

## **Final Technical Report**

**Project Title:** Sorghum to Ethanol Research

**Award Number:** DE-FG36-08GO88029

**Recipient:** National Sorghum Producers Association  
4201 N. Interstate 27  
Lubbock, TX 79403

**Project Location(s):**

|                                      |                                |
|--------------------------------------|--------------------------------|
| National Bioenergy Center            | Irrigation Research Foundation |
| National Renewable Energy Laboratory | 40161 Highway 59               |
| 1617 Cole Boulevard MS 3322          | Yuma CO 80759                  |
| Golden CO 80401                      |                                |

**Project Period:** 6/1/2008 thru 6/30/2010

**Date of Report:** September 28, 2010

**Written by:** Jeff Dahlberg, Ph.D. and Ed Wolfrum, Ph.D.

**Program Manager:** Jeff Dahlberg, Ph.D.

**Principal Investigators:** Ed Wolfrum, Ph.D., Brent Bean, Ph.D., and William Rooney, Ph.D.

**Subcontractors:**

|                                      |                                |
|--------------------------------------|--------------------------------|
| National Bioenergy Center            | Irrigation Research Foundation |
| National Renewable Energy Laboratory | 40161 Highway 59               |
| 1617 Cole Boulevard MS 3322          | Yuma CO 80759                  |
| Golden CO 80401                      |                                |

**Cost-Sharing Partners:**

|                                     |                                    |
|-------------------------------------|------------------------------------|
| Texas AgriLife Research & Extension | Colorado Department of Agriculture |
| Amarillo, TX                        | Lakewood, CO                       |

|                                     |             |
|-------------------------------------|-------------|
| Texas AgriLife Research & Extension | ICM         |
| College Station, TX                 | Colwich, KS |

Ceres  
Thousand Oaks, CA

**DOE Project Team:**

DOE-HQ contact: John Ferrell  
DOE Field Project Officer: Steve Thomas  
DOE Contract Specialist: Jon Olsen  
DOE Project Engineer: Dan Warneke

**Acknowledgment:**

This material is based upon work supported by the Department of Energy under Award Number DE-FG36-08GO88029 entitled “Sorghum to Ethanol Research.”

Disclaimer: This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

## **Executive Summary**

The development of a robust source of renewable transportation fuel will require a large amount of biomass feedstocks. It is generally accepted that in addition to agricultural and forestry residues, we will need crops grown specifically for subsequent conversion into fuels. There has been a lot of research on several of these so-called “dedicated bioenergy crops” including switchgrass, miscanthus, sugarcane, and poplar. It is likely that all of these crops will end up playing a role as feedstocks, depending on local environmental and market conditions. Many different types of sorghum have been grown to produce syrup, grain, and animal feed for many years. It has several features that may make it as compelling as other crops mentioned above as a renewable, sustainable biomass feedstock; however, very little work has been done to investigate sorghum as a dedicated bioenergy crop. The goal of this project was to investigate the feasibility of using sorghum biomass to produce ethanol. The work performed included a detailed examination of the agronomics and composition of a large number of sorghum varieties, laboratory experiments to convert sorghum to ethanol, and economic and life-cycle analyses of the sorghum-to-ethanol process. This work showed that sorghum has a very wide range of composition, which depended on the specific sorghum cultivar as well as the growing conditions. The results of laboratory- and pilot-scale experiments indicated that a typical high-biomass sorghum variety performed very similarly to corn stover during the multi-step process required to convert biomass feedstocks to ethanol; yields of ethanol for sorghum were very similar to the corn stover used as a control in these experiments. Based on multi-year agronomic data and theoretical ethanol production, sorghum can achieve more than 1,300 gallons of ethanol per acre given the correct

genetics and environment. In summary, sorghum may be a compelling dedicated bioenergy crop that could help provide a major portion of the feedstocks required to produce renewable domestic transportation fuels.

## Introduction/Background

There has been significant research on the use of dedicated bioenergy crops such as switchgrass [*Panicum virgatum*], Miscanthus [*Miscanthus giganteus*], sugarcane [*Saccharum officinarum*], corn [*Zea mays*], and various trees species, such as poplar [*Populus trichocarpa*]; however, very little discussion is given to the potential for any annual crops as a dedicated biomass feedstock. Most of that research has concentrated on using stover and grain as sources of feedstock. High tonnage annual crops that could be part of crop rotation systems within producer's farming operations offer unique advantages as compared to some of the other agricultural crops being proposed for renewable fuel production.

Sorghum [*Sorghum bicolor* (L.) Moench] is an excellent example of an annual crop that could be both a short term and long term solution for our need for a renewable, sustainable biomass feedstock. Sorghum is unique among the crops being discussed as feedstocks for renewable energy in that it can be used in all the various processes being discussed and debated for biofuel production; starch-to-ethanol, sugar-to-ethanol, and cellulosic/lignocellulosic-to-biofuel. Sorghum is a C<sub>4</sub> plant that is drought tolerant and is typically found on marginal agricultural lands, though the crop responds very well to both irrigation and fertilizer.

