarter

- Breeding and Genetics (Mike Casler and Rob Mitchell)
  - ✓ Prepare for the 2016 field season.
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✓ Fertilize and apply herbicides, as needed, to all field plots.

# • Feedstock Quality Analysis (Bruce Dien and Akwasi Boateng)

- ✓ Determine chemical composition for first of two harvest year sample set for switchgrass grown in Arlington, Wisconsin. Determine differences based upon variety, fertilizer loading, and harvest maturity.
- ✓ Begin to optimize pretreatment conditions for before mentioned switchgrass samples for enzymatic conversion to sugars at low solids loading.
- ✓ Finalize manuscripts on field studies looking at the impact harvest time has on conversion, both biochemical and thermochemical.

# • Pathology and Entomology (Tiffany Heng-Moss and Gary Yuen)

- ✓ Complete the arthropod survey publication.
- ✓ Begin writing the electronic feeding monitoring and choice study publication.

# • Publications / Presentations/Proposals Submitted

- ✓ Dien, B.S., W.F. Anderson, M. Lamb, P.J. O'Bryan & P.J. Slininger. (2016). Invited presentation: Field productivities of Napier grass for production of sugars and ethanol. 252nd ACS National Meeting in Philadelphia, PA to be held August 21-25, 2016.
- ✓ Ramstein, G.P., J. Evans, S.M. Kaeppler, R.B. Mitchell, K.P. Vogel, C.R. Buell & M.D. Casler. (2015). Accuracy of genomic prediction in switchgrass improved by accounting for linkage disequilibrium. Genes, Genomes, Genetics. doi: 10.1534/g3.115.024950.
- ✓ Serapiglia, M.J., A.A. Boateng, D.K. Lee & M.D. Casler. (2016) Switchgrass harvest time management can impact biomass yield and nutrient content. Crop Science 56: 1-11 doi: 10.2135/cropsci2015.08.0527.

# Objective 2. Sustainable Feedstock Production Systems

The Sustainable Feedstock Production Systems objective focuses on conducting comparative analyses of the productivity potential and the environmental impacts of the most promising perennial grass bioenergy crops and management systems using a network of 14 fields strategically located across the Central United States. The overarching goal is to produce a



quantitative assessment of the net energy balance of candidate systems and to optimize perennial feedstock production and ecosystem services on marginally productive cropland while maintaining food production on prime land.

# Iowa State University

## Report on Activities - Armstrong Farm System Plots

- ✓ In April 2016, we submitted a manuscript (currently under review) to *Agriculture*, *Ecosystems and Environment* on the effects that seed mixture and biochar application have on establishing biofuel plantings. We measured species emergence, cover, and peak and post-frost biomass over four years in three seeding mixtures with or without a biochar application at a rate of 9.3 Mg ha⁻¹. We found that seed mix choice had significant effects on nearly every variable measured, with switchgrass monocultures outperforming the two more diverse mixtures by the third year of the experiment, despite initial establishment failure. In contrast, the high diversity plots exhibited poor sown species recruitment in the establishment year, largely due to high weed pressure in a drought year, but continued to improve over time. Biochar application had no consistent effect on plant biomass or community composition, and affected a single community trait. Our results suggest that perennial bioenergy plantings may be most productive through selection of one or a few high-yielding grass species, rather than through biochar application.
- ✓ As plots established over the three years observed (stand years 2-4), sown species cover increased (Fig. 1). Biochar application had no effect on the relative cover of sown grasses, forbs, or unsown weedy species in early summer. In contrast, species seed mix impacted both sown grass and weedy species cover. Sown forb cover, measurable only within high diversity (HD) plots, also increased from 2013 to 2015. Sown grass cover in switchgrass plots, which initially was low (<5% in 2013), increased to >30% by 2015. Grass cover in low diversity (LD) and HD plots also increased, but more slowly than in switchgrass plots.
- ✓ Between 2014 and 2015, post-frost sown plant yields increased by 36 percent, weed biomass decreased by 85 percent, and switchgrass plots yielded more 38 percent sown biomass and 14 percent less weedy species biomass than LD plots. In 2015 (Fig. 2), HD plots had the lowest estimated total (3.9 Mg ha⁻¹) biomass, while switchgrass plots had three times more total biomass (12.0 Mg ha⁻¹). Yields from LD plots were in the middle, with a total biomass of 8.7 Mg ha⁻¹.



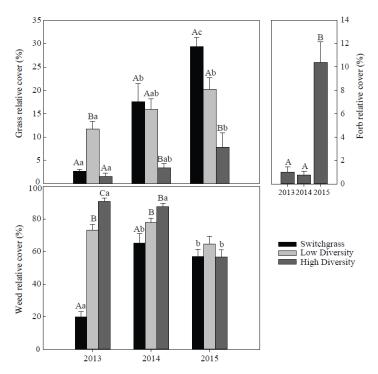


Fig 1. Relative cover in late May-early June, divided into grasses, forbs (high diversity plots only), and weeds, from 2013 to 2015. Capital letters represent significant differences within a year across the three species mixtures, and lowercase letters represent significant differences within a species mixture across the three years. Note: Relative cover may not add up to 100% due to the presence of bare ground.

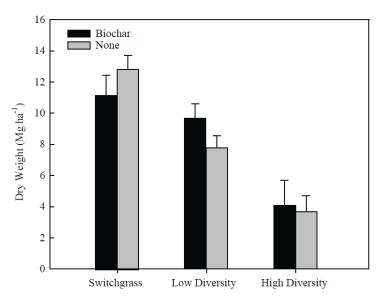


Fig 2. Post-frost yields for 2015 for each seed mix and by biochar treatment.

- Report on Activities Related to the Biochar Rate Trials (Boyd Farm)
- 6 Quarterly Progress Report: February 2016 April 2016



An incubation study was used to determine the impact of biochar application rate on NH<sup>4+</sup> to NO<sup>3-</sup> ratios and potential mineralizable nitrogen (PMN). Soil samples were collected in Fall 2015 from the Boyd field plots (biochar rate trial) by depth (0-5, 5-15 and 15-30 cm). All samples were air dried, passed through a 2mm sieve and analyzed for 2M KCl extractable nitrate and ammonium both before and after incubation. The aerobic incubation was for 28 days at 30<sup>o</sup> C under 60% water filled pore space (WFPS). The results show a consistent increase in soil NH<sup>4+</sup> to NO<sup>3-</sup> ratio with depth for both incubated and non-incubated soils (Figure 1). Soils collected from the field plots generally showed decreasing NH<sup>4+</sup> to NO<sup>3-</sup> ratios with increasing biochar application rate (Fig. 3). However, after the 28-day incubation the NH<sup>4+</sup> to NO<sup>3-</sup> ratio showed a variable response to rate of biochar application.

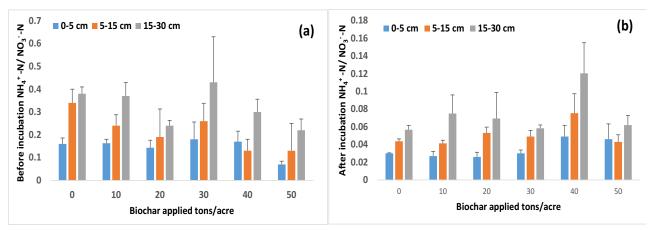


Fig 3. Effect of biochar application rate on soil  $NH_4^+$  -N to  $NO_3^-$ -N ratios by different depths measured before (a) and after (b) a 28 day incubation.

Potentially mineralizable N (PMN) is determined by the difference between the sum of NH<sup>4+</sup>-N and NO<sup>3-</sup>-N measured before and after the 28-day incubation. A significant depth effect on PMN was observed but no clear biochar effect on PMN was found (Table 1).

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Table 1: Effect of biochar on soil Potential Mineralizable N (PMN) by depth for the Boyd field trial.

Biochar tons/acre	PI		
	0-5 cm	5-15 cm	15-30 cm
0.0	37.95 (5.65)*	21.44 (2.13)	16.9 (0.38)
10	36.26 (2.04)	25.3 (0.65)	17.32 (0.97)
20	40.64 (2.21)	17.48 (3.69)	19.41 (5.56)
30	35.5 (2.75)	20.83 (3.53)	15.95 (1.39)
40	40.13 (2.6)	23.87 (0.67)	17.57 (1.18)
50	37.2 (0.8)	27.34 (1.94)	17.67 (1.4)

<sup>\*</sup> Standard error of mean

# **Update of Activities on the Long Term Rotation Plots (Sorenson Farm)**

In spring 2016, prior to planting, surface soil cores (0-15 cm) were taken from all 208 plots to evaluate the effects of biochar and biochar age on soil properties. All plots were tilled in Spring 2015 after biochar applications and before planting. This work is part of a larger study that is investigating the impact of biochar and diverse bioenergy crop rotations on soil quality. Biochar age is defined in this study as time since the biochar was incorporation into the soil environment (i.e. "1 yr" means that biochar was applied in 2015; "2 yr" biochar was applied in 2014; "3 yr", biochar was applied in 2013; and "4 yr", biochar was applied in 2012). Soil cores were collected using a hydraulic probe, with the top 2 cm of every core discarded to minimize the effects of tillage, resulting in a final core volume of 347.5 cm<sup>3</sup>. Sample analysis is ongoing to determine bulk density, pH, EC, total C, total N, and Mehlich III extractable P and K. Soil bulk density is the only parameter determined at this time. The results show a decrease in bulk density with biochar application relative to controls; statistical analysis of the age effect has not yet been conducted.

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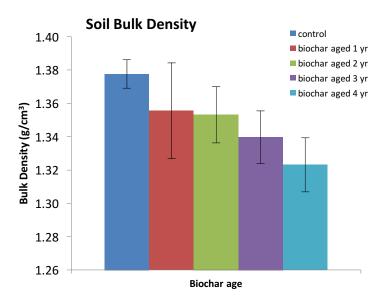


Fig 4. Effect of biochar treatments and biochar age on soil bulk density (g/cm³) for intact soil cores (347.5 cm³) collected from the long-term rotation plots. Error bars indicate standard error.

# • Update on greenhouse study of biochar impacts on GHG emissions

A paper is being prepared for submission to GCB Bioenergy that will report on potential trade-offs in environmental benefits resulting from biochar applications. In this study, we examined pollution trade-offs (NO<sub>3</sub> leaching vs. N<sub>2</sub>O emissions) in parallel greenhouse and field studies resulting from application of mixed wood biochar. Here we report on results of the greenhouse study which compared the effects of fresh and aged biochar and the use of NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> fertilizers on NO<sub>3</sub><sup>-</sup> leaching and N<sub>2</sub>O emissions. Decreased NO<sub>3</sub> leaching from soil columns amended with fresh biochar corresponded with higher N<sub>2</sub>O emissions (Fig. 5); and higher soil moisture in both fresh and aged biochar-amended soils corresponded with higher N<sub>2</sub>O emissions (Fig. 6). Biochar also slightly increased soil moisture and N<sub>2</sub>O emissions in the field study, but the effect of biochar on CO<sub>2</sub> and N<sub>2</sub>O emissions was not significant. Thus, biochars enhanced retention of water and nitrate, both of which are beneficial to crop production, but also can promote emissions of greenhouse gases like N<sub>2</sub>O. Overall the results suggest that application of biochar to agricultural soils may involve trade-offs among soil water retention, NO<sub>3</sub> leaching, and N<sub>2</sub>O emission. Further research is needed to determine whether these trade-offs are significant at the field scale, under what conditions they occur, and whether there are management options that mitigate any adverse effects.

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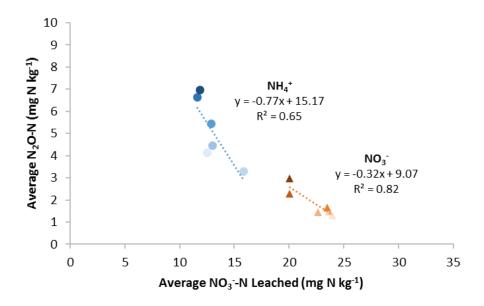


Fig 5. Total  $N_2O$  emissions and  $NO_3$  leached from control and fresh biochar-amended soil columns, with  $N_2O$  emitted and  $NO_3$  leached averaged for each fresh biochar application rate (darker points represent higher biochar application rates;  $NH_4$  fertilized treatments shown in blue circles and  $NO_3$  fertilized treatments shown in orange triangles) (n = 6).

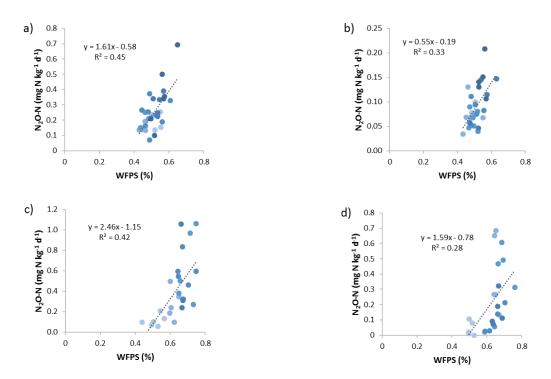


Fig 6. Soil  $N_2O$  emissions rate and water-filled pore space (WFPS, % volume) averaged over all sampling dates from columns with a) fresh biochar and  $NH_4^+$  fertilizer, b) fresh biochar and  $NO_3^-$  fertilizer, c) aged biochar and  $NH_4^+$  fertilizer, and d) aged biochar and  $NO_3^-$  fertilizer, in mg of N per kg of soil (96 Mg ha<sup>-1</sup> aged biochar application rate excluded) (*not to same scale*).



#### Plans for Next Quarter

The 2016 field season has begun and our focus is on maintaining the field plots on the Armstrong, Boyd, and Sorenson farms and collecting the necessary 2016 field data. Soil moisture modeling using the newly developed biochar module in the APSIM cropping systems model is underway and we anticipate significant progress in our efforts to model soil moisture dynamics for the systems plots on the Armstrong Farm. This will be a test of the new biochar module in the APSIM model, which was recently built by the ISU team. Once the biochar APSIM model is fully calibrated and validated it will be used to assess crop response to biochar, management, soils and climate for the various field trials.

