Sorghum to Ethanol Research Initiative

Cooperative Research and Development Final Report

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CRADA number: **CRD-08-291**

CRADA Title: **Sorghum to Ethanol Research Initiative**

Parties to the Agreement: **National Sorghum Producers**

Joint Work Statement Funding Table showing DOE commitment:

Estimated Costs	**NREL** Shared Resources
Year 1	$ 293,500
Year 2	$ 513,184
Year 3	$ 69,316
TOTALS	$ 876,000

Abstract of CRADA work:

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 the 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 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 portion of the feedstocks required to produce renewable domestic transportation fuels.

Summary of Research Results:

Work on this project was performed in a number of different areas: compositional analysis & Near-infrared (NIR) model development, laboratory-scale pretreatment, enzymatic hydrolysis, and fermentation, pilot-scale pretreatment, enzymatic hydrolysis, and fermentation, techno-economic analysis, and education and outreach. These are summarized below.

Compositional Analysis & NIR Model Development

The goals of this portion of this work 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). Over 100 different sorghum varieties 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, 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[1] and the typical uncertainties[2] 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/α-amylase method[3]. 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).

[1] "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 C hemistry* 2010 ***58*** (16), pp 9043–9053.

[2] "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.

[3] Total Starch Assay Procedure(Amlyoglucosidese/a-amylase method) AOAC Method 996.11, AACC Method 76.13, available at

www.megazyem.com/downloads/data/K-TSTA.pdf

The results of the compositional analysis showed large variation in composition across the different cultivars. Data from this will be available on the DOE Compositional 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[4]. 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.

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 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[5]. 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_o values of 2.76 – 2.83. The second set was treated at R_o 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 ^{o}C $\pm$ 0.5 ^{o}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 and process intermediates were measured using standard NREL Laboratory Analytical Procedures (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

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[4] 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.

[5] 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.

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.

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.

Lab-scale enzymatic hydrolysis and 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. 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. 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.

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.

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[6] and was used without any modification. 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.

Educational and outreach programs

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 policy makers. The Furrow magazine also published an article on sorghums potential in the bioenergy field.

[6]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

Subject Inventions listing: NONE

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