Compositional analysis is critical to a agricultural crop's evaluation as a potential feedstock for bioenergy conversion and the goal of this project was to investigate the feasibility of sorghum as a dedicated bioenergy feedstock and the tremendous variation in sorghum that can be exploited to produce more efficient conversion feedstocks. The work performed included a detailed examination of the composition of a large number of

sorghum varieties, the development of a rapid analysis method using near-infrared spectroscopy coupled to wet chemical biomass analysis, laboratory- and pilot-scale pretreatment, saccharification and fermentation experiments to understand how sorghum performs as a cellulosic ethanol feedstock, and techno-economic and life-cycle analyses of the cellulosic ethanol production process. The expected outcomes where a comprehensive listing within the DOE's Biomass Feedstock Composition and Property Database of forage, hay, and sweet sorghums sorghums, creation of NIR calibration models and curves to be used to calibrate NIR technology to optimize selection tools for use by sorghum plant breeders, identification of potential candidate sorghum feedstocks for further research pilot plant evaluation, life-cycle analyses to be used by EPA and other regulatory agencies to evaluate the potential of sorghum as a biomass feedstock, and educational components to highlight research results to producers, policy makers, and other interested parties.

## **Results & Discussion**

### ***1. Compositional Analysis & NIR Model Development***

The goals of this portion of the project were (1) to better understand the compositional variability in sorghum and (2) to develop a high-throughput method to determine the composition of large numbers of sorghum cultivars. Such a tool would be very valuable for researchers in this area.

All sorghum samples were provided by the National Sorghum Producers (Lubbock, TX) and Texas A&M University (TAMU, College Station, TX). One hundred different sorghums were analyzed, including commercially-available cultivars and experimental lines. All analytical methods followed standard NREL Laboratory Analytical Procedures (LAPs), available at [www.nrel.gov/biomass/analytical\\_procedures.html](http://www.nrel.gov/biomass/analytical_procedures.html), and are based on

the classical dietary fiber methods. In summary, all feedstock samples were subjected to a multi-step analytical procedure involving two-stage solvent extraction of the samples followed by two-stage acid hydrolysis of the extracted biomass. The structural carbohydrates were measured as their monomeric forms in the analytical hydrolyzate. We also measured lignin (both acid soluble and acid-insoluble), and ash. Recent work has reviewed the history<sup>1</sup> and the typical uncertainties<sup>2</sup> of these methods.

One significant modification to the standard methods was made because of the presence of starch in several samples. As mentioned above, structural carbohydrates are hydrolyzed to their monomers and measured. All glucose present in the analytical hydrolyzate is apportioned to structural glucan. Typically, glucan and cellulose are used interchangeably. However, any starch present in the material will be also hydrolyzed to glucose, along with any native cellulose. That is, the methods we used cannot distinguish between structural starch and cellulose; both are hydrolyzed to glucose. We noticed that a number of samples had atypically high glucan values, which indicated the presence of starch. We measured the starch present using the standard amyloglucosidase/ $\alpha$ -amylase method<sup>3</sup>. Thus, three related values are reported: structural starch, glucan, and cellulose. The glucan value is the sum of the structural starch and cellulose values; both the cellulose and starch fractions can be converted to ethanol (albeit with very different chemical transformation pathways).

The results of the compositional analysis showed large variation in composition

---

<sup>1</sup> “Compositional Analysis of Lignocellulosic Feedstocks. 1. Review and Description of Methods”, Justin B. Sluiter, Raymond O. Ruiz, Christopher J. Scarlata, Amie D. Sluiter and David W. Templeton, *Journal of Agricultural and Food Chemistry* 2010 **58** (16), pp 9043–9053.

<sup>2</sup> “Compositional Analysis of Lignocellulosic Feedstocks. 2. Method Uncertainties”, David W. Templeton, Christopher J. Scarlata, Justin B. Sluiter and Edward J. Wolfrum, *Journal of Agricultural and Food Chemistry* 2010 **58** (16), pp 9054–9062.

<sup>3</sup> Total Starch Assay Procedure(Amyloglucosidase/ $\alpha$ -amylase method) AOAC Method 996.11, AACC Method 76.13, available at [www.megazym.com/downloads/data/K-TSTA.pdf](http://www.megazym.com/downloads/data/K-TSTA.pdf)

across the different cultivars. Table 1 shows representative data on the composition of 22 commercially available sorghum hybrids grown at the Texas Agrilife Research Station in Bushland, TX in 2007. Table 2 shows the mean averages of all the samples evaluated in this research with high and low values for each compositional character. Data from this will be available on the DOE's Biomass Feedstock Composition and Property Database as it is formatted and uploaded to the web site.

The development of a rapid calibration model was performed in collaboration with Dr. William Rooney at TAMU. We used multivariate statistical algorithms to correlate near-infrared (NIR) reflectance spectra with compositional analysis data. The resulting model is used to predict the composition of subsequent samples based solely on their NIR spectra. This is a well-known and robust technique, and the subject of previous NREL research<sup>4</sup>. A calibration model for sorghum is currently in regular use at TAMU. The underlining data needed to build NIR calibration curves are publically available and copyrighted.

The first manuscript describing agronomic and compositional analysis results of commercial sorghum hybrids is being written. A second manuscript discussing the compositional variety of experimental hybrid lines and a third manuscript discussing the development of the NIR calibration model development are both currently in preparation.

## ***2. Lab-Scale Pretreatment Experiments***

Two varieties of forage sorghum Sugargraze Ultra and Sweeter 'N Honey BMR were initially identified by research supported by the Colorado Department of Agriculture as

---

<sup>4</sup> Wolfrum, E.J.; Sluiter, A.D. (2009). "Improved Multivariate Calibration Models for Corn Stover Feedstock and Dilute-Acid Pretreated Corn Stover." *Cellulose* (16:4); pp. 567-576.