The following studies are currently on-going:

- ✓ Laboratory analysis (pH, EC, total C, total N, and Mehlich III extractable P and K) of the 208 soil samples collected in spring 2016 from the long-term rotation plots is ongoing to assess biochar and crop rotation impacts on soil quality.
- ✓ Laboratory study to develop methodology to distinguish between labile and recalcitrant biochar fractions and to quantify the C:N ratio of the labile fraction.
- ✓ Laboratory incubation study using an<sup>15</sup>N tracer designed to assess biochar impacts on soil N cycling.
- ✓ Greenhouse study designed to determine how different types of biochar and biochar aging influence plant available water and water use efficiency.
- ✓ Field and laboratory study designed to assess the viability of the Saxton and Rawls pedotransfer functions for predicting hydraulic properties of biochar amended soils.

# Purdue University

- Laboratory analyses continue on plant biomass including: C, N, cellulose, hemicellulose, lignin, sugar and starch analysis of samples collected at all sites.
- Analysis of soil samples for P and K continues in the studies where P and K nutrition are relevant.
- Greenhouse gas data sets are being assembled and summarized. Comparisons include sorghum, maize, Miscanthus, and switchgrass, all with and without N (150 kg/ha/yr). We also include a native prairie control without N.
- Plot work for the 2016 growing season has begun. This includes application of fertilizers and herbicides as appropriate.



- We continue to sample water flow from the bioenergy plots at the Water Quality Field Station. Water samples are analyzed for nitrate and other contaminants. These biomass systems include dual-purpose sorghum, continuous maize with residue removal, Shawnee switchgrass, and Miscanthus, all managed with best management practices. Controls include a native, drained prairie and a corn-soybean rotation. Planting of the annual systems on these plots has begun
- Because we have 12 site-years (three locations x 4 years) we have terminated the sorghum N rate trial on marginal lands. The analysis of these biomass samples continues and data compilation is underway.
- With reduced funding in 2016 and the no cost extension year, we have suspended studies at the "Throckmorton East" site. This site includes flumes for measuring surface run-off and sediment/nutrient losses. However, we will plant the annual systems (maize, sorghum) into their respective plots, and maintain the perennial biomass systems currently in place (Liberty switchgrass, Miscanthus, poplar).

## University of Illinois

- To minimize weed pressure, all CenUSA plots were burned on March 20, 2016. Also, Pre-emergent herbicide was applied on March 25, 2016 and Post-emergent broadleaf herbicide was sprayed on April 19.
- All CenUSA plots were fertilized with 3N rates (0N, 50N and 100N) on May 5, 2016.





Fig 7. Factor analysis plots (Left: 2012 plots; Right: 2013 plots on May 10, 2016)

# University of Minnesota



# Accomplishments

We completed the biomass sample grinding for both the Becker and Lamberton factor plots and are preparing to send samples to USDA-ARS, Lincoln, Nebraska for analyses. We submitted biomass samples to the University of Minnesota Research and Analytical Laboratory for total tissue N analysis (via combustion), and we have been working on statistical analyses and summarizing results for years 2012-2015 at Becker and 2013-2015 at Lamberton. We also applied early-season herbicide to the Becker factor plot in April 2016.

#### Plans for Next Quarter

We will apply broadleaf herbicide to the grass-only factor plots at Lamberton, and we will be fertilizing as appropriate, beginning in mid-late May 2016. The Becker plot will receive split applications of fertilizer due to excessive drainage on loamy sand. We are awaiting results of the tissue N analyses and will continue working on statistical analyses and summarizing results.

We also plan to showcase the Lamberton factor plots as part of an Organic Crops Field Day in July. While these plots have not been managed organically, switchgrass and other native perennial crops are receiving increased attention in Minnesota after the passage of a 2015 law requiring perennial vegetative buffers on all public ditches and waterways.

## USDA-ARS, Lincoln

## Accomplishments

- ✓ Completed fertilization and herbicide treatments on all plots.
- ✓ Installed anchors for greenhouse gas (GHG) sampling.
- ✓ Initiated GHG sampling.

#### • Factor Analysis Plots

- ✓ Yield data for 2012-2015 is being summarized.
- ✓ Samples collected in 2012, 2013, 2014, & 2015 have been processed and are being scanned and predicted.
- ✓ Plots are being prepared for 2016 field season.

#### System Analysis Plots

- ✓ Samples collected in 2012, 2013, 2014, & 2015 are being scanned & predicted.
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- ✓ Triticale cover crop has been terminated & corn has been planted.
- ✓ GHG samples from 2013-2015 are being summarized.
- ✓ VOM & elongated leaf height data are being summarized.
- ✓ Fields have been prepared for 2016 field season, and the harvest height study and field-scale harvest data is being evaluated.
- The Crop/Livestock/Bioenergy Production System Demonstration site in eastern
  Nebraska was leveraged to get additional funding through the new SDSU NIFA-CAP to
  increase sampling intensity and graze this site in 2016-2019. Cover crops were sampled
  and terminated and corn planted on the site.
- The field-scale herbaceous perennial feedstock research and demonstration site in cooperation with Vermeer Manufacturing near Pella, IA was harvested in early May.

#### • Plans for Next Quarter

- ✓ Scan and predict biomass samples forwarded from other locations.
- ✓ Finalize the scanning & predicting of 2012, 2013, 2014, & 2015 Nebraska biomass samples.
- ✓ Analyze and summarize field data.
- ✓ Collect samples for GHG emissions at scheduled intervals.
- ✓ Submit manuscripts on CenUSA projects.

#### USDA-ARS, Madison

#### 1. Planned Activities

Prepare for 2016 season.

## 2. Actual Accomplishments

- Completed fertilization and herbicide treatments on all plots.
- Completed soil sampling and manure applications.

# 3. Plans for Next Quarter

Prepare for first harvests of 2016.



#### 4. Publications, Presentations, and Proposals Submitted

- Bonin C.L. & E.A. Heaton. (2016). Seeding mixture choice, not biochar application, impacts establishing plant community attributes in native perennial biofuel plantings. Submitted to Agriculture, Ecosystems and Environment (in review).
- Graber, E.R., L. Tsechansky, R.B. Fidel, M.L. Thompson & D.A. Laird. (2016). Determining Acidic Groups at Biochar Surfaces via the Boehm Titration. In: Singh, B., Camps-Arbestain, M., Lehmann, J. (Eds). Methods of Biochar Analysis. CSIRO Publishing, Melbourne, Chapter 8 (in press).
- Mitchell, R., M. Schmer, B. Anderson, V. Jin, K. Balkcom, K. Kiniry, A. Coffin & P. White. (2016). Dedicated energy crops and crop residues for bioenergy feedstocks in the Central and Eastern USA. Bioenergy Research 9:384-398. DOI 10.1007/s12155-016-9734-2.
- Serapiglia, M.J., A.A. Boateng, D.K. Lee, M.D. Casler. (2016) Switchgrass harvest time management can impact biomass yield and nutrient content. Crop Science 56: 1-11 doi: 10.2135/cropsci2015.08.0527.
- Dierking, R.M., D. Allen, S.M. Brouder & J.J. Volenec. (2016). Yield, biomass composition, and N use efficiency during establishment of four *Miscanthus* × giganteus genotypes as influenced by N management. Biomass Bioenergy (in press).
- Presented information to the University of Nebraska Extension Conference on March 16, 2016.
- Presented information to the GAO Bioenergy Review, USDA-ARS-NLAE, Ames, IA, 13 April, 2016.
- Accepted an invitation to serve on the organizing committee and present CenUSA information to the World Bioenergy Congress and Expo to be held in Rome in June, 2016.
- Accepted an invitation to give a keynote address to the World Bioenergy Congress and Expo to be held in Rome in June, 2016.
- Grant submitted: Co-PI: Evaluation of stability of heterosis for biomass yield in prairie cordgrass on marginal land, North-Central Sun Grant. PI: D.K. Lee, University of Illinois.



• Grant submitted: Co-PI: Growing bioenergy crops on marginally productive croplands: implications on erosion and water quality parameters, *North-Central Sun Grant*. PI: Humberto Blanco, University of Nebraska.

#### **Objective 3. Feedstock Logistics**

The Feedstock Logistics objective focuses on developing systems and strategies to enable sustainable and economic harvest, transportation and storage of feedstocks that meet agribusiness needs. The team also investigates novel harvest and transport systems and evaluates harvest and supply chain costs as well as technologies for efficient deconstruction and drying of feedstocks.

## **Iowa State University**

#### 1. Planned Activities

- Continued analysis of data collected during drying experiments and validation of empirical drying prediction models developed from laboratory and field experiments.
- Continued development and evaluation of prototype real-time biomass moisture sensor for switchgrass and corn stover.

## 2. Actual Accomplishments

- A journal paper titled *Estimating dry matter and composition change due to rainfall during field drying of switchgrass and corn stover* has been submitted for review.
- Drying experiments for different maturity stages of switchgrass have been completed and the results analyzed to refine the empirical models for switchgrass and corn stover drying developed. The analysis of results found that solar radiation (r = 0.46) was the most important factor which affected the drying rate of switchgrass during the harvesting period in August and September, followed by vapor pressure difference (VPD) with a Pearson correlation coefficient of 0.40. Wind speed (r =-0.22) and swath density (r = 0.26) were negatively correlated with drying rate, suggesting that increase in wind speed and swath density decreased the drying rate of switchgrass. During the harvesting period in October and November, VPD (r=0.67) influenced the drying rate the most, followed by solar radiation (r = 0.45). Wind speed (r = -0.29) and swath density (r = 0.30) were again negatively correlated with drying rate during later maturity stages. Empirical drying rate models were developed for all maturity stages of switchgrass from August to November. Separate night time equations for corn stover were also developed which accounted for rewetting by due at night.



• Research on the development of sensors capable of predicting moisture content and bulk density of biomass feedstocks based on the dielectric measurements continued during this quarter. The development and design of the electronics for real-time biomass moisture sensor is continuing. At present research is being conducted on the control and operation of High Speed A/D convertors and D/A converters and the integration of these with FPGA filtering algorithms to determine signal attenuation and phase shift.

# 3. Explanation of Variance

No variance in planned activities has been experienced.

## 4. Plans for Next Quarter

Research activities planned during next quarter include:

- Continued Analysis of data collected during drying experiments and validation empirical drying prediction models developed from laboratory and field experiments.
- Continued development and evaluation of prototype real-time biomass moisture sensor for switchgrass and corn stover.

# 5. Publications, Presentations, and Proposals Submitted

Estimating dry matter and composition change due to rainfall during field drying of switchgrass and corn stover. Khanchi, A. & S.J. Birrell. Submitted for review.

# **University of Wisconsin**

#### 1. Planned Activities

Our objectives for the quarter included:

- Complete work on modifying an experimental high-density baler and conduct initial field evaluation;
- Compress large-square bales and quantify pressure-density relationship;
- Continue to assess the economic viability of the various grass harvest and processing options using the Integrated Biomass Supply Analysis and Logistics Model (IBSAL);
- Conduct an evaluation of how bale density affects twine tension in large square bale; and
- Submit an additional two manuscripts for publication review.

#### 2. Actual Accomplishments



An experimental baler from an independent inventor has been obtained. This baler does not use the typical reciprocating plungerhead approach to densification. It is intended to produce much greater biomass bale density than conventional approaches. Redesign and modifications were completed and the baler was tested in reed canarygrass and switchgrass in April. Although portions of very high density bales were formed, there were many performance issues related to feeding and crop flow that prevented steady-state evaluation of the machines performance. Crop flow issues were identified and redesign is underway to address these deficiencies.

The bale press intended to compress large square bales to double density was redesigned and modifications are almost complete. Biomass bales will be recompressed with the improved test fixture this coming summer. The goal is to collect force, energy and density data to model the recompression process of large square bales and compare to data collected on recompressing large round bales.

The large square bale remains the dominant package for biomass crops. Plastic twine is used to secure material in the bale and limit re-expansion. As bale density increases, larger diameter twine is required, which adds to harvest costs. The cost of twine can exceed \$3 per dry ton, which is almost 25percent of the total cost of baling. Twine failure and broken bales anywhere in the logistics system adds considerably to the delivered costs due to the additional inefficient handling required to deal with broken bales. We have developed a system using bending beam load cells to measure twine tension as the bale is made. The system was used when harvesting overwintered reed canarygrass, switchgrass and sorghum across a wide variety of bale densities. Models of twine tension as a function of bale density have been developed.

## 3. Explanation of Variance

Work continues on manuscripts, but progress is slow due to difficulties with integrating our data with IBSAL.

#### 4. Plans for Next Quarter

Our efforts in the next year will include:

- Complete redesign and modifications to the experimental high-density baler and conduct further field evaluation;
- Compress large square bales and quantify pressure-density relationship;
- Continue work on twine tension for large square bales;
- Conduct an outdoor storage study of large square bales covered with breathable film;



- Have our intern work on assessing the economic viability of the various grass harvest and processing options using IBSAL, and
- Complete manuscripts for publication review.

# 5. Publications, Presentations, and Proposals Submitted

• None this quarter.

# Objective 4. System Performance Metrics, Data Collection, Modeling, Analysis and Tools

This objective provides detailed analyses of feedstock production options and an accompanying set of spatial models to enhance the ability of policymakers, farmers, and the bioenergy industry to make informed decisions about which bioenergy feedstocks to grow, where to produce them, what environmental impacts they will have, and how biomass production systems are likely to respond to and contribute to climate change or other environmental shifts.

We focus on four overarching tasks:

- Task 1. Adapt existing biophysical models to best represent data generated from field trials and other data sources
- Task 2. Adapt existing economic land-use models to best represent cropping system production costs and returns
- Task 3. Integrate physical and economic models to create spatially explicit simulation models representing a wide variety of biomass production options
- Task 4. Evaluate the life cycle environmental consequences of various bioenergy landscapes.

# **Iowa State University**

#### 1. Planned Activities

The first two broad tasks under the System Performance objective are to adapt existing biophysical models to best represent field trials and other data and to adapt existing economic land-use models to best represent cropping system production costs and returns.

