# Quarterly Update

Biomass

National Bioenergy Center Sugar Platform Integration Project

Biomass Program—Sustainable Fuels, Chemicals, Materials, and Power

April/June 2004, #3


The Sugar Platform Integration Project focuses on integrating the processing steps involved in enzyme-based lignocellulose conversion technology. This project supports the U.S. Department of Energy's efforts to foster development, demonstration, and deployment of "sugar platform" biorefineries that produce inexpensive commodity sugars and fuel ethanol, as well as a variety of other fuel and chemical products, from abundant renewable lignocellulosic biomass.

The National Renewable Energy Laboratory manages this project for DOE's Office of the Biomass Program. Information on the Biomass Program is available at Biomass Program

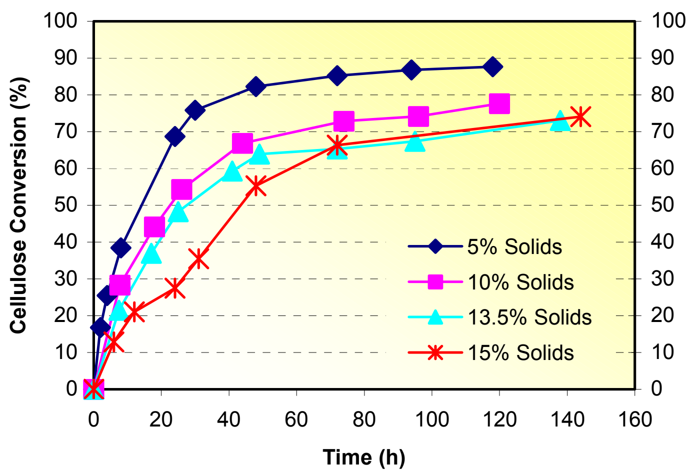
To discuss information in this update or for further information on the Sugar Platform Integration Project, contact Dan Schell at NREL, phone (303) 384-6869, email dan_schell@nrel.gov

**Process Integration Interim Gate Review Meeting.** An interim stage review meeting for the Process Integration Project has tentatively been scheduled for September 15th and will be held this year in Washington, D.C. As in the past, this meeting will be open to all interested parties and when plans are finalized more information will be made available.

## R&D Progress

**Differentially Pretreated Corn Stover Samples Produced for Surface Characterization.** To understand how pretreated biomass surface characteristics affect enzymatic cellulose digestibility, a series of dilute acid pretreated corn stover (PCS) samples have been produced using low, moderate, and high pretreatment severities. These materials, which have been supplied to NREL's Fundamentals and Advanced Concepts Project for analysis using the program's newly acquired Biomass Surface Characterization equipment, were generated in the pilot-scale (1 ton/d) pretreatment system using process realistic solids concentrations ranging from 25% to 35% w/w. The severity of pretreatment was altered by changing temperature and sulfuric acid loading; residence time through the continuous reactor was maintained at approximately 1 min. All of the pretreatment conditions achieved good solubilization of hemicellulose to sugars (>70%). Visually, the PCS solids produced at the lowest severity were much more heterogeneous than the other samples and contained some large and still mostly intact corn stover particles. In contrast, the PCS solids produced at the highest severity conditions were comprised of small, apparently highly degraded particles; this material had a mud-like consistency. These three samples have significantly different susceptibilities to enzymatic cellulose hydrolysis, which suggests that they are good candidates to study using the program's nascent biomass surface characterization tools. The PCS produced at the moderate severity conditions exhibits the highest enzymatic cellulose digestibility (90% under standard assay conditions); the material produced at the high severity condition is somewhat less digestible (85%), and the material produced at the low severity condition is the least digestible (70%). Surface characterization of these samples will attempt to identify structural features and changes therein that may correlate with enzymatic cellulose digestibility and ultimately lead to a greater understanding of the nature of biomass recalcitrance.

**Progress Made on Carrying out Enzymatic Cellulose Saccharification at High Solids Concentrations.** To realize the economic benefits of high solids processing and the subsequent increase in sugar concentrations that such an approach affords, effective enzymatic cellulose saccharification must be achieved at solids concentrations of 25% or even higher. We have a FY04 year-end goal to achieve 80% cellulose saccharification at 25% solids concentration. This would produce an effective glucose concentration of 14%-15%, which combined with hemicellulosic sugars produced by pretreatment would enable total soluble sugar concentrations in the range of 25% to be achieved. Being able to produce and use such concentrated sugar streams will significantly reduce both capital and operating costs. Approaches based on fed-batch saccharification and new reactor concepts that facilitate high solids processing are being pursued to try to reach this goal.




Recent work has shown that when conventional stirred tank reactors are operated at high solids concentrations, mixing and heat transfer limitations reduce saccharification yields. The figure to the left illustrates how increasing solid concentrations decreases cellulose conversion during saccharification. However, effective saccharification has recently been demonstrated in static, uniformly heated shake flasks at solids concentrations up to 22%. We expect that this level of performance can be achieved in agitated vessel using an appropriate fed-batch feeding regime that limits the amount of insoluble solids in the reactor to maintain effective mixing and heat transfer. A parallel approach is to use an alternative reactor design that mitigates the problems experienced with agitated reactors. We have designed a novel, bench-scale reactor that should be able to operate at very high solids levels. A prototype small-scale (5 L) reactor is currently being fabricated and initial experimental work to test its efficacy is anticipated to begin in July.

**Successful Demonstration of On-line Monitoring of a Pretreatment Process.** On-line spectra of the composition of pretreated corn stover were obtained during a recent pretreatment run carried out in the continuous 1 ton/d pilot-scale pretreatment reactor. On-line monitoring of the pretreated biomass was performed using a Nicolet Avatar 360 Fourier Transform Infrared (FTIR) spectrometer coupled to a diamond composite probe inserted into the pretreatment reactor's flash tank. This system was able to collect spectra on pretreated corn stover materials entering the flash tank. Compositional information can be obtained by applying appropriate data analysis techniques to such spectra.