Table 1. Compositional analysis of 22 commercially available sorghum forage and sorghum/sudangrass hybrids grown in the Bushland trials in 2007.

| Hybrid Designation | Ash  | Protein | Lignin | Struct-<br>ural<br>Starch | Cellulose   | Glucan      | Xylan       | Total<br>Mass<br>Closure | Total<br>Struct-<br>urals | Total<br>Solubles |
|--------------------|------|---------|--------|---------------------------|-------------|-------------|-------------|--------------------------|---------------------------|-------------------|
| 07CMP001           | 10.5 | 2.5     | 13.9   | 0.0                       | 31.0        | 31.0        | 17.4        | 97.1                     | 74.9                      | 22.1              |
| 07CMP002           | 9.9  | 0.0     | 14.4   | 0.0                       | 32.4        | 32.4        | 17.4        | 99.1                     | 74.1                      | 25.0              |
| 07CMP003           | 9.4  | 1.9     | 11.3   | 0.0                       | 35.3        | 35.3        | 19.9        | 93.9                     | 78.2                      | 15.7              |
| 07CMP004           | 8.8  | 0.0     | 13.3   | 0.0                       | 29.1        | 29.1        | 17.3        | 94.5                     | 68.4                      | 26.0              |
| 07CMP005           | 9.1  | 0.0     | 14.2   | 1.4                       | 30.1        | 31.5        | 17.2        | 99.7                     | 73.0                      | 26.8              |
| 07CMP006           | 9.0  | 0.0     | 12.9   |                           | 30.4        | 30.4        | 17.4        | 97.6                     | 67.0                      | 30.6              |
| 07CMP007           | 8.2  | 5.0     | 10.2   | 21.1                      | 21.4        | 42.5        | 14.9        | 99.0                     | 80.7                      | 18.3              |
| 07CMP009           | 9.9  | 2.0     | 11.3   | 20.2                      | 20.3        | 40.5        | 13.2        | 98.3                     | 75.7                      | 22.6              |
| 07CMP010           | 8.9  | 2.8     | 13.1   | 6.8                       | 27.4        | 34.2        | 16.0        | 98.9                     | 75.3                      | 23.6              |
| 07CMP011           | 10.0 | 3.3     | 10.6   | 19.2                      | 20.0        | 39.3        | 15.3        | 97.4                     | 77.0                      | 20.3              |
| 07CMP012           | 11.8 | 2.3     | 12.5   | 14.6                      | 22.1        | 36.7        | 13.7        | 94.4                     | 73.8                      | 20.6              |
| 07CMP013           | 8.0  | 0.0     | 12.5   |                           | 43.4        | 43.4        | 12.7        | 96.6                     | 77.1                      | 19.5              |
| 07CMP014           | 8.5  | 2.1     | 10.6   |                           | 39.9        | 39.9        | 15.7        | 94.6                     | 78.2                      | 16.4              |
| 07CMP015           | 8.9  | 3.1     | 9.5    | 13.8                      | 29.0        | 42.8        | 13.8        | 96.9                     | 78.5                      | 18.4              |
| 07CMP016           | 9.8  | 2.5     | 12.3   | 17.0                      | 23.5        | 40.4        | 14.3        | 98.7                     | 78.6                      | 20.1              |
| 07CMP018           | 8.5  | 2.4     | 9.9    | 23.6                      | 18.8        | 42.4        | 13.4        | 99.0                     | 76.7                      | 22.3              |
| 07CMP020           | 9.1  | 3.1     | 12.5   | 21.7                      | 18.8        | 40.5        | 13.1        | 99.2                     | 77.3                      | 21.9              |
| 07CMP021           | 8.9  | 1.9     | 14.0   | 8.0                       | 26.6        | 34.6        | 17.9        | 95.5                     | 76.5                      | 18.9              |
| 07CMP022           | 9.4  | 2.7     | 14.2   | 6.2                       | 27.6        | 32.3        | 16.9        | 96.5                     | 76.2                      | 20.3              |
| 07CMP030           | 8.3  | 3.1     | 13.2   | 7.7                       | 25.5        | 33.2        | 15.6        | 96.4                     | 74.9                      | 21.4              |
| 07CMP032           | 10.5 | 0.0     | 9.7    | 17.3                      | 21.9        | 39.2        | 12.7        | 95.7                     | 70.6                      | 25.2              |
| 07CMP049           | 7.4  | 0.0     | 15.9   | 0.0                       | 33.6        | 33.6        | 18.0        | 92.3                     | 74.9                      | 17.4              |
| Averages           | 9.2  | 1.8     | 12.4   | 10.5                      | <b>27.6</b> | <b>36.6</b> | <b>15.6</b> | 96.9                     | 75.3                      | 21.5              |
| Low                | 7.4  | 0.0     | 9.5    | 0.0                       | <b>18.8</b> | <b>29.1</b> | <b>12.7</b> | 93.9                     | 67.0                      | 15.7              |
| High               | 11.8 | 5.0     | 15.9   | 23.6                      | <b>43.4</b> | <b>43.4</b> | <b>19.9</b> | 99.7                     | 80.7                      | 30.6              |

Table 2. Average, low, and high values for compositional analysis of 100 sorghum samples used in this research.