#### 2. Actual Accomplishments

A paper submitted to the NAREA association journal concerning tradeoffs between food, fuel and water quality in a watershed with limited availability of land has been accepted for publication. We assess empirically how agricultural lands should be used to produce the



highest valued outputs, including food, energy, and environmental goods and services. We explore the efficiency trade-offs associated with allocating land between food and bioenergy. We use a set of market prices and non-market environmental values to value the outputs produced by these crops. Finally, we explore the degree to which using marginal land for energy crops is an approximately optimal rule.

Working with colleagues from Purdue, we have submitted a set of four papers for consideration to the Journal of the American Water Resources Association related to water quality and watershed scale modeling of biofuel crop adoption. In a policy focused overview paper, the implications and value of watershed-based modeling of the productive potential and water quality impacts associated with multiple cellulosic biofuel cropping systems are discussed. The three companion papers study large-scale conversion of cropland to switchgrass, *Miscanthus* or corn stover removal, targeting of those three biofuel cropping systems to cropland landscapes identified as marginal based on slope or other criteria, and/or a large scale evaluation of cellulosic biomass potential and water quality impacts of switchgrass and corn stover removal across the Upper Mississippi and Ohio-Tennessee River Basins. The potential for policy design to improve the performance of these systems based on the findings of these modeling studies is identified.

From October 11-12, 2015 a workshop funded by the NSF on the food, energy, water nexus was held at Iowa State University. A white paper has been completed entitled *Research* needs and Challenges in the FEW System: Coupling Economic Models with Agronomic, Hydrologic, and bioenergy Models for Sustainable Food, Energy, and Water Systems. The paper is available at <a href="http://www.card.iastate.edu/publications/synopsis.aspx?id=1246">http://www.card.iastate.edu/publications/synopsis.aspx?id=1246</a>.

## 3. Explanation of Variance

No variance has been experienced.

#### 4. Plans for Next Quarter

We hope to get review comments back on the four submitted papers soon and begin revisions. Also, based on the findings of the scenarios undertaken in the three watershed studies additional scenarios are being developed to evaluate the water quality tradeoffs associated with the simultaneous use of stover and perennial feedstocks.

#### 5. Publications, Presentations, and Proposals Submitted

Valcu, A,. C.L. Kling & P. Gassman. The Optimality of Using Marginal Land for Bioenergy Crops: Tradeoffs between Food, Fuel, and Environmental Services. Northeast Agricultural and Resource Economics Association Journal. Forthcoming 2016.



Kling, C.L., I. Chaubey, R. Cibin, P.W. Gassman & Y. Panagopoulos. Policy Implications from Multi-Scale Watershed Models of Biofuel Crop Adoption across the Corn Belt. Submitted to the Journal of the American Water Resources Association, May 2016.

#### **University of Minnesota**

#### 1. Planned Activities

- Task 3. Integrate physical and economic models to create spatially-explicit simulation models representing a wide variety of biomass production options.
- Task 4. Evaluate the life cycle environmental consequences of various bioenergy landscapes.
- Task 5. Employ the modeling systems to study the design of policies to cost effectively supply ecosystem services from biomass feedstock production.

## 2. Actual Accomplishments

This quarter, we focused on InMAP air quality model maintenance and on preparing our manuscript on the life cycle air quality impacts of switchgrass production and its potential implications for Renewable Fuel Standard mandated biofuels. We plan to submit this manuscript next quarter. We also provided revisions to a manuscript on the social cost of nitrogen.

## 3. Explanation of Variance

No variance has been experienced.

#### 4. Plans for Next Ouarter

Next year includes continued work on Tasks 4, and 5.

# 5. Publications, Presentations, and Proposals Submitted

None.

#### **Post-Harvest**

# Objective 5. Feedstock Conversion and Refining: Thermo-chemical Conversion of Biomass to Biofuels

The Feedstock Conversion and Refining Objective will perform a detailed economic analysis of the performance of a refinery based on pyrolytic processing of biomass into liquid fuels and will



provide biochar to other CenUSA researchers. The team concentrates on two primary goals:

- Estimating energy efficiency, GHG emissions, capital costs, and operating costs of the proposed biomass-to-biofuels conversion system using technoeconomic analysis;
- Preparing and characterizing Biochar for agronomics evaluations.

#### 1. Planned Activities

# Techno-economic Analysis

Estimate the Minimum Product Selling Prices (MPSP), Maximum Investment Cost (MIC), and the uncertainty of the prices of the different lignin-derived chemicals.

# • Prepare and Characterize Biochar

The manuscript entitled "Aluminum and iron biomass pretreatment and impacts on biochar anion exchange capacity" will be completed. Laboratory research will assess pyrolysis and feedstock and temperature influences on the production of biochar-zero valent iron composites. A miscible displacement experiment will be designed and imitated to test the hypothesis that BC-ZVI are effective for dechlorination of trichloroethylene in soils.

# 2. Actual Accomplishments

#### • Techno-economic Analysis

In this period, we developed uncertainty prices of lignin-derived chemicals from the solvent liquefaction of lignocellulosic biorefinery lignin streams. The lignocellulosic biorefinery converts corn stover to ethanol via fermentation, and it primarily combusts the unconverted lignin into power. In this analysis, a fraction of the lignin is converted into chemicals using a solvent liquefaction process. The techno-economic uncertainty of this process is characterized by uncertainty in the fermentation and solvent liquefaction process performance.

Figure 8 shows a diagram of the corn stover fermentation to ethanol with lignin to power and chemicals via solvolysis concept design. The process is similar to the fermentation with pyrolysis concept investigated during the last reporting period. The fast pyrolysis process is replaced here with solvent liquefaction (solvolysis). Solvolysis employs a pressurized solvent at temperatures of 200 - 400 °C to convert biomass into a mostly liquid bio-crude product. Solvolysis often employs water as the operating fluid in which case it is also known as hydrothermal processing. However, Iowa State University is working on the use of various solvents which confer operational and product quality



advantages compared to water. As shown in Figure 8, the solvolysis process would take the lignin stream recovered after solid separation. This stream has a high moisture content of over 45 wt. %. Although it can be combusted, solvolysis could yield higher value chemicals from this stream.

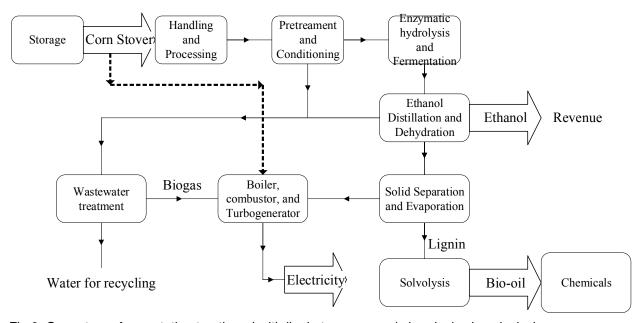


Fig 8. Corn stover fermentation to ethanol with lignin to power and chemicals via solvolysis

We obtained experimental data for lignin solvolysis describing the potential yields of various chemicals as shown in Figure 9. Figure 9 includes GC-MS relative content measurements of various chemicals measured from lignin solvolysis bio-oil treated with ethanol (B1), formic acid + ethanol at a 3:1 ratio (B2), and formic acid alone (B3). As shown, vanillin relative content in the bio-oil varies between 7.45 and 11.78 wt. %. Several other high-valued chemicals are included in the "Other" category. For this period, we focused on p=HydroxyBenzoic acid, p-HydroxyBenzaldehyde, vanillic, syringic acid, vanillin, syringaldehyde, p-Coumaric acid, and ferulic acid.

The baseline model for this study builds upon the National Renewable Energy Laboratory's enzymatic hydrolysis and fermentation model<sup>2</sup>. Key assumptions for this

<sup>&</sup>lt;sup>1</sup> Ouyang, X., Huang, X., Zhu, Y. & X. Qiu (2015). Ethanol-Enhanced Liquefaction of Lignin with Formic Acid as an in Situ Hydrogen Donor. *Energy & Fuels* 29, 5835–5840. Available on line http://pubs.acs.org/doi/abs/10.1021/acs.energyfuels.5b01127

<sup>&</sup>lt;sup>2</sup> Humbird, D., Davis, R., Tao, L., Kinchin, C., Hsu, D., Aden, A., & D. Sexton. (2011). Process design and economics for biochemical conversion of lignocellulosic biomass to ethanol: dilute-acid pretreatment and



model include: feedstock capacity of 2000 dry metric tons per day; ethanol yield of 79 gallons per ton; and feedstock price of \$58 per ton. The feedstock properties are based on the NREL report, and cellulose, hemicellulos, lignin are represented by glucan, xylose, and lignin respectively in the model. The solvolysis model is based on ISU work on hydrothermal processing.<sup>3</sup>

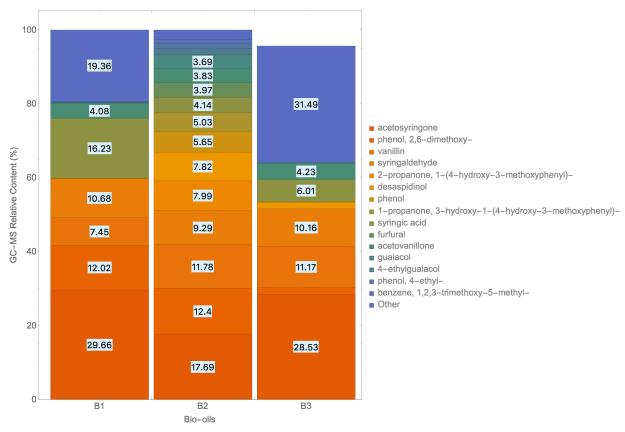


Fig 9. GC-MS relative content of key chemicals found in bio-oil samples from lignin solvolysis treated with ethanol (B1), formic acid+ethanol (B2), and formic acid (B3).

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enzymatic hydrolysis of corn stover.(No. NREL/TP-5100-47764). National Renewable Energy Laboratory (NREL), Golden, CO. Available online <a href="http://www.nrel.gov/docs/fy11osti/47764.pdf">http://www.nrel.gov/docs/fy11osti/47764.pdf</a>.

<sup>&</sup>lt;sup>3</sup> Ou, L., Thilakaratne, R., Brown, R. C., & M.M. Wright. (2015). Techno-economic analysis of transportation fuels from defatted microalgae via hydrothermal liquefaction and hydroprocessing. *Biomass and Bioenergy*, *72*, 45-54. Available online <a href="http://www.sciencedirect.com/science/article/pii/S0961953414005248">http://www.sciencedirect.com/science/article/pii/S0961953414005248</a>.



We varied several feedstock and process parameters from the ethanol + solvolysis model to determine the overall uncertainty range. Table 2 shows the min, median, and max values employed to develop triangular distributions of the parameters. We collected up to 10,000 samples from these distributions calculated the minimum ethanol selling price (MESP) for the various chemicals, and the minimum product selling price (MPSP) for the chemicals based on a fixed ethanol price.

Table 2. Uncertainty triangular distribution parameters for feedstock properties and conversion to ethanol.							
Weight %	Min	Median	Max				
Glucan Content	0.245	0.351	0.456				
Xylan Content	0.137	0.195	0.254				
Lignin Content	0.110	0.158	0.205				
Pretreatment Glucan to Glucose Conversion	0.067	0.100	0.133				
Enzymatic Hydrolysis Glucan to Glucose Conversion	0.833	0.944	1.055				
Xylan to Xylose Conversion	0.909	0.977	1.045				
Glucose to Ethanol Conversion	0.384	0.435	0.486				
Xylose to Ethanol Conversion	0.384	0.422	0.460				
Chemical Yields in Weight %				Price (\$/Ton)			
Lignin to p-HydroxyBenzoic Acid Yield	0.015	0.018	0.022	353,080			
Lignin to p-HydroxyBenzaldehyde Yield	0.023	0.029	0.035	426,520			
Lignin to Vanillic Yield	0.015	0.019	0.022	2,028,400			
Lignin to Syringic Acid	0.020	0.025	0.030	6,110,000			
Lignin to Vanillin	0.163	0.203	0.244	93,520			
Lignin to Syringaldehyde	0.144	0.180	0.216	3,783,200			
Lignin to p-Coumaric Acid	0.009	0.011	0.013	50,940,000			
Lignin to Ferulic Acid	0.013	0.017	0.020	12,572,000			

Figure 10 shows the minimum ethanol-selling price (MESP) based on the sale of a specific lignin-derived chemical. As shown, p-HydroxyBenzoic Acid yields an MESP of between \$1.5 and 4 per gallon assuming the chemical is sold at its market value (listed in Table 1). Negative MESPs indicate the facility makes sufficient profits from the chemicals alone to be profitable at a 10% internal rate of return (IRR). Although these results appear promising, lignin solvolysis is a developing technology and these yields have not been demonstrated at commercial scale.



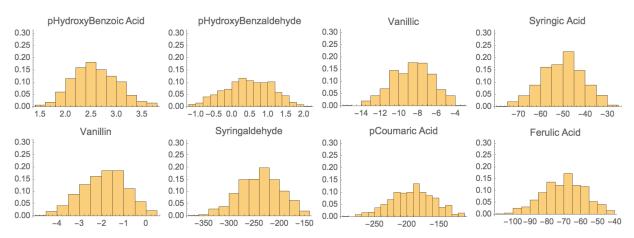


Fig 10. Minimum Ethanol-Selling Price (\$/gallon) vs. Probability for Corn Stover to Ethanol and Ligninderived Chemicals

Figure 11 shows the minimum product-selling prices (MPSP) of selling the ligninderived chemicals with a fixed ethanol price of \$2.05 per gallon. As shown, MPSPs range from under \$30,000 per ton such as vanillin to over \$1.2 million per ton as is the case for p-Coumaric Acid. The probability distributions are based on the simulated uncertainty in ethanol and chemical yields.

