Quarterly Update

National Bioenergy Center Biochemical Platform Integration Project

Biomass

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

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The Biochemical Processing Integration Task 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 "biochemical 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 Biochemical Processing Integration Task, contact Daniel Schell at NREL, phone (303) 384-6869, email dan_schell@nrel.gov

28th Symposium on Biotechnology for Fuels and Chemicals.

The Symposium this year was highly successful and set a new attendance record. This reflects the increasing global interest in renewable materials and biotechnology aimed at producing ethanol and other biochemicals/materials. We wish to thank everyone who visited our posters during the meeting.

R&D Progress

Progress Being Made in Identifying and Quantifying Water Extractives in Corn Stover. Water extraction is one of the initial steps in compositional analysis of biomass feedstocks. Previous work has shown that 10% - 30% of the dry weight of corn stover is made up of water extractable material. It is likely that other agricultural residues and herbaceous feedstocks contain significant levels of extractives. About half of the extractable materials in stover are glucose, fructose, and sucrose and the other half are unknown. These unknown compounds may have value or they may be a liability. Last year we placed a subcontract with Dr. C. Kevin Chambliss from Baylor University to identify these unknown materials. Dr. Chambliss' group has now developed procedures to identify and quantify 29 compounds in the water-soluble extracts of corn stover. These techniques were used to characterize extractives in six geographically different corn stover samples. Soluble carbohydrates accounted for about half of the water extracted material as expected. A quarter of the water extractives were aliphatic organic acids including 2% - 5% each of aconitic and citric acid. Another fraction that accounts for about 15% of the water extractives is still under investigation but is expected to be sugars ether-linked to aromatic compounds. The remainder of the water extractives consists of trace quantities of salts and aromatic organic acids.

Sucrose-Derived Sugars Contained in Some Feedstocks Survive Dilute Acid Pretreatment. Agricultural and herbaceous feedstocks may contain appreciable levels of soluble sugars. We have measured sugar (i.e., sucrose, fructose, and glucose) contents as high as 10% (w/w) in corn stover. Recent work investigated the survivability of sucrose and its hydrolysis products, fructose and glucose, in dilute sulfuric acid solutions at conditions typically used to pretreat lignocellulose biomass. Solutions containing 25 g/L sucrose with 0.1% to 2.0% (w/w) sulfuric acid concentrations were treated at temperatures of 160°C to 200°C for 3 to 12 minutes. Sucrose was observed to completely hydrolyze at all treatment conditions. However, substantial concentrations of fructose and glucose were detected and glucose was found to be significantly more stable than fructose. We previously assumed that these sugars were lost during dilute acid pretreatment. Since some of the sugars survive, they could provide an additional carbon source for production of ethanol and other bio-based products.

Improved Conditioning of Dilute Acid Pretreated Biomass. This task is continuing work to assess and reduce sugar losses that occur during lime ("overliming") conditioning of dilute acid pretreated biomass – a process required to make fermentable hydrolysates. Previous work demonstrated an approximate 10%-12% loss of glucose and xylose following overliming conditioning. Preliminary results from a follow-on study suggest that lower temperature overliming may eliminate sugar losses. Although, hydrolysate conditioned at lower temperatures is not as fermentable as one conditioned at higher temperatures, better overall ethanol production is achieved because the lower conversion yield of sugars to ethanol is more than compensated for by the greater amount of sugars present in the conditioned hydrolysate. We are investigating other modifications of the process that may be able to further improve overall ethanol yields.

Developing New Methods to Accurately Measure Lignin in Corn Stover. Recent work has shown high lignin mass balance closure around pretreatment suggesting that compositional measurements of lignin in either the raw and/or pretreated stover are inaccurate. We are investigating the use of analytical pyrolysis to measure lignin instead of the normal analytical technique based on measuring the residue left after concentrated acid hydrolysis, which is defined as acid-insoluble lignin. Preliminary results reveal that lignin-like non-structural material is present in pretreated stover samples. The normal compositional analysis methods would report this material as extractives in the feedstock, but report it as lignin in pretreated material. This fact may explain the high lignin mass balance closures seen in previous work where the amount of lignin in pretreated stover was being over estimated. Work will continue to improve lignin quantitation and to further identify the source and structures of the extractable lignin-like material and develop improved methods for compositional analysis of lignin and lignin-like compounds.

Related Activities

New Patent Issued Describing Cellulase Enzymes. This patent (U.S. No. 7,059,993) represents the most recent of a number of patents that have been issued to NREL on the topic of improved cellulases for biomass conversion. The current invention, entitled "Thermal Tolerant Cellulase from *Acidothermus cellulolyticus,*" provides for the enzyme GuxA, a thermal tolerant cellulase that has catalytic domains similar to glycosyl hydrolase families 6 and 12. This enzyme was discovered in a thermophilic actinomycete originally isolated from decaying wood found in an acidic, thermal pool in Yellowstone National Park. A number of thermostable enzymes have now been characterized from this biomass degrading organism. One cellulase enzyme produced by this organism, the endoglucanse E1, known to display maximal activity at 75^{o} - 83^{o}C, was described in U.S. Patent No. 5,275,944. The E1 enzyme displays a high degree of synergism with the exocellulase CBH1 from *Trichoderma reesei* and has been successfully expressed in a wide variety of hosts. The current invention describes an enzyme, GuxA, which has two catalytic domains identified as belonging to the GH6 and GH12 families. The GH6 domain family includes a number of cellobiohydrolases, or exoglucanses, that degrade cellulose substrates using an inverting mechanism. The GH12 domain family includes a number of endoglucanases that degrade cellulose using a retaining mechanism. Being a member of the GH6 and GH12 family of proteins identifies GuxA as potentially having both exoglucanase and endoglucanase activity.

Biochemical Processing Integration Task Information. Web-based information on the process integration project, including presentations made at the most recent stage gate interim review meeting, can be found at the following link (Process Integration Project Information). A discussion of how Stage Gate management is used in the Biomass Program is also available at this site (Stage Gate Management).

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