| 100  | Ash  | Lignin | Whole<br>Starch | Structural<br>Starch | Cellu-<br>lose | Glucan | Xylan | Galac-<br>tan | Arab-<br>inan | Total | Struc-<br>tural | Sol-<br>uble |
|------|------|--------|-----------------|----------------------|----------------|--------|-------|---------------|---------------|-------|-----------------|--------------|
| Ave. | 6.7  | 13.7   | 9.7             | 4.7                  | 28.6           | 32.7   | 16.4  | 0.9           | 2.2           | 97.7  | 74.0            | 23.8         |
| Low  | 2.3  | 9.5    | 0.0             | 0.0                  | 14.7           | 21.9   | 10.8  | 0.5           | 1.2           | 92.3  | 53.7            | 11.0         |
| High | 11.8 | 20.6   | 42.8            | 24.6                 | 43.4           | 47.4   | 22.5  | 1.8           | 4.7           | 104.6 | 87.6            | 44.0         |

potential candidates for evaluation. They were planted, grown, harvested, baled and stored at the Irrigation Research Foundation in Yuma, CO. The sorghum was knife milled to 6 mm particle size. Corn stover (*Zea mays*, Pioneer 33A14) was grown and harvested in Wray, CO, in 2002 and tub ground in the field and knife-milled to pass a 6 mm screen. Both the corn stover and sorghum materials were impregnated with sulfuric acid prior to pretreatment. Pretreatment experiments were performed in a 4-L (2-L working volume) ZipperClave® batch reactor (Autoclave Engineers). This reactor was chosen for its ability to operate with smaller feedstock quantities (~100g dry) at solids loadings as high as 50% (wt/wt). The ZipperClave® reactor uses direct steam injection for heating the biomass and depressurizes by relieving head space pressure in approximately 15 to 20 seconds through a throttle valve to a condenser. The ZipperClave® reactor uses a modified anchor-type impeller with customized lifting wedges which sweep the reactor bottom and provide lifting of the biomass under high solids loading conditions.

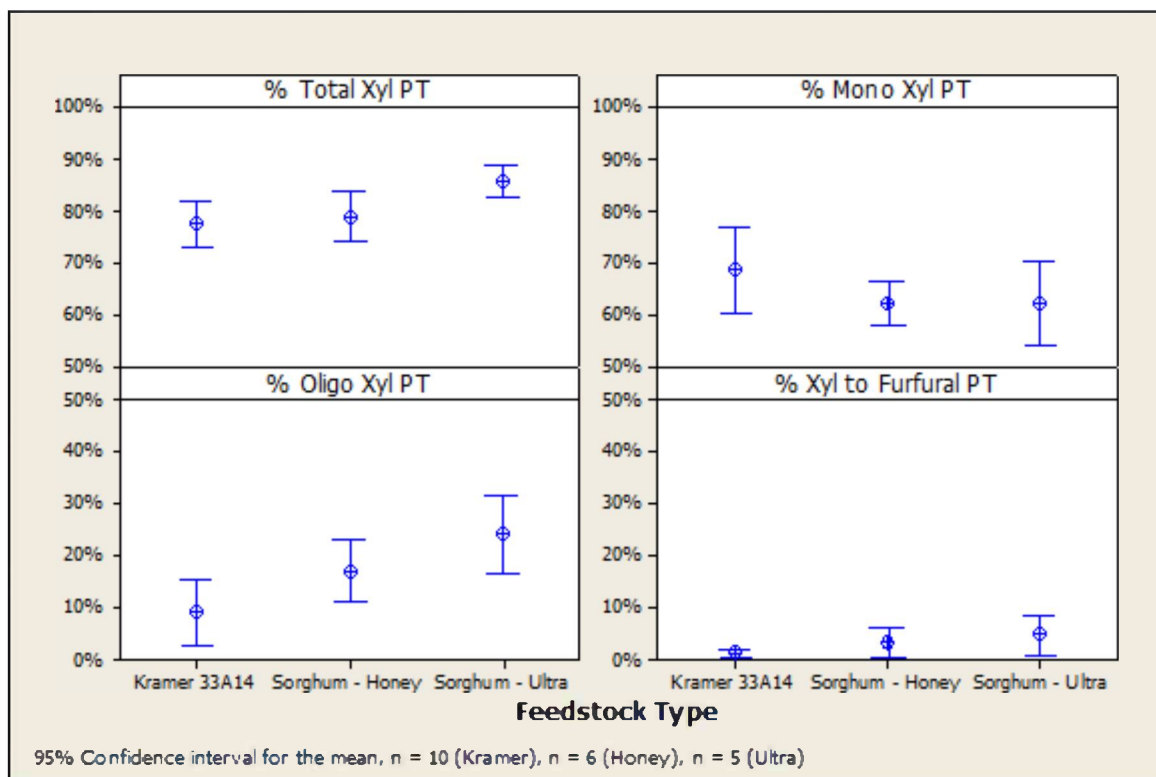
Pretreatment experiments were conducted at a range of severity conditions<sup>5</sup>. The severity range for corn stover pretreatments was 2.17 - 3.67. Two sets of experiments were performed on the sorghum samples. The first set was treated at  $R_0$  values of 2.76 – 2.83. The second set was treated at  $R_0$  values of 2.83 – 3.35. Enzymatic hydrolysis was carried out using Genencor GC220 cellulase (Rochester, NY) at 17mg of enzyme per gram of cellulose; 1.5wt% solids;  $48^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ ; in 125ml Erlenmeyer flasks with 50ml working volume sealed with screw caps; 0.75g of washed pretreated solids were added to 50ml of citrate buffer at pH 4.8. The solids loading and volume used in enzymatic saccharification were slightly modified from NREL's LAP. The composition of feedstock