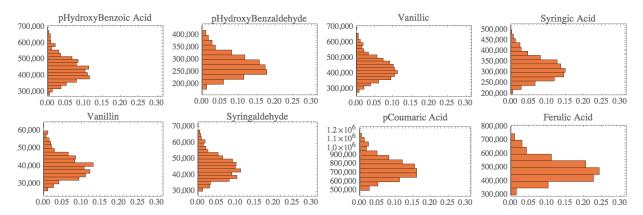


Fig 11. Minimum Product-Selling Prices probabilities of various lignin-derived chemicals.

These results indicate the range of potential ethanol and chemical prices from an integrated lignocellulosic ethanol and chemicals biorefinery. Future work will focus on developing a detailed model of the production and recovery of the specialty chemicals to reduce the uncertainty in the expected yields and selling prices.



#### **Prepare and Characterize Biochar**

The manuscript entitled Accelerated aging of biochars: Impact on anion exchange capacity has been published: The manuscript entitled Aluminum and iron biomass pretreatment and impacts on biochar anion exchange capacity is nearly completed and recent discussion among authors may call for additional experimental. Research on biochar-zero valent iron composites is continuing and a manuscript entitled "Feedstock and temperature influences in the production of biochar-zero- valent iron composites" has been prepared for Green Chemistry and is final editing. A meeting will soon be called among the authors to finalize this manuscript.

A miscible displacement experiment designed to test the hypothesis that biochar-zero valent iron (BC-ZVI) composites can remove trichloroethylene from water better than zero valent iron alone has begun. Due to the high hydraulic conductivity of BC-ZVI previously produced, another approach to produce such material from lignin and magnetite has been initiated. Biochars were produced by this approach which involved compression molding precursor materials to ensure thorough mixing and subsequent pyrolysis. Characterization of the resulting material has begun.

X-ray diffractometry revealed that BC-ZVI can be produced from lignin and magnetite by one-step pyrolysis, with diffraction evidence for ZVI depicted by the reflections of α-Fe. Residual magnetite is detected in BC-ZVI produced from a mixture of 30 percent lignin and 70 percent magnetite, with nearly complete reduction of magnetite achieved in biochar produced from 50 percent lignin/ 50 percent magnetite (Fig. 12). BC-ZVI produced from 30 percent lignin / 70% magnetite also exhibited evidence of residual wüstite, which is unstable at low pressure and temperature and is expected to transform to magnetite and ZVI in time.

Additionally, XRD (Fig. 12) and Raman IR spectroscopic evidence (Fig. 13) for graphite illustrate the graphitizing nature of lignin and secondary char formation by the reduction of carbonaceous gases by reduced forms of Fe during high temperature pyrolysis. The presence of characteristic disorder (D) and graphite ring stretch (G) bands in the Raman spectra reveal that carbon transformed in pyrolysis is graphitic, however the high D/G band intensities (Table 3) reveal that this graphite is highly disordered.

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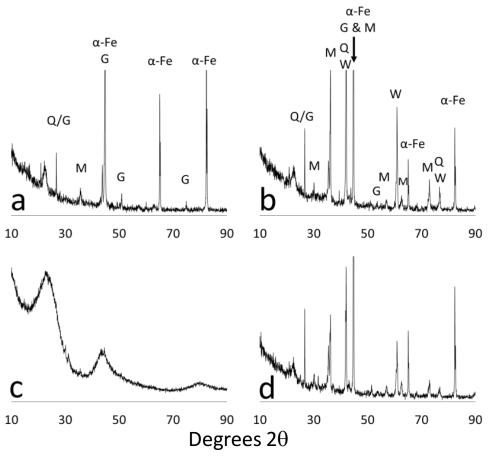


Fig 12. XRD patterns of BC-ZVI produced from lignin and magnetite and control biochar produced from lignin by pyrolysis of at 900°C. Letter identifiers describe feedstock preparation:  $\mathbf{a} = 50\%$  lignin / 50% magnetite, **b** = 30% lignin / 70% magnetite (pressed), **c** = control biochar from lignin, **d** = 30% lignin / 70% magnetite (unpressed). Mineral phases identified include Q=quartz, M=magnetite, G=graphite, W= Wüstite, and  $\alpha$ -Fe=ZVI.

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Table 3. XRD peaks used to identify mineral phases found in 900°C BC-ZVI. Symbols used in the table:  $^{\circ}$  2 $\Theta$  is the two-theta diffraction angle, d(Å) is the basal spacing of crystal structures in Angstroms, and I(f) is the percent intensity of the reflection, relative to the most intense peak. Source JADE version 9.0.

G	iraphite		(	Quartz		M	agnetite		Z۱	/I (α-Fe)		١	Nüstite	
° 20	d(Å)	l(f)	° 2 <del>0</del>	d(Å)	l(f)	° 2 <del>0</del>	d(Å)	l(f)	° 2 <del>0</del>	d(Å)	l(f)	° 2 <del>0</del>	d(Å)	l(f)
26.506	3.36	100	20.86	4.255	20	30.095	2.967	30	44.67	2.027	100	36.267	2.475	50
44.6	2.03	50	26.644	3.343	100	35.423	2.532	100	65.033	1.433	20	42.091	2.145	100
54.652	1.678	80	36.542	2.457	10	43.059	2.099	20	82.352	1.17	30	61.121	1.515	60
77.4	1.232	30	39.474	2.281	10	53.379	1.715	10	99.001	1.013	10	73.197	1.292	20
83.394	1.158	50	42.444	2.128	10	56.936	1.616	30	116.47	0.906	10	77.029	1.237	20
86.907	1.12	20	50.137	1.818	10	62.539	1.484	40	137.142	0.8275	10	91.871	1.072	10
101.6	0.994	40	59.982	1.541	10	73.929	1.281	10				103.19	0.983	10

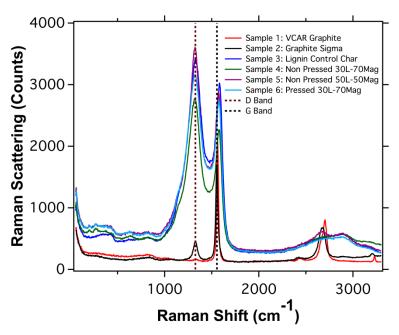


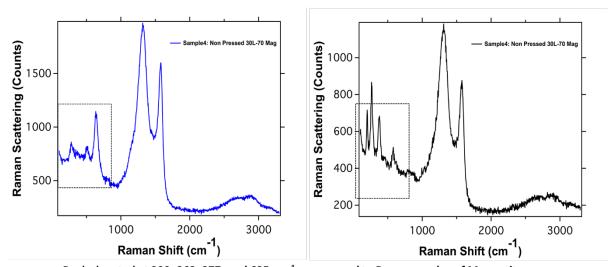
Fig 13. Raman spectra of 900°C BC-ZVI derived from lignin and magnetite and graphite standards.

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Table 4. Raman analysis of carbon in 900°C BC-ZVI.								
Sample	D Band	G Band	D/G Height Ratio	D/G Area Ratio				
	Location	Location	Location	Location				
VCAR Graphite	1332 cm <sup>1</sup>	1576 cm <sup>1</sup>	0.113 ± 0.002	0.03 ± 0.02				
Graphite Sigma 1	1326 cm <sup>1</sup>	1554 cm <sup>1</sup>	$0.02 \pm 0.03$	$0.3 \pm 0.1$				
Lignin Control Char	1329 cm <sup>1</sup>	1576 cm <sup>1</sup>	$1.2 \pm 0.8$	$3 \pm 5$				
Non pressed 30L – 70 Magnetite	1318 cm <sup>1</sup>	1569 cm <sup>1</sup>	$1.2 \pm 0.9$	$3 \pm 5$				
Non pressed Lignin 50 - 50	1322 cm <sup>1</sup>	1567 cm <sup>1</sup>	$1.2 \pm 0.9$	$3 \pm 4$				
Pressed 30L – 70 Magnetite	1322 cm <sup>1</sup>	1571 cm <sup>1</sup>	$1.2 \pm 0.8$	3 ±6				

The effect of mixing of precursor materials is seen by the loss of magnetite character for 900°C BC-ZVI in pressed versus unpressed material (Fig. 14). Greater intensity of the wüstite reflections in the XRD pattern of pressed versus unpressed material indicates greater transition of magnetite to wüstite in the pressed samples. This transformation is seen in the Raman spectra which reveal complete loss of magnetite vibrational modes in 900°C BC-ZVI produced from pressed material. Greater mixing and dispersion of magnetite particles within a lignin matrix effected better conversion of magnetite to ZVI.



Peaks located at 200, 262, 377, and 635 cm<sup>-1</sup> correspond to Raman modes of Magnetite (FeFe<sub>2</sub>O<sub>4</sub>), this was observed is some replicates in sample 4: Non Pressed 30L-70 Magnetite but not in Sample 5: Pressed 30L-70 Mag

Fig 14. Raman spectra of pressed and unpressed 900°C BC-ZVI derived from lignin and magnetite.



#### 3. Explanation of Variance

Delay on the submission of "Aluminum and iron biomass pretreatment and impacts on biochar anion exchange capacity" to *Nature Chemistry* has been due to the necessity of producing a quality manuscript as the rejection rate of this journal is very high. The necessity of producing BC-ZVI with appropriate **physical** and chemical characteristics effected the need to start anew with BC-ZVI production, however has led to a more attractive pathway to producing such material due to the low cost of feedstock materials and reduced chlorinated effluent and concomitant PAH production in the process.

#### 4. Plans for Next Quarter

# • Techno-economic Analysis

Chemical recovery and purification systems, operating costs and capital costs will be investigated. Process models for chemical recovery with a focus on higher value chemicals will be developed. A manuscript on the costs of lignin-derived chemicals from an integrated refinery will be prepared.

# • Prepare and Characterize Biochar

Both manuscripts discussed will be submitted for publication and a third manuscript based on BC-ZVI derived from lignin and magnetite and its performance in a miscible displacement experiment will be prepared.

#### 5. Publications, Presentations, and Proposals Submitted

See Above

## **Objective 6. Markets and Distribution**

The Markets and Distribution objective recognizes that a comprehensive strategy that addresses the impacts to and requirements of markets and distribution systems will be critical to the successful implementation and commercialization of a regional biofuels system derived from perennial grasses grown on land unsuitable or marginal for the production of row crops. To create this comprehensive strategy, the team focuses on two unifying approaches:

- The study and evaluation of farm level adoption decisions, exploring the effectiveness of policy, market and contract mechanisms that facilitate broad scale voluntary adoption by farmers; and
- Estimate threshold returns that make feasible biomass production for biofuels.
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#### 1. Planned Activities

- Continue work on a spatial model of biomass supply.
- Continue work on the economic feasibility of grasses by modelling the cost optimization problem of a unique plant under different market structures. Initial findings suggest that agriculture residues will not be available in sufficient quantities to meet the mandate at relatively low prices, and this will provide an opportunity for perennial grasses.

## 2. Actual Accomplishments

- **Spatial Model of Biomass Supply**. Ongoing. Hayes and Jacobs are working with industry partner DuPont to identify optimal market segmentations for biomass collection systems and contracting. This work is expected to be complete during summer of 2016.
- Economic Feasibility of Grasses. Ongoing.

# 3. Explanation of Variance

None. All activities are moving forward according to the project schedule.

# 4. Plans for Next Quarter

During the next quarter our team will continue work on planned activities outlined above.

## 5. Publications, Presentations, and Proposals Submitted

None.

## **Objective 7. Health and Safety**

The production of bioenergy feedstocks will have inherent differences from current agricultural processes. These differences could increase the potential for workforce injury or death if not properly understood and if effective protective counter measures are not in place.

The Health and Safety team addresses two key elements in the biofuel feedstock supply chain:

- The risks associated with producing feedstocks; and
- The risks of air/dust exposure.

## 1. Task 1. Managing Risks in Producing Biofeedstocks

## • Planned Activities



Final calculations of risk between production systems will be completed and potential areas for model improvement will be identified for future efforts. A technical paper will be written for the International Society of Agricultural Safety and Health professional improvement committee. The preparation of a peer review journal article that shares the results of the model will begin.

## • Actual Accomplishments

✓ The Monte Carlo output frequency distribution of the difference in risk ranges from the comparative risk assessment model indicates that corn production systems will produce a higher likelihood of worker injury more often than biofuel switchgrass production systems (Fig. 15). Corn productions systems had a higher likelihood of human injury (82%) than biofuel switchgrass production systems (18%) over a 10-year life cycle. The zero is where worker injury risk between corn and biofuel switchgrass production systems is equal.

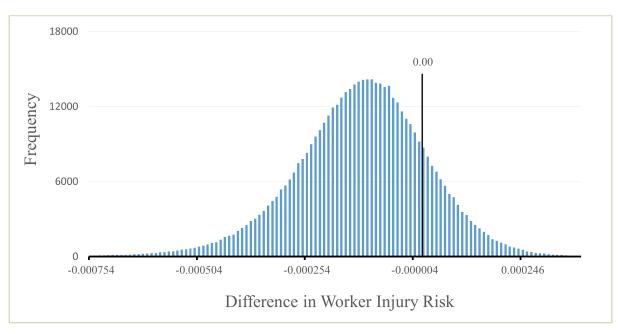


Fig 15. Difference in worker injury risk output frequency distribution from the comparative risk assessment model based on final calculation using 500,000 iterations.

The research conducted was an initial estimate of the difference in worker injury risk for agricultural workers in corn and biofuel switchgrass production systems. One improvement would be the collection of more detailed data for exposures and injuries that would yield a more accurate estimated of worker injury risks. The availability of



that type of detailed agricultural exposure and injury data is a limiting factor. It is not expected that the availability of detailed agricultural data will occur soon but the model will accept it when it becomes available. An important future test would be to validate this model by assessing the risk between two agricultural systems with abundant exposure and injury data sources.

✓ A technical paper proposal was accepted for presentation at the International Society of Agricultural Safety and Health conference 2016. The technical paper has completed the review process and the presentation will occur in Lexington, KY during June.

The initial stages of the journal selection and manuscript preparation have begun. Publishing the findings of this project task will be completed with this manuscript.

## • Explanation of Variance

None to report.