In this work, the FTIR spectrometer was configured to measure the near-infrared (NIR) overtone and mid-infrared fingerprint regions extending from 400 $cm^{-1}$ to 7800 $cm^{-1}$. The collected spectra showed a variety of mid-infrared spectral features, including bands between 1000 $cm^{-1}$ and 1500 $cm^{-1}$ resulting from carbon-carbon and carbon-oxygen bond vibrations, bands near 1750 cm-1 resulting from carbonyl bond stretching vibrations associated with acetic acid, and sugar hydroxyl O-H bond stretching bands near 3300 $cm^{-1}$. The signal-to-noise ratio was only modest in the NIR overtone region, and future work will examine the use a liquid nitrogen cooled detector to improve upon this.

With this accomplishment, we can now begin to construct a Projection to Latent Structures (PLS) model that will enable us to determine pretreated corn stover composition from on-line spectra. Ultimately, this methodology will enable us to perform real time monitoring and control of pretreatment processes, e.g., so that we can predict sugar yields in real time when seeking to maximize hemicellulose sugar yield and/or enzymatic cellulose digestibility. Despite this tremendous progress, a sustained development effort is still needed to bring this goal to fruition.

**Work Continues on Developing Improved Biomass Sugars Analysis Methods.** A new subcontracted effort to develop improved methods for measuring biomass sugars using High Performance Liquid Chromatography (HPLC) was placed with Virginia Polytechnic Institute and State University. Professor Foster Agblevor, the subcontract principal investigator, will develop, test and rigorously validate new HPLC methods that achieve better resolution of all biomass sugars and significantly improve quantification of minor biomass sugars. Professor Agblevor has already demonstrated the applicability of these new methods for analyzing sucrose and fructose, two sugars that are important constituents of corn stover biomass that are not quantifiable using existing standard methods. Once validated, the methodology will be transferred to NREL and standard analysis protocols will be written up for publication through the American Society for Standards and Materials. Ultimately, by improving analytical data quality that enables more accurate feedstock characterization and higher quality carbon mass balances across pretreatment and saccharification/fermentation processes, this work will facilitate industry's biomass technology deployment efforts.

**New and Improved Laboratory Analytical Procedures Issued for Biomass Compositional Analysis.** Eight new or improved standard Laboratory Analytical Procedures (LAPs) currently being used at NREL to support all Sugar Platform research activities have been posted on the new Biomass Program Web site (Analytical Procedures). These updated standard methods apply to a wider variety of biomass types, improve method precision and accuracy, and expand the summative analysis to include more components of lignocellulosic biomass. The procedures are:

- Preparation of samples for compositional analysis
- Determination of ash in biomass
- Determination of total solids in biomass
- Determination of extractives in biomass
- Determination of structural carbohydrates and lignin in biomass
- Determination of protein in biomass
- Determination of sugars, by-products and degradation products in liquid process samples
- Guidelines for rounding and significant figures when reporting analytical data.

These new or improved LAPs are *draft* methods, and a dedicated email address has been set up at the web site to collect comments and suggestions from other laboratories (industry, university, and government laboratories) using these methods. The ultimate goal is to establish a portfolio of validated universal consensus methods for accurately analyzing, with confidence, a wide variety of biomass feedstocks and biomass-derived materials.

**Many Pretreated Corn Stover Samples Generated from a Broad Range of Conditions Produced for Porosity Measurements.** Over the last year, corn stover has been pretreated in the pilot-scale reactor using a broad range of dilute-acid pretreatment conditions. Accordingly, a wide variety of pretreated corn stover (PCS) samples has been produced, including many that differ significantly in their susceptibilities to enzymatic cellulose hydrolysis; extents of enzymatic conversion under standard assay conditions range from 69% to 96%. In an effort to understand how structural features are influencing cellulose conversion, porosity measurements will be made on a subset of over 30 of these PCS samples recently provided to the Fundamentals and Advanced Concepts project. Their goal is to identify structural features and changes therein, such as in porosity, that correlate with enzymatic cellulose digestibility and ultimately lead to a greater understanding of the nature of biomass recalcitrance. This is a good example of how the Process Integration project, in addition to supplying samples for external Biomass Program stakeholders, continues to generate timely, process relevant samples to facilitate and interface with ongoing Fundamentals work.

## Related Activities Biomass

**Corn Stover Anatomical Fractions Being Quantified.** In the Fundamentals and Advanced Concepts Project related work on corn stover is underway. Since the composition of different functional parts of a corn plant are known to be compositionally distinct, it is important to understand what fraction of the plant mass is represented by each plant organ. This, of course, may vary from one variety to another. Dissection of five plants of a single variety shows that grain represents 38% of the biomass (dry weight basis); leaves, 3%; sheaths 6%; stalks, 37%; cobs and ear husks, 8% each. Then considering stover constituents only (i.e., excluding grain), the stalk represents 59% of the biomass; leaves, 5%; sheaths, 11%; cobs and husks, 13% each. Of the stalk fraction, 69% is internode and 31% is node. Of the internode fraction, 72% is rind and 28% is pith. This type of data will be invaluable when reviewing various harvesting and pretreatment scenarios, as well as to guide work in the Fundamentals and Advanced Concepts Project.

**Enzyme Sugar Platform Project Information.** Web-based information on the ESP project including our recent presentations at stage gate review meetings can be found at the following link (ESP Project Information). A discussion of how Stage Gate management is used in the Biomass Program is also available at this site.

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