---

<sup>5</sup> Overend, R.P., Chornet, E., 1987. Fractionation of Lignocellulosics by Steam-Aqueous Pretreatments. *Philosophical Transactions of the Royal Society of London Series a-Mathematical Physical and Engineering Sciences*, 321, 523-536.

and process intermediates were measured using standard NREL Laboratory Analytical Procedures ([www.nrel.gov/biomass/analytical\\_procedures.html](http://www.nrel.gov/biomass/analytical_procedures.html)). In addition the liquor samples were analyzed for density as well as monomeric and total xylose and glucose concentrations. Solids were analyzed for the fraction of insoluble solids (FIS) remaining following pretreatment. The FIS measurement was used to close the mass balance and determine the fraction of xylan and glucan solubilized in pretreatment and enzymatic hydrolysis. The acid concentration of the liquor remaining in the acid impregnated feedstocks was determined by titration with a solution of NIST traceable 1.0 N NaOH (J.T. Baker, Phillipsburg, NJ, USA). Pretreatment mass balance and component yield calculations were performed by accounting for the total dry mass and component masses entering and exiting the reactor. The FIS measurement was used to determine the solids content of the pretreated slurries, which was in turn used to calculate the total mass of solids recovered. Xylose component yields were calculated from the concentration of monomeric xylose, total xylose, and furfural in the slurry liquor as well as condensate samples. Figure 1 shows an interval plot of monomeric xylose yield, oligomeric xylose yield, total xylose yield, and xylose degraded to furfural for all three materials.

Each interval includes data across the full range of severities tested. These data show that for each feedstock similar monomeric xylose yields, soluble oligomeric xylose yields, and xylose degraded to furfural was observed. For the acid concentrations and severities studied, nearly 60%-70% monomeric xylose, 15%-20% soluble oligomeric xylose, and 5% xylose degradation to furfural was observed for corn stover, and the



**Figure 1.** Plot of monomeric, oligomeric, total xylose; and xylose to furfural from DAP. Each interval includes data across the full range of severities presented in Fig. 1. The data is comparable across each feedstock. (Kramer = corn stover)

sorghum forages Ultra and Honey. It is important to note that the severity in which a maximum yield is observed for corn stover is different than the severity in which a maximum yield is observed for either sorghum cultivars. The maximum monomeric xylose yield during pretreatment was achieved at a severity of 2.76 for corn stover and 3.35 for Ultra and Honey sorghum. The maximum oligomeric xylose yield was achieved at severities of 2.17, 2.76, and 2.82 for corn stover, Honey, and Ultra sorghum respectively. This suggests that corn stover hemicellulose begins to depolymerize at less severe pretreatment conditions than sorghum hemicellulose.

Pretreatment and enzymatic hydrolysis yields for both xylose and glucose for two sorghum cultivars that were not statistically significantly different from corn stover within the severity range tested. Total DAP xylose yields of 85% and EH yields of 90% were achieved from forage (Ultra) sorghum, BMR (Honey) sorghum, and corn stover feedstocks. Both sorghum cultivars exhibited maximum xylose and glucose yields over a narrower range of pretreatment severities than for corn stover. The data suggest the cell wall structure of the sorghum cultivars may require a more narrow range of pretreatment severities to optimize pretreatment than for corn stover. There was no observed digestibility increase for Honey sorghum, the BMR mutant, compared to Ultra although the lignin content of the Honey cultivar was measurably lower. Due to the potential for high yielding mass/acre/year crops of sorghum, and the knowledge that sorghum appears to be as reactive as corn stover to DAP and enzymatic hydrolysis, sorghum cultivation could be utilized as a U.S. bioenergy crop. A manuscript describing these results in more detail is in preparation.

### 3. *Lab-Scale Fermentation Experiments*

Laboratory scale enzymatic hydrolysis and fermentation experiments were performed to compare the performance of biomass sorghum and corn stover. Prior to enzymatic hydrolysis, the two feedstocks were subjected to dilute acid pretreatment and then pH adjusted using two methods, neutralization and conditioning to a pH of 5.0. Two different total solids loadings of 15% and 20% were targeted in the enzymatic hydrolysis and Novozymes Cellic Ctec enzyme was used. The results of the saccharification experiments are summarized in Table 3. Overall, the enzymatic hydrolysis involving the corn stover feed stock produced 8-13% more glucose than the sorghum feedstock at identical conditions. However, the cellulose conversion (calculated as fraction of cellulose present in the pretreatment material released as glucose after saccharification) was the same for both materials. No significant effect of either the neutralization method or total solids loading during saccharification was seen.

The hydrolyzed slurries were transferred into bioreactors and fermented by *Zymomonas mobilis* 8b. The results of these experiments are summarized in Table 4. The fermentation produced 18% more ethanol from the corn stover feedstock at a 20% solids loading and 29% more ethanol at the 15% solids loading. This is to be expected as more fermentable sugar was available post-hydrolysis and therefore, present at the start of fermentation. Although the corn stover feedstock produced a greater ethanol titer, the process yields of the different feedstock fermentations were quite similar at approximately 90%. Overall, the digestibility and fermentability of the corn stover and sorghum feedstocks were very similar, although corn stover yielded more sugars from enzymatic hydrolysis and therefore, more ethanol from fermentation.