## Plans for Next Quarter

The technical paper will be presented at the International Society of Agricultural Safety and Health conference in Lexington, Kentucky in June 2016. The preparation of the manuscript for a peer review journal article that shares the results of the model should be completed and submitted to the selected journal.

## Publications, Presentations, and Proposal Submitted

Ryan, S.J., C.V. Schwab & G.A. Mosher. (2016). Comparing worker injury risk in corn and switchgrass production systems: Results from a probabilistic risk assessment model. International Society for Agriculture Safety and Health. International Meeting Normal, Illinois. ISASH Paper No. 16-03. ISASH Urbana, IL 61801.

## 2. Task 2 – Assessing Primary Dust Exposure

#### Planned Activities

Receive approval for modifications to the human subjects study.

## • Actual Accomplishments

Approval was not obtained at the time of this report.

# • Explanation of Variance

Extra efforts are needed to complete this part of the task.

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#### Plans for Next Quarter

Receive approval for modifications to the human subjects study.

## Publications, Presentations, and Proposal Submitted

No publication, presentations or proposal submitted from this task.

#### **Education and Outreach**

## **Objective 8. Education**

The Education Objective seeks to meet the future workforce demands of the emerging bioeconomy through two distinct subtasks, as follows:

- To develop a shared bioenergy curriculum core for the Central Region.
- To provide interdisciplinary training and engagement opportunities for undergraduate and graduate students

Subtask 1 is curriculum development. Subtask 2A is training undergraduates via a 10-week summer internship program modeled on the highly successful NSF REU (research experience for undergraduates) program. Subtask 2B is training graduate students via a two-week summer intensive program modeled on a highly successful industry sponsored intensive program in biorenewables the team led in 2009. Subtask 2C is training graduate students via a monthly research webinar. The next portion of this report is broken into subtasks.

## **Subtask 1: Curriculum Development**

#### 1. Planned Activities

Module 3. Harvesting

Bring edited lessons online to Moodle site.

Module 13. Preprocessing of biomass feedstocks

Refine and add content to existing rough draft.

- Module 14. Biochemical conversion
  - ✓ Complete draft of initial Moodle lessons.



✓ Complete content draft of anaerobic digestion.

# Module 15. Thermochemical conversion

- ✓ Complete draft content lessons for gasification and pyrolysis.
- ✓ Identify audio/video clips from past Cenusa presentations and other sources that can be used in the Moodle module

## Module 17. Introduction to Conversion

- ✓ Complete draft content.
- ✓ Begin drafting Moodle lesson.

# 2. Actual Accomplishments

# Module 3. Harvesting

Materials summarizing research findings from Objective 3. Logistics were obtained from Kevin Shinners at UW-Madison and will be integrated into the existing module materials in the next quarter.

## **Module 9. Enterprise Budgeting**

Cost estimation tool was obtained from Keri Jacobs (Objective 4).

#### Module 14. Bioconversion

Draft of content of additional lesson of anaerobic digestion was created with Daniel Andersen at Iowa State University. Arrangements to capture footage of two operating anaerobic digester systems and interview facility operators have been made. Written and voice script for the pretreatment, hydrolysis and fermentation lessons in Moodle were created.

#### **Module 15. Thermochemical Conversion**

Obtained summary research results from Robert Brown (Objective5) on pyrolysis and gasification.

# Module 16. Biofuel Quality of Perennial Warm Season Grasses

Completed initial draft of content

## 3. Explanation of Variance



# Module 3. Harvesting

It was decided to update the module lesson with research findings from the Objective 3 prior to making the materials available on the Moodle site. Content for an additional lesson on field capacity and efficiency of harvesting perennial grasses will be created.

# Module 13. Preprocessing of Biomass Feedstocks) and Module 17. Introduction to Conversion

Work on these modules was suspended during this quarter to focus on activities in Module 14. Work on modules 13 & 17 will continue in the fourth quarter.

# 4. Plans for Next Quarter

# Module 3. Harvesting

Complete all edits to the existing Moodle lessons and submit to industry partner for review prior to submittal for publication.

# Module 13. Preprocessing of Biomass Feedstocks

Refine and add content to existing rough draft.

#### Module 14 Biochemical Conversion

- ✓ Complete edits of Moodle lessons for pretreatment, hydrolysis and fermentation.
- ✓ Complete filming and editing activities for anaerobic digestion lesson and make all parts of the lesson script available for creation of the Moodle lesson.

## Module 15 (Thermochemical Conversion)

Integrate video content into Powerpoint content script.

## • Conversion Modules 17 (Introduction to Conversion)

Complete draft content.

## 5. Publications, Presentations, and Proposals Submitted

None to report this period.

# Subtask 2A: Training Undergraduates via Internship Program

## 1. Planned Activities



- Continue to promote the undergraduate internship program and encourage application submissions through the application deadline.
- Centrally vet and rank applications based on the letters of interest, academic achievement, previous research experience, and letters of recommendation.
- Send pool of likely candidates to faculty hosts for review late March 2016.
- Conduct phone interview of highly ranked students in early April.
- Make offers and complete cohort by end of April.
- Begin to arrange travel for accepted students.
- Secure housing for students who will be placed with faculty mentors at partner institutions.

## 2. Actual Accomplishments

- Robust promotion of the program yielded a pool of highly-qualified applications by deadline.
- Central vetting and ranking of the applications completed late March 2016.
- Pool of likely candidates packaged and given to faculty hosts for review and responses received early April as requested.
- Student phone interviews with Raj Raman took place the second and third weeks of April.
- First offers were extended mid-April, second offers were extended the third week of April, and a cohort of 11 students was finalized late April.
- The 2016 CenUSA Bioenergy Internship cohort consists of 11 students who will be placed at Iowa State University, University of Nebraska-Lincoln, Purdue University, and the University of Wisconsin, Madison. Gender diversity is 6 women, 5 men, one Hispanic/Latino, one Asian.
- All non-selected candidates were notified as soon as selections were final.

## 3. Explanation of Variance

None.

## 4. Plans for Next Quarter



- Finalize all logistics; student travel, lodging at Iowa State and all partner institutions (University of Wisconsin Madison, University of Nebraska Lincoln, and Purdue University), and administration of stipends.
- Provide mentor training using a 15-minute video (created by Raj Raman). We will share the link with the internship mentors (faculty/grad student/post doc) in mid-May, followed by a combined face-to-face (for ISU-based mentors) and virtual (via Zoom for partners) meeting to clarify any questions and concerns.
- Launch the program on June 1, 2016 with the arrival of the students. Run the orientation at Iowa State the following four days, then send students to lab placements for start date on June 6, schedule weekly meetings (June 6 Aug 1) with student interns to discuss progress, face-to-face for ISU students and virtual (via Zoom) for partner-placement students.
- Iowa State University's Research Institute for Studies in Education (RISE) will administer a pre-program survey to assess students on June 2. This provides a baseline for program evaluation.
- The ISU-based interns will participate in a team-building canoe trip on the Des Moines River on Saturday, June 4.
- All partner-placed students will return to Iowa State in the last week of the program in preparation for the close of the program. All students participate in the closing celebration brunch with faculty and graduate student mentors and ISU REU-wide poster session on Thursday, August 4.

## 5. Publications, Presentations, and Proposals Submitted

None to report in this period.

## Subtask 2B – Training Graduate Students via Intensive Program

## 1. Actual Accomplishments

None as this was strictly a PY2 and a PY4 program activity. No forward planning is required.

# **2. Explanation of Variance** None.

## 3. Plans for Next Quarter



None as this was strictly a PY2 and a PY4 program activity. No forward planning is required.

# 4. Publications, Presentations, and Proposals Submitted

None.

## Subtask 2C – Subtask 2C – Training Graduate Students via Monthly Research Webinar

#### 1. Planned Activities

This series will no longer be offered, however, graduate students will be invited to participate in critical project meetings as objectives disseminate findings in this final year.

## 2. Actual Accomplishments

None as this was strictly a PY1 - PY4 program activity. No forward planning is required.

## 3. Explanation of Variance

None.

## 4. Plans for Next Quarter

None as this was strictly a PY1 - PY4 program activity. No forward planning is required.

## 5. Publications, Presentations, and Proposals

Raman, D. R., B.N. Geisinger, M.R. Kemis & A. de la Mora. 2015. Key actions of successful summer research mentors. *Higher Education* DOI: 10.1007/s10734-015-9961-z.

## **Objective 9. Extension and Outreach**

The Outreach and Extension Objective serves as CenUSA's link to the larger community of agricultural and horticultural producers and the public-at-large. The team delivers science-based knowledge and informal education programs linked to CenUSA Objectives 1-7.

The following teams conduct the Outreach and Extension Objective's work:

# Extension Staff Training/eXtension Team/Educational Materials/Communications Team



This team concentrates on creating and delivering professional development activities for Extension educators and agricultural and horticultural industry leaders, with special emphasis on materials development (videos, publications, web posts, etc.).

## Producer Research Plots/Perennial Grass Team

This team covers the areas of:

- Production, harvest, storage, transportation.
- Social and community impacts.
- Producer and general public awareness of perennial crops and biochar agriculture.
- Certified Crop Advisor training.

## Economics and Decision Tools Team

The Economics and Decision Tools Team focuses on the development of crop enterprise decision support tools to analyze the economic possibilities associated with converting acreage from existing conventional crops to energy biomass feedstock crops.

# Health and Safety Team

This team integrates its work with the Producer Research Plots/Perennial Grass and the Public Awareness/Horticulture/eXtension 4-H and Youth teams (See Objective 7. Health and Safety).

#### Public Awareness/Horticulture/eXtension/4-H and Youth Team

This team focuses on two separate areas:

- Youth Development. The emphasis is on developing a series of experiential programs for youth that introduce the topics of biofuels production, carbon and nutrient cycling, and biochar as a soil amendment.
- **Broader Public Education/Master Gardener.** These programs acquaint the non-farm community with biofuels and biochar through a series of outreach activities using the Master Gardener volunteer model as the means of introducing the topics to the public.

## Evaluation/Administration Team

This team coordinates CenUSA's extensive extension and outreach activities. The team is also charged with developing evaluation mechanisms for assessing learning and behavior change resulting from extension and outreach activities, compiling evaluation results and



preparing reports, and coordination of team meetings.

# Extension Staff Training/eXtension Team/Educational Materials/Communications Team

## 1. Planned Activities

- Finish production and editing of the plant breeding video and post to Vimeo, YouTube and eXtension sites.
- Publish and distribute two BLADES newsletters: February and April 2016 Editions
- Continue production and editing of the 7-10 minute CenUSA legacy video that
  documents the CenUSA project. Add additional video clips to the legacy video
  including student poster session at annual meeting, biochar, crop harvesting and
  economics. Summarize concise list of achievements over the project's 5 years and
  add intro and ending transitions and voice overs
- Continue maintenance of CenUSA eXtension Index.
- Publish one case study.
- Use eXtension Farm Energy Social Media sites to broadcast information from CenUSA.

## 2. Actual Accomplishments

## • CenUSA Website.

The CenUSA website had 1,252 visitors this quarter. These visitors logged a total of 3,909 pageviews during 1,687 sessions. Pageviews are the total number of pages that visitors looked at during their time on the site. A session qualifies as the entire time a user is actively engaging with the site. If activity ceases for an extended period of time, and the user returns, a new session is started.

#### CenUSA eXtension Website

- ✓ Continued updating and maintenance of CenUSA eXtension website (<a href="http://articles.extension.org/pages/72584/resources-from-cenusa-sustainable-production-and-distribution-of-bioenergy-for-the-central-usa">http://articles.extension.org/pages/72584/resources-from-cenusa-sustainable-production-and-distribution-of-bioenergy-for-the-central-usa</a>).
- ✓ The CenUSA eXtension web site received 3,909 page views by 1,252 users this quarter. Pageviews are the total number of pages that visitors looked at during their time on the site. A session qualifies as the entire time a user is actively



engaging with the site.

- ✓ Google Analytics for CenUSA articles/fact sheets posted on eXtension Farm Energy Site
  - 4,871 pageviews by 3,503 users; 3,247 of those were new sessions, averaging
     1.2 pages per session; average time on page is 5.08
  - Traffic sources are 86 percent search engines ("organic" Google, etc.), 10
     percent direct traffic and 4 percent referring sites. Efforts continue to optimize publications for search engines
  - The Top 10 states accessing CenUSA articles were California, Texas, Michigan, New York, Pennsylvania, Illinois, North Carolina, Colorado, Minnesota and Iowa with use throughout the US and world; England and Ontario continue to top the international use.
- Vimeo. During this quarter, the 50 CenUSA videos archived on Vimeo have had 380 plays or views. The 50 videos also had 6,265 loads; 5,088 of those loads came from our videos embedded on other sites. When a video is loaded, people see the video but they do not click "play." The means the video was saved to their hard drive (users usually do this because they have limited internet connectivity which does not allow for live streaming of video). Once the video is downloaded, it is available on their computer to watch at their convenience. Embedded videos were played 159 times.
- YouTube. CenUSA videos are also posted on YouTube, and those videos have been viewed 1353 times between February 1 and April 1, 2016. 863 views were from the United States. Demographic analytics report an audience that is 83 percent male and 17 percent female. Our viewers ranged in age from 13-65+. The top 3 represented age groups were 25-34 (31%), 35-44 (18%), and 55-64 (17%). Users find our videos through various avenues, which are referred to as 'traffic sources'. Our top 4 traffic sources for this quarter include: YouTube search, YouTube suggested videos, referrals from other web sites and direct URL usage. Thirty-four percent of our views came from users accessing videos suggested by YouTube. YouTube search accounted for 32 percent of our views. Referrals from outside YouTube (Google Search or access through external web sites) account for 18 percent of video views. Views from direct URL usage accounted for 4 percent of video views.
- Twitter. Twitter traffic consists of followers who subscribe to our account and "follow" our tweets (announcements). Followers can "favorite" a tweet, or retweet it to share with their own followers. They can also "mention" us by tagging CenUSA bioenergy's twitter account in their own tweets. During this quarter, our tweets were



retweeted a total of 108 times. Followers tagged CenUSA tweets as a favorite 232 times, and mentioned us 71 times. CenUSA bioenergy also has 802 followers currently, up from 743 followers last quarter.

