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 the content of 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

R&D Progress

NREL Contributes to Development of a Rapid Xylose Measurement

The ability to rapidly measure sugar concentrations is desirable and necessary when monitoring the health and progress of a fermentation. This is challenging when multiple sugars are present, for example, when fermenting sugars present in lignocellulosic biomass. Yellow Springs Instruments (YSI) makes a device that uses enzyme electrode technology to measure glucose and product (e.g., ethanol, lactic acid, etc.) concentrations in about one minute. YSI recently developed a technique for measuring xylose, and we tested the technique during fermentation of dilute-acid-pretreated corn stover hydrolysates. The xylose concentration measurements from the YSI instrument compared well to measurements performed by high performance liquid chromatography. We will present these results in a joint NREL/YSI poster at the Society of Industrial Microbiology annual meeting in August 2008.

Sucrose Hydrolysis Study Published in Bioresource Technology

In collaboration with personnel from Colorado State University (CSU), we recently published a report on sucrose hydrolysis entitled "Modeling sucrose hydrolysis in dilute sulfuric acid solutions at pretreatment conditions for lignocellulosic biomass." Authors of this paper were Shane Bower (CSU), Ranil Wickramasinghe (CSU), Nick Nagle (NREL) and Daniel Schell (NREL). Because agricultural and herbaceous feedstocks may contain appreciable levels of sucrose, the goal of this study was to evaluate the survivability of sucrose and its hydrolysis products, fructose and glucose, in dilute sulfuric acid solutions at conditions typically used for dilute acid pretreatments. Sucrose was hydrolyzed at all treatment conditions tested, which spanned temperatures of 160°C to 200°C, sulfuric acid concentrations of 0.1% to 2.0% (w/w), and treatment times of 3 to 12 min. However, we detected large concentrations of fructose and glucose at many treatment conditions, with glucose exhibiting much greater stability than fructose. Different mathematical approaches were examined to fit kinetic parameters for acid-catalyzed thermal degradation of these sugars. The results show that, depending on the treatment conditions, up to 75% of the total available glucose and fructose survive pretreatment. Therefore, these sugars could provide an additional carbon source for the production of ethanol and other bio-based products. These findings were published in the journal *Bioresource Technology*, Vol. 99, p. 7354.

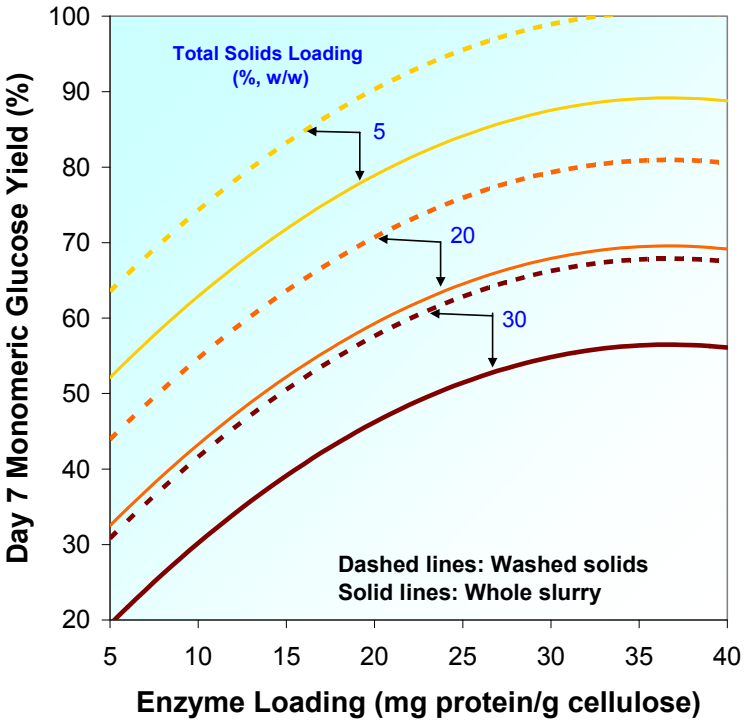

Figure 1. Effect of solids and enzyme loading on monomeric glucose yields during enzymatic hydrolysis of dilute acid pretreated corn stover solids with (whole slurry) and without (washed solids) the presence of background hydrolysate liquor.