Table 3. Total sugar production and calculated cellulose conversion at end of 120 hour enzymatic hydrolysis. No statistically significant difference in cellulose conversion was seen between the two materials.

| Condition                        | Total Solids (%) | Total Monomeric Glucose (g/L) | Total Monomeric Xylose (g/L) | Cellulose Conversion (%) |
|----------------------------------|------------------|-------------------------------|------------------------------|--------------------------|
| Sorghum, Ammonia Conditioned     | 20.0             | 69.7                          | 31.6                         | <b>92.4</b>              |
| Sorghum, Ammonia Neutralized     | 20.1             | 69.0                          | 31.7                         | <b>90.0</b>              |
| Sorghum, Ammonia Neutralized     | 15.1             | 50.9                          | 23.7                         | <b>92.3</b>              |
| Corn Stover, Ammonia Conditioned | 20.6             | 84.7                          | 47.6                         | <b>88.6</b>              |
| Corn Stover, Ammonia Neutralized | 20.3             | 84.0                          | 46.9                         | <b>91.7</b>              |
| Corn Stover, Ammonia Neutralized | 15.5             | 62.9                          | 34.8                         | <b>91.4</b>              |

Table 4. Initial and Final major sugar concentration (glucose and xylose), final ethanol concentration, and calculated ethanol yields of the laboratory-scale fermentation experiments for corn stover and sorghum materials. The fermentation organism was a genetically-modified *Zymomonas mobilis* 8b. No significant difference in ethanol yield between the sorghum and corn stover materials was seen.

| Condition                        | Total Solids @ EH | Initial sugar conc. (g/L) | Final sugar conc. (g/L) | Final ethanol conc. (g/L) | Ethanol Yield (%) |
|----------------------------------|-------------------|---------------------------|-------------------------|---------------------------|-------------------|
| Sorghum, Ammonia conditioned     | 20.0              | 99.6                      | 7.6                     | 40.6                      | <b>92.4</b>       |
| Sorghum, Ammonia Neutralized     | 20.1              | 99.6                      | 5.4                     | 41.7                      | <b>90.0</b>       |
| Sorghum, Ammonia Neutralized     | 15.1              | 78.8                      | 2.1                     | 32.4                      | <b>92.3</b>       |
| Corn Stover, Ammonia Conditioned | 20.6              | 124.9                     | 18.9                    | 47.8                      | <b>88.6</b>       |
| Corn Stover, Ammonia Neutralized | 20.3              | 124.7                     | 15.9                    | 49.1                      | <b>91.7</b>       |
| Corn Stover, Ammonia Neutralized | 15.5              | 90.0                      | 3.2                     | 41.7                      | <b>91.4</b>       |

A manuscript describing these results in more detail is in preparation.

#### **4. *Pilot-Scale Saccharification and Fermentation***

Pilot-scale saccharification and fermentation runs were performed in the NREL biochemical pilot plant. A brief outline of the pilot-scale run plan is discussed below. In brief, the mixed Sorghum lot was pretreated in the vertical pretreatment reactor and fed continuously to a 1500-L fermentation vessel. The pH of the slurry was adjusted to 5 with ammonium hydroxide, and then enzymatic hydrolysis was initiated with the addition of enzyme. Following approximately 4 days of enzymatic hydrolysis, the vessel was inoculated with a 10% (v/v) inoculum of *Z. mobilis* 8b. The fermentation was completed in about 3 days, at which time the vessel contents were sterilized and sent to the plant's neutralization tank. These runs were successful, and the data are currently being compiled and a manuscript describing these results in more detail is in preparation.

#### **5. *Techno-Economic & Life-Cycle Analysis***

The compositional analysis and feedstock conversion data generated from this project has been incorporated into a techno-economic analysis (TEA) model for cellulosic ethanol production. This use of laboratory- and pilot-scale data in TEA models has been used to guide the research and development of lignocellulosic biofuels production processes at NREL for over two decades. The TEA model consists of a process simulation model developed in Aspen PLUS (Cambridge MA). Data from the laboratory- and pilot-scale experiments are used to determine yields in the various unit operations within the process model. The model outputs are material and energy balances for the cellulosic ethanol process, and the required sizes of the various unit operations (tanks, mixers, fermenters, etc.). The model outputs are used to determine the capital and operating costs for the



overall plant. These calculations are done in a spreadsheet.

A preliminary technoeconomic analysis of the biochemical production of ethanol from three different sources of sorghum biomass, namely forage sorghum, sweet sorghum bagasse, and grain sorghum stover, was performed based on the 2002 NREL Biochemical Cellulosic Ethanol Process<sup>6</sup> and was used without any modification. The minimum ethanol selling price (MESP) for each biomass feedstock was calculated using a discounted cash flow rate of return analysis (DCFRROR) and was determined to be \$2.89/gal for forage sorghum, \$3.48/gal for sweet sorghum bagasse, and \$2.78/gal for grain sorghum stover, all in 2007 dollars. This is very close to the corn stover case of \$2.85/gal. The differences in MESPs are attributed mainly to the equivalent ethanol yields from the feedstocks. Note that these estimates are based on the process model documented in the 2002 NREL Design Report. We have made significant changes to the process model and economic assumptions since this model was first developed; this model does not represent the current state of technical and economic understanding of the cellulosic ethanol conversion process. Thus, the MESP values should only be understood in a comparative sense: forage sorghum, grain sorghum and corn stover all have similar MESP values.