- **Facebook**. By the end of April 2016, CenUSA's Facebook page had 233 likes, up from 229 the previous quarter. Our most liked post from this quarter received 17 likes. The highest daily reach of the quarter had a total reach of 214 individuals.
- **BLADES Newsletter**. The CenUSA communications team published one newsletter this quarter (March 2016). The newsletter was sent to 858 people with 281 unique opens. This puts our newsletter open rate at 33% which is slightly higher than the industry average (32%). It featured 6 e-stories
  - ✓ Energy Company Total Makes Investment in Renmatix (<a href="http://blades-newsletter.blogspot.com/2016/02/energy-company-total-makes-investment.html">http://blades-newsletter.blogspot.com/2016/02/energy-company-total-makes-investment.html</a>)
  - ✓ Second Generation Biofuels Reduce Emissions (<a href="http://blades-newsletter.blogspot.com/2016/02/second-generation-biofuels-reduce.html">http://blades-newsletter.blogspot.com/2016/02/second-generation-biofuels-reduce.html</a>)
  - ✓ Healthy Soil, Healthy Plants (<a href="http://blades-newsletter.blogspot.com/2016/02/healthy-soil-healthy-plants.html">http://blades-newsletter.blogspot.com/2016/02/healthy-soil-healthy-plants.html</a>)
  - ✓ Cultivating Higher Yielding Scientists (<a href="http://blades-newsletter.blogspot.com/2016/02/cultivating-higher-yielding-scientists.html">http://blades-newsletter.blogspot.com/2016/02/cultivating-higher-yielding-scientists.html</a>)
  - ✓ The RFS: What's the Real Impact to Food and Fuel Prices? (<a href="http://blades-newsletter.blogspot.com/2016/02/the-rfs-whats-real-impact-to-food-and.html">http://blades-newsletter.blogspot.com/2016/02/the-rfs-whats-real-impact-to-food-and.html</a>)
  - ✓ CenUSA Bioenergy to Launch MOOC (<a href="http://blades-newsletter.blogspot.com/2016/02/cenusa-bioenergy-to-launch-mooc.html">http://blades-newsletter.blogspot.com/2016/02/cenusa-bioenergy-to-launch-mooc.html</a>)
- Work continued on the CenUSA Legacy Video.
- Published Plant Breeding video and published to Vimeo, YouTube and eXtension sites (see: <a href="https://proxy.qualtrics.com/proxy/?url=https%3A%2F%2Fvimeo.com%2F157206700">https://proxy.qualtrics.com/proxy/?url=https%3A%2F%2Fvimeo.com%2F157206700</a>

%26gt&token=KE6QlBQz%2BQuo2kfrp34Dk9ThVRyWtNJW4szEgCkBWS4%3D.

# 3. Explanation of Variance

The February newsletter was delayed due to difficulty arranging interviews, and interns struggling to meet deadlines. Since the February Newsletter was pushed back to March, the April Newsletter was purposely delayed until May to maintain a bi-monthly schedule.



## 4. Plans for Next Quarter

- Finish new fact sheet or research summary on new "Willingness to Produce" data from Richard Perrin's study.
- Publish and distribute one BLADES newsletter (May).
- Finish production and editing of 7-10 minute CenUSA legacy video that documents the CenUSA project.
- Continue maintenance of CenUSA eXtension Index.

## 5. Publications, Presentations, Proposals Submitted

- BLADES Newsletter, March. 2015 (<a href="http://blades-newsletter.blogspot.com/p/march-2016.html">http://blades-newsletter.blogspot.com/p/march-2016.html</a>).
- Case Study: Renmatix Processes Biomass into Sugars for Industrial Use; authored by Susan Harlow and peer reviewed by Robert Brown
   (<a href="https://proxy.qualtrics.com/proxy/?url=http%3A%2F%2Farticles.extension.org%2Fpages%2F73640&token=WIfYHv7tuzXRMd2p%2FSn%2FYKNJpMdr1SmtSn%2FYV9Dni7s=">https://proxy.qualtrics.com/proxy/?url=http%3A%2F%2Farticles.extension.org%2Fpages%2F73640&token=WIfYHv7tuzXRMd2p%2FSn%2FYKNJpMdr1SmtSn%2FYV9Dni7s=">https://proxy.qualtrics.com/proxy/?url=http%3A%2F%2Farticles.extension.org%2Fpages%2F73640&token=WIfYHv7tuzXRMd2p%2FSn%2FYKNJpMdr1SmtSn%2FYV9Dni7s=">https://proxy.qualtrics.com/proxy/?url=http%3A%2F%2Farticles.extension.org%2Fpages%2F73640&token=WIfYHv7tuzXRMd2p%2FSn%2FYKNJpMdr1SmtSn%2FYV9Dni7s=">https://proxy.qualtrics.com/proxy/?url=http%3A%2F%2Farticles.extension.org%2Fpages%2F73640&token=WIfYHv7tuzXRMd2p%2FSn%2FYKNJpMdr1SmtSn%2FYV9Dni7s=">https://proxy.qualtrics.com/proxy/?url=http%3A%2F%2Farticles.extension.org%2Fpages%2F73640&token=WIfYHv7tuzXRMd2p%2FSn%2FYKNJpMdr1SmtSn%2FYVYV9Dni7s=">https://proxy.qualtrics.com/proxy/?url=http%3A%2F%2FyKNJpMdr1SmtSn%2FYVYV9Dni7s=">https://proxy.qualtrics.com/proxy/?url=http%3A%2F%2FyKNJpMdr1SmtSn%2FYVYV9Dni7s=">https://proxy.qualtrics.com/proxy/?url=http%3A%2F%2FyKNJpMdr1SmtSn%2FYVYV9Dni7s=">https://proxy.qualtrics.com/proxy/?url=http%3A%2F%2FyKNJpMdr1SmtSn%2FYVYV9Dni7s=">https://proxy.qualtrics.com/proxy/?url=https://proxy.qualtrics.com/proxy/?url=https://proxy.qualtrics.com/proxy/?url=https://proxy.qualtrics.com/proxy/?url=https://proxy.qualtrics.com/proxy/?url=https://proxy.qualtrics.com/proxy/?url=https://proxy.qualtrics.com/proxy/?url=https://proxy.qualtrics.com/proxy/?url=https://proxy.qualtrics.com/proxy/?url=https://proxy.qualtrics.com/proxy/?url=https://proxy.qualtrics.com/proxy/?url=https://proxy.qualtrics.com/proxy/?url=https://proxy.qualtrics.com/proxy/?url=https://proxy.qualtrics.com/proxy/?url=https://proxy.qualtrics.com/proxy/?url=https://proxy.qualtrics.com/proxy/?url=https://proxy.qualtrics.com/proxy/?url=ht
- Revised research summary: *Biofuel Quality Improved by Delaying Harvest of Perennial Grass* with images and info about ecosystem services of the practice (http://articles.extension.org/pages/73615).

## Producer Research Plots/Perennial Grass/Producer and Industry Education Team

#### 1. Planned Activities

#### Indiana

- ✓ Collect last samples from 2015 to be ground, enter the data and send the samples and data to Rob Mitchell at the University of Nebraska, Lincoln (Objective 2).
- ✓ Harvest the residue from Roann plots by mid-April 2016.
- ✓ Use of the Roann and Trafalgar plots to measure switchgrass growth and development with different rates of nitrogen will be discussed and a protocol developed.
- ✓ Contact the Purdue Exhibit Center and discuss installation of a switchgrass plant box so root growth can be observed and a segment of a grassland drill at



Trafalgar.

#### Iowa

✓ Fertilize on-farm demonstration switchgrass plots.

## Minnesota

- ✓ Process samples from the two Minnesota demonstrations plots: the John Weis Farm in Elko and Southwest Research and Outreach Center in Lamberton.
- ✓ Schedule a field day for the summer of 2016.
- ✓ Maintain and fertilize the plots.

## Nebraska.

- ✓ Apply nitrogen fertilizer to both CenUSA on-farm sites.
- ✓ Complete land rental payment to growers.
- ✓ Make plans with University of Nebraska Agricultural Research and Development Farm Manager to see contour prairie strips in a crop clinic demonstration area. Three strips will be planted include:
  - o Liberty Switchgrass.
  - o Low Diversity Pollinator mix.
  - o Roundup Ready Alfalfa.
- ✓ Apply nitrogen fertilizer to State Fair switchgrass bioenergy demonstration area and remove 2015 biomass.
- ✓ Plan, schedule, develop marketing materials for youth programming using the C6 BioFarm App.

## 2. Actual Accomplishments

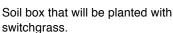
#### Indiana

- ✓ Review Curriculum for K-12 programming.
- ✓ Last collected samples from 2015 were ground, data entered and sent to Rob Mitchell at the University of Nebraska.
- ✓ Residue from Roann plots was harvested in mid-April.



- ✓ Use of the Roann and Trafalgar plots to measure switchgrass growth and development with different rates of nitrogen were discussed and a protocol was developed.
- ✓ The Purdue Exhibit Center contacted Truax and received a segment of a drill that was assembled and will be put in place in May 2016.
- ✓ The Purdue Exhibit Center constructed a planting box so individuals can view root growth and development.







Grass drill segment that will have components labeled.

Fig 16. These hands-on articles will be installed alongside the exhibit boards that deal with native grass rooting and seeding at the Indiana FFA Leadership Center.

## Iowa

- ✓ Plots were burned off on March 8 (SE Research Farm) and March 28, 2016 (Phil Winborn Farm).
- ✓ Nitrogen fertilizer was applied to the plots on March 22 (SE Research Farm) and March 29 (Phil Winborn Farm).

#### • Minnesota

✓ Process samples from the two Minnesota demonstrations plots: the John Weis Farm in Elko and Southwest Research and Outreach Center in Lamberton.



Samples were ground and prepared for shipment to Nebraska for further analysis.

✓ A Field day was scheduled for July 6, 2016 at the Southwest Research and Outreach Center.

## Nebraska

- ✓ Applied nitrogen fertilizer to both on-farm demonstration sites in April 2016.
- ✓ Made land rental payments to both CenUSA cooperators.
- ✓ Applied nitrogen fertilizer to State Fair switchgrass bioenergy demonstration area and removed 2015 biomass.
- ✓ Planned and marketed youth programs that will use C6 BioFarm App that was developed as part of CenUSA. Over 30 days of teaching are scheduled during the summer.

# 3. Explanation of Variance

Minnesota plot maintenance and fertilization moved back to May 2016 due to cold and wet conditions in MN in April 2016.

## 4. Plans for Next Quarter

#### • Indiana

- ✓ Collect switchgrass samples at Roann and Trafalgar to measure plant growth and development.
- ✓ The Purdue Exhibit Center will put in place the planting box and drill at Trafalgar.
- ✓ Plan a conference to discuss bioenergy grasses and what was learned at Roann and Trafalgar.
- ✓ Preparation for a conference in October, 2016 as a CENUSA final output delivery.

#### Iowa

- ✓ Fertilize switchgrass demonstration plots.
- ✓ Discuss and share the perennial grass decision tool in agronomy newsletter sent to growers and landowners in Southeast Iowa.



#### Minnesota

- ✓ A field day is planned for July 6, 2016 at the Southwest Research and Outreach Center in Lamberton where the CenUSA demo and factor plots will be discussed. CenUSA Extension staff will contact Minnesota NRCS and SWCD staff to invite them to come and learn about the decision tool and possible use of bioenergy grasses to fulfill the new Minnesota buffer law.
- ✓ Do general maintenance and fertilization of the demonstration plots.

#### Nebraska

- ✓ Maintain CenUSA research and demonstration plots.
- ✓ Maintain prairie bioenergy strips.

## 5. Publications, Presentations, Proposals Submitted

None submitted.

## Economics and Decision Tools

#### 1. Planned Activities

- Finalize prototype U of MN Crop Budget Tool (<a href="http://cropbudget.apec.umn.edu/">http://cropbudget.apec.umn.edu/</a>) (Exhibit 2).
- Continue to promote use of Ag Decision Maker Perennial Grass Tool <a href="http://www.extension.iastate.edu/AgDM/crops/html/a1-29.html">http://www.extension.iastate.edu/AgDM/crops/html/a1-29.html</a>.

# 2. Actual Accomplishments

- The U of MN Crop Budget Tool is finalized.
- A CenUSA Extension Economist is collaborating with University of Minnesota staff
  on a project focusing on the HUC12 Seven Mile Creek watershed, around 25,000
  acres, west of St. Peter MN. The focus is on stakeholder engagement, looking at how
  to introduce biomass production while maintaining or improving environmental
  measures such as water quality and carbon. The project leader is Nick Jordan in the
  Agronomy Department.
- Other project staff have developed a touch-screen Geodesign tool that groups of stakeholders can sue to draw in various land use and conservation practices wherever they chose in the watershed. The Geodesign tool has been tested and used in monthly



stakeholder meetings in February, March and April, reaching a total of 15 stakeholders.

- CenUSA Extension team member Bill Lazarus is developing a spreadsheet tool to estimate the community economic impacts from constructing and operating a hypothetical biomass processing plant using one of several processes which would be at different scales. He used the CenUSA switchgrass decision tool, along with Objective 5 Co-Project Director Robert Brown's NREL report to develop the IMPLAN input data that will feed into the economic impact calculations. He is planning to compare switchgrass and corn stover as feedstock.
- Continued outreach and promotion of the CenUSA Switchgrass Decision Tool. In the months of February April, 2016, ninety-four individuals accessed the CenUSA Switchgrass pdf about the Decision Tool
   (<a href="http://www.extension.iastate.edu/AgDM/crops/pdf/a1-29.pdf">http://www.extension.iastate.edu/AgDM/crops/pdf/a1-29.pdf</a>) and sixty-nine individuals completed the Decision Tool Spreadsheet
   (<a href="http://www.extension.iastate.edu/AgDM/crops/html/a1-29.html">http://www.extension.iastate.edu/AgDM/crops/html/a1-29.html</a>, click on xls link in box on right side of screen). This brings May-December 2015 totals for Decision Tool outreach to:
  - ✓ 363 individual downloads of the CenUSA Switchgrass pdf.
  - ✓ 405 individual completions of the CenUSA Switchgrass Decision Tool.
- Worked with CenUSA Extension materials team to develop a Research Summary publication to add to the CenUSA portfolio of materials that describes the survey completed by CenUSA Extension team member Richard Perrin. "Analysis of survey responses from 1043 farmers and landowners in the CenUSA region." Analysis revealed that about 10 percent of farm operators would produce switchgrass for biofuel at a price of \$60/ton; 25 percent would do so at \$75/t and the mean price necessary for production was \$84/t. Landowners responses to the prospect of leasing out their land for someone else to produce switchgrass for biomass indicated they were less willing to lease than to produce themselves, with \$80/t necessary to convince the first 10 percent to lease, and a mean willingness to lease land for switchgrass production of \$95/t.