Understanding Performance of a Commercial Enzyme Product on Dilute-Acid- Pretreated Corn Stover

We recently completed a study investigating the ability of a commercial enzyme preparation to enzymatically hydrolyze dilute-acid-pretreated corn stover solids at various total solids loadings. Enzyme performance was evaluated on washed solids after removing background sugars and compounds produced during pretreatment as well as on whole slurries where these compounds remained. Figure 1 shows monomeric glucose yields after 7 days of hydrolysis as a function of enzyme and total solids loading. At the same total solids loading, the cellulose content in whole slurries is about one-half the value in washed solids because of the dissolved soluble compounds present in whole slurry. Figure 1 clearly shows several trends. Yields increase with increasing enzyme loading, but performance plateaus at an enzyme loading of about 30 mg protein/g cellulose. High total solids loadings have a significant negative impact on yield. For example, during hydrolysis of washed solids at an enzyme loading of 20 mg/g, the monomeric glucose yield is 90% at a 5% solids loading, but decreases to 55% at a 30% solids loading. This result demonstrates significant end-product inhibition from higher sugar concentrations produced at the higher solids loadings. Finally, at the same total solids loading yields in whole slurry are lower than on washed solids even though the cellulose content is half the value of that found in washed solids. This latter observation means that glucose concentrations produced during hydrolysis of whole slurries are lower than glucose concentrations produced during hydrolysis of washed solids, which should lead to lower end-product inhibition in whole slurries. The contrary result suggests that other inhibitory compounds are present in the liquor of whole slurries. We previously showed that other sugars (xylose and arabinose), acetic acid, furfural and HMF present in dilute-acid-pretreated hydrolysates are inhibitory. Improvement in pretreatment technology and new enzymes with reduced inhibition, faster hydrolysate rates and higher specific activities are needed to aid rapid development of bio-based lignocellulosic conversion technology.

Related Activities

New Publication on Molecular Simulation of Cellulase Action

In cooperation with university collaborators, Biological Processing Fundamentals area recently published the results of molecular dynamics simulations in a paper entitled "Molecular Simulation Evidence for Processive Motion of *Trichoderma reesei* Cel7A During Cellulose Depolymerization." Authors of this paper are Clare McCabe, Xiongce Zhao, Tauna R. Rignall, William S. Adney, and Michael E. Himmel. Cellobiohydrolase I (Cel7A) from *Trichoderma reesei* is one of the most active enzymes in the hydrolysis of cellulose and is believed to hydrolyze cellulose in a "processive" manner. Cel7A is a multi-domain enzyme, consisting of a large catalytic domain with an active site tunnel and a small cellulose binding module joined to each other by a 27-residue linker peptide. The exact mechanism of depolymerization of cellulose by Cel7A is not fully understood. This paper presents free energy calculations for the linker peptide from extensive molecular dynamics simulations focused on understanding processive catalysis. The authors found that the linker displays two stable states at lengths of 2.5 nm and 5.5 nm during compression/extension, with a free energy difference of 10.5 kcal/mol between the two states. The length-dependent free energy increases dramatically when the linker stretched beyond 6 nm or compressed to less than 2 nm. The switching between these two stable states supports the hypothesis that the linker peptide has the capacity to store energy in a manner similar to a spring. These findings are available on-line in the journal *Chemical Physics Letters*.

Biochemical Processing Integration Task Information

Web-based information on the process integration project, including presentations made at review meetings, is found at the following links (Process Integration Project Information, http://obpreview07.govtools.us/biochem/). A discussion of how the Biomass Program uses Stage Gate management is available at the following site (Stage Gate Management).

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