Additional data obtained from feedstock composition analysis, bench-scale and pilot plant conversion studies has been incorporated into an updated process simulation model. This model is currently under review by DOE, and results from this updated model are not yet publicly available. A manuscript describing these results in more detail is in

---

<sup>6</sup>Technical Report NREL/TP-510-32438 (2002) “Lignocellulosic Biomass to Ethanol Process Design and Economics Utilizing Co-Current Dilute Acid Prehydrolysis and Enzymatic Hydrolysis for Corn Stover”, A. Aden, M. Ruth, K. Ibsen, J. Jechura, K. Neeves, J. Sheehan, B. Wallace, L. Montague, A. Slayton, and J. Lukas

preparation.

The compositional data produced in this work has been incorporated into a life-cycle analysis LCA study which will include several herbaceous and woody dedicated bioenergy crops. This work will build on recent published work<sup>7</sup>, and will be published in mid-2011.

## **6. Outreach and Education**

Educational and outreach programs were initiated throughout the proposal timeline. The research team participated in and planned the International Conference on Sorghum for Biofuels that was held in Houston, Texas on August 19-22, 2008 (see <http://www.ars.usda.gov/meetings/Sorghum/agenda.htm>; verified Sept. 21, 2010).

Various newspaper and magazine stories were also developed and published outlining the work that was undertaken. Outreach activities also included presentations at various bioenergy meetings to highlight research results, as well as poster presentations at other meetings. One highlight of the outreach program was the meeting held in conjunction with the Board meeting of the United Sorghum Checkoff Program in December of 2009 in which USCP Board members, the Secretary of Agriculture for Colorado, members of DOE, and of the press were invited to learn about the various research programs that were involved in the Sorghum to Ethanol Research program. Sorghum Growers magazine has highlighted the research in two articles and is focusing on a third article to appear next year. The magazine touches 35,000 sorghum producers and is a major educational tool for the sorghum community in its efforts to outreach with producers, scientists, and

---

<sup>7</sup> “Life Cycle Environmental Impacts of Selected U.S. Ethanol Production and Use Pathways in 2022”, David D. Hsu, Daniel Inman, Garvin A. Heath, Edward J. Wolfrum, Margaret K. Mann and Andy Aden, *Environmental Science & Technology* 2010 **44** (13), pp 5289–5297

policy makers. The Furrow magazine also published an article on sorghums potential in the bioenergy field. A list of various publication and news articles is listed in Appendix 1.

## **Conclusions**

In the Department of Energy's "The Ideal Biomass Crop," three crops were outlined: Corn, a short-rotation Coppice, and Perennial Grass. This research has allowed us to add a forth column for sorghum (Table 5). In reviewing this table and modifying it to reflect current status of agronomics, seed industry support, genomics and known pests and diseases, it is evident that sorghum could and should play an important role in shaping the feedstock debate for biofuel conversion. Though sorghum is not a perennial grass, its role as an annual in a crop rotation farming scheme would provide a relatively low risk option for producers wishing to participate in the production of feedstocks for renewable fuel production.

Yield data from 2007 and replicated over several years have shown that forage sorghums and sorghum/sudangrass hybrids can produce excellent yields of high tonnage biomass that would be suitable for use in the conversion of these feedstocks into biofuels. These forages would thrive in various geographic regions in the U.S. including high moisture regions such as the south and southeast, to limited irrigated fields in the western U.S. and more importantly are currently grown throughout the U.S. for forage and silage production. Sorghum has known genetics and agronomics that are understood by producers and would make for a more rapid acceptance as a biofuels feedstock. Prior to this research, little was known about the compositional makeup of sorghum feedstocks and as this research shows, sorghum has a tremendous amount of variability in its compositional makeup. Over 100 samples of various sorghums were evaluated in the

Table 5. The Ideal Biomass Crop (modified from DOE Ideal Biomass Crop Table) with a column added for sorghum.

| The “Ideal” Biomass Crop?    | Corn     | Short-Rotation Coppice <sup>1</sup> | Perennial Grass | Sorghum   |
|------------------------------|----------|-------------------------------------|-----------------|-----------|
| C4 photosynthesis            | *        | -                                   | *               | *         |
| Long canopy duration         | -        | *                                   | *               | *         |
| Recycles nutrients to roots  | ?        | -                                   | *               | ?         |
| Low input                    | -        | *                                   | *               | *         |
| Known Agronomics             | *        | ?                                   | ?               | *         |
| Sterile (noninvasive)        | -        | *                                   | ?               | *         |
| Winter standing              | -        | *                                   | *               | -         |
| Easily removed               | *        | -                                   | ?               | *         |
| High water-use efficiency    | -        | -                                   | *               | *         |
| Drought tolerant             | -        | -                                   | ?               | *         |
| Known pests or diseases      | *        | ?                                   | ?               | *         |
| Uses existing farm equipment | *        | -                                   | *               | *         |
| Seed Industry                | *        | -                                   | ?               | *         |
| Sequenced                    | -        | -                                   | -               | *         |
| Photoperiodism               | -        | -                                   | ?               | *         |
| Sweet stalked                | -        | -                                   | -               | *         |
| Grain-Ethanol                | *        | -                                   | -               | *         |
| Known Genetics               | *        | -                                   | -               | *         |
| ESTs                         | Many     | ?                                   | ?               | 220,000   |
| GSS                          | Many     | ?                                   | ?               | 600,000   |
| Trace Files                  | ?        | ?                                   | ?               | 6,800,000 |
| SSRs                         | Many     | ?                                   | ?               | Over 200  |
| SNPs                         | Many     | ?                                   | ?               | 2,000     |
| Reproduction mode            | Outcross | Outcross                            | Outcross        | Self      |