# 3. Explanation of Variance

None.

## 4. Plans for Next Quarter



- Present the community economic analysis to the Seven Mile Creek watershed stakeholder group on June 23, 2016.
- Continue promotion, outreach and education for the Switchgrass Decision Tool.
- Finalize Research Summary about producer willingness to produce/sell perennial biomass.

# 5. Publications, Presentations, Proposals Submitted

See above.

# Health and Safety

See Objective 7.

#### Public Awareness/Horticulture/eXtension/4-H and Youth Team

# • Youth Development

#### 1. Planned Activities

## • Indiana

- ✓ Continue to finish edits on curriculum and supporting materials and launch web portal for access to all Purdue developed CenUSA Youth Extension and education materials.
- ✓ Complete draft of journal article focused on 4-H Renewable Energy Science Workshops.
- ✓ Continue collaboration in Indiana State 4-H and Indiana Corn Board to create "Teens Teaching..." model for Bioenergy. Training for first teams of teens to be held next quarter with supporting materials for the teaching teams under development currently.
- ✓ Continue work on electronic companion materials for demonstration plot signage.

## Iowa

Iowa: Host CenUSA C6 sessions at:

- ✓ Dordt College STEM Festival Feb 20, 2016.
- ✓ Cedar Rapids STEM Festival Feb 23.



- ✓ AGWSR ISU Visit March 14.
- ✓ Tama County Family STEM Festival April 7.
- ✓ Drake STEM Festival April 14.
- ✓ ISU AGEDS Teaching Activity April 18.
- ✓ Johnston Middle School STEM Day April 22.
- ✓ Ankeny Middle School ISU Visit April 28.

## 2. Actual Accomplishments

#### • Indiana

- ✓ Thirty-three teachers (8 males and 25 females) participated in a training on CenUSA Youth bioenergy programs at the HASTI Annual Conference, Feb 2016 (Hoosier Association of Science Teachers Inc.)
- ✓ Work continued on curriculum, app finalization, online learning modules, and planning for May and June events and workshops and partnership with 4-H.

#### Iowa

Conducted C6 Outreach for a total of 562 participants (547 youth and 15 adults):

- ✓ Dordt College STEM Festival Feb 20.
- ✓ Cedar Rapids STEM Festival Feb 23.
- ✓ AGWSR ISU Visit March 14.
- ✓ Tama County Family STEM Festival April 7.
- ✓ Drake STEM Festival April 14.
- ✓ ISU AGEDS Teaching Activity April 18.
- ✓ Johnston Middle School STEM Day April 22.
- ✓ Ankeny Middle School ISU Visit April 28.

A subset of the participants (243 participants) completed the survey process for C6 to gage learning and attitudes (Table 5).

Learning and attitudes about carbon, stem careers and C6 app



Table 5. Learning and attitudes about carbon, stem careers and C6 app.						
	Yes	No				
Did you learn something about Carbon?	228	15				
Did you learn something about careers in STEM?	198	45				
Would you like to download the C6 app and play it again?	107	123				

# 3. Explanation of Variance

The C6 BioFarm Coordinator has accepted a new job and her last day with CenUSA was May 6, 2016. A new intern has been hired to fulfill June and July 2016 duties, but some events originally planned for the summer had to be cancelled due to lack of staffing availability.

## 4. Plans for Next Quarter

#### Indiana

- ✓ Finish edits on curriculum and supporting materials and launch web portal for access to all Purdue developed CenUSA Extension and education materials.
- ✓ Work towards completion of edits on CenUSA Biofuels curriculum, and plan for fall workshop on same topic.
- ✓ Continue collaboration with Indiana State 4-H and Indiana Corn Board to create "Teens Teaching..." Model for Bioenergy. Training for first teams for teens to be held in May 2016.
- ✓ Continue development of supporting materials for "Teens Teaching..." model for Bioenergy.
- ✓ External review of curriculum and electronic companion materials for demonstration plot signage for publication requirements.
- ✓ Conduct Trainings/Workshops
  - Teens Teaching Teens May, 2016.
  - 4-H Academy Renewable Energy Workshop June, 2016.
  - 4-H Round Up Career Day Session June, 2016.

#### Iowa



Host CenUSA C6 sessions at the following events:

- ✓ Calhoun County Workshop, June 14, 2016.
- ✓ Marshall County Agriculture Day, June 21.
- ✓ Linn County Fair, June 23.
- ✓ Taste of Camp Workshop, June 24.
- ✓ Iowa 4-H Youth Conference, June 28.
- ✓ Iowa 4-H Youth Conference.
- ✓ Dallas County Fair, July 7.
- ✓ Calhoun County Fair, July 10.
- ✓ Middle School Teacher Workshop, July 11.
- ✓ North Carolina Teacher Workshop, July 14.
- ✓ Washington County Fair, July 18.

# 5. Publications, Presentations, Proposals Submitted

- Proposal submitted for presentation at National Energy Education Workshop (Washington D.C., June 7, 2016).
- Workshop planned for July 11, 2016 to educate teachers about how to implement C6 BioFarm in their classroom
- Abby Stanek will begin her thesis research and writing in July 2016, which
  focuses on knowledge increases for youth and educators by using C6 BioFarm in
  the classroom
- Learning STEM through Bioenergy: Lessons from the Plants; *Invigorate your lessons with a comprehensive curriculum focused on biofuels from plants that provides a foundation for teaching fundamental STEM concepts and making connections to a range of diverse careers*. Abstract submitted (4/15/16) for presentation at 2017 National Science Teachers Association (NSTA) National Conference.

## Broader Public Education/Master Gardener Program

The Master Gardener program segment of the CenUSA Extension task concluded last

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quarter. However, the project team is in the process of summarizing the data from their 4-years of biochar demonstration gardens and is preparing an Extension Publication on the topic.

#### Evaluation and Administration

#### 1. Planned Activities

- Collect information from CenUSA Extension teams and prepare reports.
- Continue support for development of CenUSA C6 Youth app, videos, and iBook materials.
- Submit abstract to National Energy Education Summit to present learnings about citizen science as a methodology to help people learn about renewable energy.
- Work with CenUSA Extension team members to finalize plans for the final year of the project.

## 2. Actual Accomplishments

- Collected and prepared reports.
- Hired summer intern to work with CenUSA C6 program.
- Worked with CenUSA Extension teams in Iowa, Nebraska, Minnesota, and Indiana to finalize goals and plans for the last year of the project.
- Submitted an abstract about CenUSA outreach programs to the National Energy Education Summit program. The abstract has been selected for presentation.

## 3. Explanation of Variance

None.

## 4. Plans for Next Quarter

- Develop survey instruments, conduct analysis of surveys completed by participants, and produce reports summarizing impact of CenUSA Extension efforts.
- Support C6 team to continue development of educational materials targeting K-12 vouth.
- Meet with all CenUSA Extension teams to continue planning and orchestrating to meet deliverables in CenUSA Work Plan.



• Collect information from CenUSA team members and prepare reports.

## 5. Publications, Presentations, Proposals Submitted

None this quarter.

## **Objective 10. Commercialization**

## Sub Objective 10A. Archer-Daniels-Midland

The Commercialization Objective was initiated in project year 4 (2015-2015) to evaluate near and long-term commercialization prospects for products produced from perennial grasses grown on marginal land. It involves two commercial partners, ADM and Renmatix, who are evaluating CenUSA feedstocks in their conversion processes.

#### 1. Planned Activities

- Low temperature, Low Pressure Conversion of Industrial Lignin Sources to Stable Intermediates. The planned research for the previous quarter was to explore pretreatments other than sodium hydroxide (NaOH) to enable low temperature, low pressure-hydrogenation (LTLP-H) of ADM, Renmatix, and POET lignin co-product streams. While NaOH pretreatment followed by LTLP-H is an effective pathway to convert lignin into a stable, liquid intermediate, sodium is problematic in upgrading with zeolite catalysts. Alternate pretreatments planned include calcium hydroxide (Ca(OH)<sub>2</sub>), ammonium hydroxide, trimethylamine (Et<sub>3</sub>N), and acetic acid.
- Conversion of Lignin-derived Phenolic Monomers to Vanillin. As described in earlier updates, thermochemical depolymerization of lignocellulosic biomass and industrial lignin streams produces high concentrations of phenolic monomers. These phenolic monomers, such as 4-vinylguaiacol (4-VG) have the potential to be upgraded to high-value products and chemicals. The plan for this quarter was to track studies on biological transformation of 4-VG to vanillin, a molecule with a market value of \$12/kg and market size of 20,000 metric tons/year.

## 2. Actual Accomplishments

• Low temperature, Low Pressure Conversion of Industrial Lignin Sources to Stable Intermediates. The ammonium hydroxide pretreatment was not used because it vaporized at low temperature making it unsuitable for the pretreatments under the conditions used and the acetic acid did not breakdown the lignin using the mild pretreatment conditions. These two alternate pretreatments were eliminated from the



list of possible pretreatments. The pretreatments used were NaOH, Et<sub>3</sub>N, and CaOH. Previous research had shown that NaOH followed by LTLP-H was effective, therefore, it was used as a model approach for comparison to the other treatments. Four industrial co-product lignin streams were pretreated followed by LTLP-H. The lignins and their moisture contents are shown in Table 6.

Table 6. Moisture content of the four lignin co-product streams.						
Lignin	Moisture (wt%)					
ADM Organosolv	12					
Renmatix Supercritical Hydrolysis (dry)	11					
Renmatix Supercritical Hydrolysis (wet)	42					
POET Enzymatic Hydrolysis	12					

Each of the lignin co-product streams were pretreated using the following protocol: a reflux consisting of either 5% NaOH, 10% Ca(OH)<sub>2</sub>, or 10 percent triethylamine (Et3N) in water solution at 125-140°C for six hours with agitation.

Mass yields of each of the pretreatments with each lignin is shown in Table 7. Both NaOH and Et<sub>3</sub>N pretreatments gave excellent yields on a dry basis (db). They ranged from 48.9-98.4 wt% db. The Ca(OH)<sub>2</sub> pretreatment was not effective and will not be used for further studies.

Proximate analysis was performed on each of the oil products after the pretreatment and LTLP-H (Table 8). The volatiles for the NaOH ranged from 37-52 wt%, whereas, the volatiles for the ET<sub>3</sub>N oil ranged from 60-72 wt%. This is an indication that these volatiles have potential to be upgraded via the catalyst.

Table 7. Comparison of mass yields weight percentage dry i	basis (wt% db) for each lighin stream and
pretreatment.	

Lignin Type	NaOH Pretreatment & LTLP-H: Oil (Insoluble) Yield (wt% db)	Ca(OH) <sub>2</sub> Pretreatment & LTLP-H: Oil (Insoluble) Yield (wt% db)	Et <sub>3</sub> N Pretreatment & LTLP-H: Oil (Insoluble) Yield (wt% db)
ADM Organosolv	87.9 (8.86)=96.7%	21.2 (79.3)=100.6%	93.0 (9.92)=102.9%
Renmatix Supercritical Hydrolysis (dry)	98.4 (6.30)=104.7%	26.7 (79.6)=106.3%	93.4 (9.13)=102.6%
Renmatix Supercritical Hydrolysis (wet)	90.9 (7.68)=98.6%	n/a	81.5 (11.3)=92.7%
POET Enzymatic Hydrolysis	88.0 (15.6)=103.7%	28.9 (69.1)=98.0%	48.9 (47.0)=95.9%



Table 8. Proximate analysis of the liquid product obtained from the co-product lignin after pretreatments using NaOH, Ca(OH)<sub>2</sub> and Et<sub>3</sub>N followed by LTLP-H.

Lignin Type

	Moisture (wt%)	Volatiles (wt%)	Fixed C (wt%)	Ash (wt%)	Moisture (wt%)	Volatiles (wt%)	Fixed C (wt%)	Ash (wt%)	Moisture (wt%)	Volatiles (wt%)	Fixed C (wt%)	Ash (wt%)
ADM Organosolv	21	42	25	12	9.4	63	22	4.9	19	60	17	3.8
Renmatix Supercritical Hydrolysis (dry)	37	37	16	11	4.0	68	26	2.0	10	63	26	1.0
Renmatix Supercritical Hydrolysis (wet)	18	52	13	18	20	54	2.0	25	2.5	72	23	2.0
POET Enzymatic Hydrolysis	19	49	10	22	8.1	56	8.0	28	4.4	67	22	4.0

Figure 17 indicates the functional group changes after NaOH pretreatment and LTLP-H of POET enzymatic hydrolysis lignin by Fourier transform infrared (FTIR) spectroscopy. The increased functional groups include carboxylic acid salts COO (1586 cm<sup>-1</sup>, 1384cm<sup>1</sup>). This was not unexpected because of the sodium (Na) in the pretreatment. However, there was also functional group decreases including ethers at wavenumbers 1280-1220 cm<sup>-1</sup>, 1140-1110 cm<sup>-1</sup>, and 1050-1000 cm<sup>-1</sup> and alcohols at 1200-1015 cm<sup>-1</sup>.

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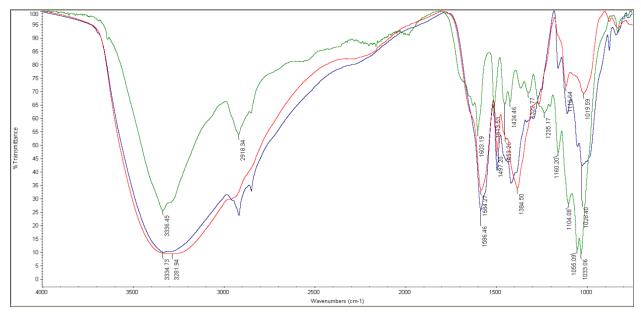


Fig 17. Fourier transform infrared spectroscopy indicating functional group changes after NaOH pretreatment and the pretreatment followed by LTLP-H using POET enzymatic hydrolysis lignin.

Functional group changes, as shown in the FTIR spectrum (Fig. 18) for ADM organosolv lignin, also indicated increases in carboxylic acid salts COO<sup>-</sup> at 1556 cm<sup>-1</sup> and 1413 cm<sup>-1</sup> due to the calcium (Ca) in the pretreatment. Decreased functional groups included carbonyls (C=O) at 1706 cm<sup>-1</sup> and ethers at 1280 -1220 cm<sup>-1</sup>, 1140-1110 cm<sup>-1</sup>, and 1050-1000 cm<sup>-1</sup> wavenumbers.

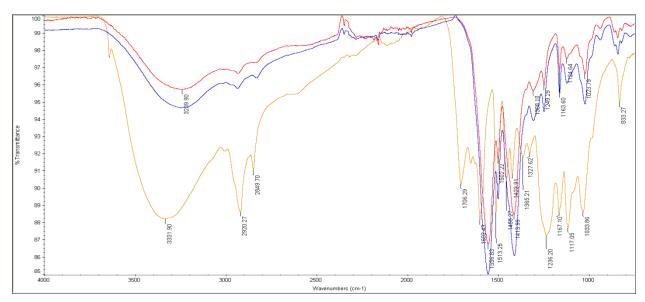


Fig 18. Fourier transform infrared spectroscopy indicating functional group changes after Ca(OH)<sub>2</sub>



pretreatment and the pretreatment followed by LTLP-H using ADM organosolv lignin.

The ET<sub>3</sub>N pretreatment and LTLP-H of Renmatix supercritical hydrolysis lignin (dry) indicated functional group increases by FTIR (Fig. 19). The increased functional groups include the aliphatic CH<sub>2</sub> and CH<sub>3</sub> at 2939 cm<sup>-1</sup> wavenumber. There is also an increase in the CH<sub>3</sub> groups attached to oxygen which is shown at 1594 cm<sup>-1</sup>.

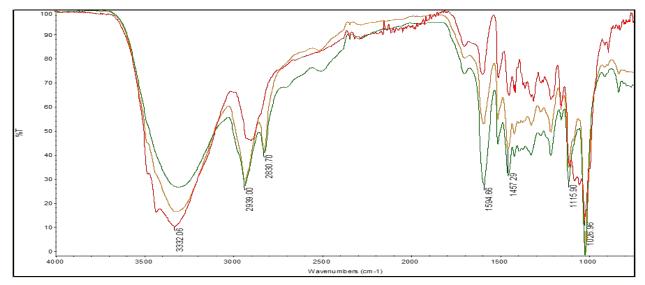
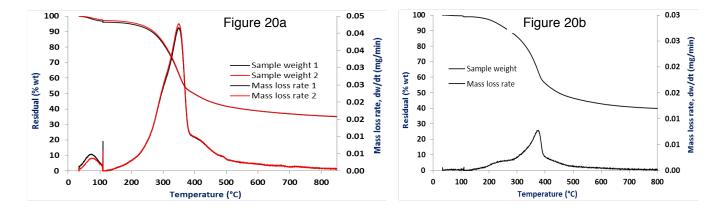


Fig 19. Fourier transform infrared spectroscopy indicating functional group changes after Et<sub>3</sub>N pretreatment and the pretreatment followed by LTLP-H using Renmatix supercritical hydrolysis lignin (dry).

Thermogravimetric analyses (TGA) along with the first derivative curve showing mass loss rate as a function of time (DTG) was also performed on each of the pretreated followed by LTLP-H lignin streams. The POET enzymatic hydrolysis lignin indicated changes in these curves after the NaOH pretreatment and LTLP-H (Fig 20a-c). The POET enzymatic hydrolysis lignin indicated mass loss at 200-350°C on the TG curve (Fig 20a). The pretreated with NaOH only sample shows mass loss from 300-400°C (Fig 20b) on the TG curve. After pretreatment with NaOH and LTLP-H the POET lignin showed the loss of moisture at 100°C and gradual mass loss from 100-700°C (Fig 20c). The DTG curves indicated the temperatures at which the mass was lost. The POET enzymatic hydrolysis lignin showed mass loss for moisture at 100°C and rapid degradation which peaks at 200-400°C (Fig 20a). The NaOH pretreated POET lignin shows degradation at 175-275°C and 300-400°C (Fig. 20b). However, on the NaOH pretreated followed by LTLP-H the first derivative curve



shows the moisture loss at 100°C, in addition to rapid degradation at 125°C and more gradual degradation at 250-350°C. These graphs give strong supporting evidence that the lignin was more monomeric in nature as there is mass loss at lower temperatures versus that of the original lignin prior to pretreatment and LTLP-H. The differences shown between the pretreated only DTG curve (Fig. 20b) and the DTG curve after pretreatment and LTLP-H (Fig. 20c) gives evidence that the hydrogenation further stabilized the oil and it did not polymerized during the thermogravimetric analyses but came off as volatiles.



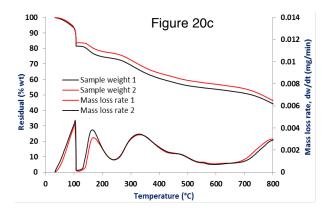
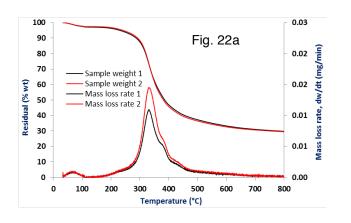


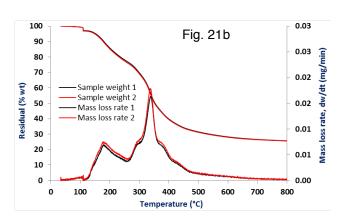
Fig 20a-c. POET enzymatic hydrolysis lignin thermogravimetric (TG) and first derivative (DTG) graphs of the enzymatic hydrolysis lignin (a), the NaOH pretreated enzymatic hydrolysis lignin (b) and the NaOH pretreated followed by LTLP-H enzymatic lignin (c).

The TG curve for the Renmatix supercritical hydrolysis lignin (wet) indicates rapid mass loss at 300-400°C (Fig 21a). After mild pretreatment using Et<sub>3</sub>N, the lignin (wet) indicated a more gradual mass loss between the temperatures of 100-400°C (Fig. 21b) with a very similar weight loss pattern after the pretreatment followed by LTLP-



H (Fig 21c). The DTG curves show the moisture loss at 100°C and rapid degradation at 350°C (Fig 21a). The ET<sub>3</sub>N pretreated supercritical hydrolysis lignin (wet) indicated two areas of degradation, as well as, the moisture loss at 100°C (Fig 21b). The graph indicates loss at 175°C and 350°C. The DTG curve after pretreatment and LTLP-H follows a similar degradation pattern (Fig 21c) as seen with the pretreated only sample. The results for the Et3N suggests that LTLP-H may not be required when using this pretreatment. Other lignins may respond differently because each lignin co-product stream is unique depending on the treatment used to extract the cellulose and hemicellulose. It is important to note that wet streams can be used for pretreatments we have researched thus promoting financial savings used for drying.





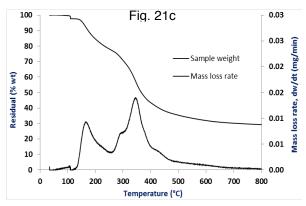


Fig 21a-c. Renmatix supercritical hydrolysis lignin (wet) thermogravimetric (TG) and first derivative (DTG) graphs of the supercritical hydrolysis lignin (a), the Et<sub>3</sub>N pretreated supercritical hydrolysis lignin (b) and the Et<sub>3</sub>N pretreated followed by LTLP-H

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In summary, the lignin treated with NaOH and Et<sub>3</sub>N followed by LTLP-H gave significant yields, 88-98 wt% db and 49-93 wt% db, respectively. Additionally, FTIR, TG, and DTG spectra indicates dramatic lignin structure modifications after pretreatment and LTLP-H.

Conversion of Lignin-derived Phenolic Monomers to Vanillin. A variety of microorganisms have been reported to produce vanillin from phenolic monomers. We have acquired and characterized several of these that were deemed likely to convert 4-VG to vanillin, including Pseudomonomas putida KT2440, Bacillus coagulans DSM1 and Lactobacillus coagulans DSM 20174. However, no production of vanillin from 4-VG was observed by these organisms, though we have confirmed our ability to detect vanillin in aqueous samples (data not shown). As opposed to using existing, undercharacterized organisms as our microbial catalyst for vanillin production from 4-VG, during this quarter we have pursued the genetic modification of standard industrial organisms, such as E. coli and S. cerevisiae, to enable the 4-VG to vanillin reaction. Specifically, we have been using the Cso2 enzyme from Caulobacter segnis, which was previously expressed and characterized in E. coli and showed a high Vmax for the production of vanillin from pure 4-VG.

Plasmid pET30b was modified to include the cso2 gene and then transformed into the selected host strain E. coli BL21\*. We verified that the cso2 was present and in the correct orientation by gene sequencing. A commercial helper plasmid pgro7 which contains the genes groES and groEL and is designed to help foreign protein fold correctly in E. coli was also transformed into our host strain.

The modified cells encoding cso2 were then characterized for their ability to convert 4-VG to vanillin. Ultra performance liquid chromatography (UPLC) was used to measure vanillin production and 4-VG consumption. Despite following previously published protocols, as well as extensively modifying these protocols, our experimental results indicate that the engineered cells neither produce vanillin nor consume 4-VG. At this time, we are focusing on verifying the presence of a translated cso2 gene product within the engineered cells.

In tandem with the above E. coli work, we also cloned the cso2 gene codon optimized for yeast into Saccharomyces cerevisiae because it is an industrially relevant species. For this cloning project, we used the DNA assembler method. The resulting engineered S. cerevisiae YSG50 was also tested for its ability to produce vanillin from 4-VG, but again the results were negative. No vanillin production and no 4-VG consumption were observed.



DNA ladder Colony 5 Colony 2
Colony 3
Colony 4 Colony 1 Colony 7



Fig 22. A gel electrophoresis of the restriction digestion product verifies that colony replicates 1,2,5,6,7, and 8 harbor a plasmid which contains cso2.

In both cases it is possible that the protein required for the biological transformation is not being produced. We are currently using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) to characterize both organisms. It should also be noted that our selection of cso2 as our enzymatic catalyst is based on a single publication (Furuya et al, New Biotechnology, 2015), whose results have not yet been replicated by other scientists.



## 3. Explanation of Variance

None occurred.

# 4. Plans for Next Quarter

- Evaluate catalysts compatible with NaOH pretreatment. Initial candidates include cobalt promoted molybdenum on silicon carbide (CoMo/SiC) and ruthenium on carbon (Ru/C) catalysts.
- Confirmation of Cso2 expression.

## Sub-Objective 2. Renmatix

## 1. Planned Activities

- **Task 10c-1.** Final report on lignin characterization by <sup>13</sup>C-NMR.
- **Task 10c-3.** Techno-economic feasibility analysis for the processing of corn stover and switchgrass.
- *Task 10c-4*:Production of larger sample of crude lignins for conversion into value added products.

# 2. Actual Accomplishments

- Task 10c-1. Lignin characterization work completed and report written.
- Task 10c-3. As mentioned in the second quarter report data from hydrolysis piloting trials on corn stover and switchgrass that were performed in the Bioflex Conversion Unit (BCU) have been compiled and a manufacturing concept has been conceived for the production of C5 and C6 monomeric sugars from switchgrass and corn stover. Activities and discussions have been carried on in order to estimate the economics for capital and operating costs. Keri Jacobs is providing assistance with determining the size of the biorefinery for the feasibility evaluation. In addition, supplementary piloting and analytical tests have been performed to evaluate water waste treatment costs.
  - ✓ Additional Activity. As mentioned in the second quarter report samples of C5 and C6 sugars that were produced in the BCU pilot plant from switchgrass were sent for testing to Bruce Dien (Objective 1) at the USDA ARS. He is testing the proof of concept for conversion of switchgrass hydrolysates to single cell oils using an oleaginous yeast strains and two-stage culture system. Lipid production was

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<sup>4</sup> https://cenusa.iastate.edu/files/cenusa\_q2\_yr\_5\_report.pdf



conducted as a two-stage process using yeast. He reports exciting results. While little has been done so far in the way of optimization of the lipid production cultures, optimization targets have been identified.

• Task 10c-4. Lignin samples from switchgrass and corn stover were produced successfully in Renmatix's BCU pilot plant and shipped to Iowa State University on May 4, 2016. These samples will be tested by Robert Brown for conversion into value added products.

# 3. Explanation of Variance

- Task 10c-1. Lignin characterization by NMR final report was delayed by equipment issues that were discussed in previous reports.
- Task 10c-3. Delay due to additional technical discussions and pilot/analytical testing.
- Task 10c-4. No variance.

## 4. Plans for Next Quarter

- Task 10c-3. A techno-economic feasibility analysis for the processing of corn stover and switchgrass to sugars and lignin will be completed this quarter based on the proposed manufacturing process.
- We will also participate in the remainder of project activities and issue a final report.

## 5. Publications / Presentations / Proposals Submitted

None this quarter.



"Our vision is to create a regional

system for producing advanced

transportation fuels derived

from perennial grasses grown on

land that is either unsuitable or

marginal for row crop production.

In addition to producing advanced

biofuels, the proposed system

will improve the sustainability

of existing cropping systems by

reducing agricultural runoff of

nutrients and soil and increasing

carbon sequestration."

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