EST=Expressed Sequence Tags

GSS=Genomic Survey Sequences

Trace Files=Part of JGI sorghum genome sequencing project

<sup>1</sup>Coppice is a grove of densely growing small trees pruned to encourage growth

project (data not show, but available on the DOE's Biomass Feedstock Composition and Property Database) and indicate a tremendous amount of variability exists in sorghum for various compositional characteristics. This data were also used to formulate NIR calibration curves for sorghum compositional makeup and will assist plant breeders in selecting various characteristics to optimize their use as a feedstock, no matter what commercial process is used to produce renewable products.

The best currently available commercial forage hybrid could produce over 850 gal acre<sup>-1</sup> of ethanol (based on average biomass yield and theoretical estimates for ethanol production) and this is without genetic improvement to optimize the compositional characteristics to maximize theoretical yield. Provided with information from the renewable industry, it is feasible based on the variability noted in the sorghum germplasm used in this study that sorghums could be tailored for the various needs of the renewable industry, whether that be for low or high lignin content, or low or high structural carbohydrate content. With its tremendous genetic variability, the sorghum sequence, a robust seed industry and good characterization of its compositional makeup, sorghum research will continue to produce hybrids that will meet the challenges of moving away from a fossil fuel dependent economy and play a leading role in our ability to become more self-sufficient in our energy needs in the future.

**Key Findings:**

- Compositional analysis of over 100 different sorghum cultivars showed a broad variability in composition. When typical compositional data are combined with yield data from previously published field trials and the DOE theoretical ethanol

yield calculator, sorghum has the potential to produce over 1300 or more g acre<sup>-1</sup> of ethanol.

- NIR technologies coupled with a sorghum standard curve developed by this research will allow plant breeders to tailor future sorghum feedstocks to whatever needs are required by the renewable industry.
- Laboratory-scale pretreatment, enzymatic hydrolysis, and fermentation yields for two sorghum cultivars that were not statistically significantly different from corn stover within the experimental conditions examined.
- Preliminary techno-economic analysis (TEA) models showed that biomass sorghums provide essentially the same economics as corn stover in the production of cellulosic ethanol.

## **Appendix 1. Manuscripts, Reports and Other Educational Pieces Based on This Work**

### Proposed Manuscripts:

1. Dahlberg, J., E. Wolfrum, B. Bean, and B. Rooney. Compositional and agronomic evaluation of annual grass biomass as a potential feedstock for renewable fuels: Sorghum [*Sorghum bicolor (L.) Moench*]. (in preparation).
2. Kuhn, E., C. Scarlata, N. Nagle, and E. Wolfrum. Laboratory Scale Dilute-Acid Pretreatment and Enzymatic Hydrolysis of Two Sorghum Cultivars. (in preparation).
3. Bench-scale Comparison of corn stover and sorghum as an ethanol feedstock. (in preparation).
4. E. Tan et al, Technoeconomic analysis of the biochemical process for the conversion of sorghum biomass and sorghum grain to ethanol. (in preparation).
5. Dighe, N., W.L. Rooney, E. Wolfrum and J.A. Dahlberg. Comparison of dietary and detergent fiber methods of estimating sorghum biomass composition. (in preparation).
6. Hoffmann, L., W. Rooney, and T Stefaniak. Effect of genotype and environment on dietary lignin concentrations in photoperiod sensitive sorghum biomass. (in preparation).
7. Packer, DJ, N. Dighe, E. Wolfrum and WL Rooney. Variation in dietary fiber composition detected in a diverse set of exotic sorghum accessions. (in preparation).

#### Articles in Popular Magazines or Newspapers:

1. Lipps, H. Fall 2008. Standing Room Only: Worldwide interest in sorghum marks Houston conference. Sorghum Growers Magazine. National Sorghum Producers.
2. Perin, A. Sept. 5, 2008. Biofuel researchers set their sights on sorghum. Houston Business Journal.
3. Arnold, A. Fall 2009. Creating a future for cellulosic ethanol. Sorghum Growers Magazine. National Sorghum Producers.
4. Reichenberger, L. Summer 2010. Sorghum steps up. The Furrow.

#### Miscellaneous Educational Pieces:

1. Dahlberg, J. Planning committee for the International Conference on Sorghum for Biofuels that was held in Houston, Texas on August 19-22, 2008 (see <http://www.ars.usda.gov/meetings/Sorghum/agenda.htm>)
2. Dahlberg, J. 2008. NSP Partnering with the National Renewable Energy Lab for research on forage, sweet & biomass sorghums. Cellulosic Ethanol Summit, 2008. Miami, FL.
3. Dahlberg, J. 2009. Powerpoint presentation entitled “Sorghum: A Promising Feedstock for Biofuels” at the Western Great Plains Sustainable Feedstock Development Partnership meeting in Fort Collins, CO.
4. Tan, E. A. Aden, and J. Dahlberg. 2010. Technoeconomic Analysis of the Biochemical Process for the Production of Ethanol with Sorghum. Abstract and poster at the 32nd Symposium on Biotechnology for Fuels and Chemicals.
5. Dahlberg, J. 2010. Biomass Economics and Biofuels Supply Pane. Next Generation Biofuels. Chicago, IL.