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

29th Symposium on Biotechnology for Fuels and Chemicals.

The 29th Symposium is almost here. If you haven't registered or made travel arrangements now is the time. The Symposium will be held in downtown Denver, CO at the Adam's Mark Hotel from April 29 – May 2, 2007. Meeting, registration and hotel information can be found at the following web site: http://www.simhq.org/meetings/29symp/index.html.

2007 Joint ACS/AIChE Rocky Mountain Regional Meeting.

This Meeting will also be held in downtown Denver, CO at the Adam's Mark Hotel from August 29 – September 1, 2007. Meeting and registration information and a link for submitting abstracts are available at the following web site: http://www.uwyo.edu/rmr2007acs-aiche. The abstract submittal system is now active. Meeting topics are given below and in particular please consider a poster and/or oral presentation for the Advances in Alternative Energy Technologies session.

- **Advances in Conventional Energy Technologies (Coal, Oil, Natural Gas)**
- **Advances in Alternative Energy Technologies (Fuel Cells, Photovoltaics, Hydrogen, Biofuels)**
- **Advances in Pollution Control and Environmental Remediation Technologies**
- **Green Chemistry and Engineering**
- **Bioprocesses for Diagnostic and Therapeutic Agents**
- **Biomaterials**
- **Electronic Materials**
- **Advances in Nanotechnology**
- **Teacher workshop**

R&D Progress

Testing Performance of Ethanologens in Dilute Acid Pretreated Corn Stover.

We recently tested the ability of a limited number of microorganisms to produce ethanol from sugars in concentrated dilute acid pretreated corn stover hydrolysates (pretreated at 30% total solids). The liquor fraction of the hydrolysate was used at 40%, 65%, and 85% of its original strength, neutralized with lime or conditioned by overliming, and then supplemented with additional glucose. We tested several glucose fermenting yeasts, a glucose/xylose fermenting yeast, and the recombinant glucose/xylose fermenting *Zymomonas mobilis* 8b. The glucose fermenting yeasts were able to effectively produce ethanol regardless of treatment. However, some of the yeasts did not perform well in medium supplemented with an additional 100 g/L of glucose. Two yeasts, a Broin commercial *Saccharomyces cerevisiae* and *S. pastorianus* (ATCC #26602) performed well in 85% hydrolysate with 200 g/L of added glucose. They achieved an ethanol yield of 70% of theoretical (based on glucose) producing approximately 90 g/L of ethanol. The glucose/xylose fermenting microorganisms were severely affected by hydrolysate concentration, producing little ethanol in 85% of full strength hydrolysate. *Z. mobilis* performed well in 65% hydrolysate achieving an ethanol yield of 67% of theoretical (based on glucose and xylose) while using 70% of the xylose.

Evaluating Alternative Hydrolysate Conditioning Technologies. Conditioning hydrolysate with lime ("overliming" process) effectively improves the fermentability of dilute acid hydrolysates, but the process causes sugar losses due to high pH degradation reactions. It also produces gypsum that may pose downstream processing problems (e.g., plating out in the distillation column reboiler). A shake flask screening study was performed that identified conditions where treatment with ammonium hydroxide did not produce the sugars losses normally seen during lime treatments. These results were confirmed in 0.5 L working volume pH-controlled fermentations using the glucose-xylose fermenting recombinant *Zymomonas mobilis* 8b, in which a 15% to 20% improvement in ethanol yields was demonstrated for ammonium hydroxide treated liquor compared to lime treated liquor (see Figure 1). In part, these improved results were achieved because the ammonium hydroxide treatment does not cause significant sugar losses so that more sugars remain available for conversion. The highest overall ethanol yield (i.e., yield based on available sugars in the unconditioned hydrolysate) of 84% of theoretical was reached when ammonia hydroxide was used to raise the pH of a pretreated corn stover hydrolysate liquor to 8.5. The treated liquor was then held at 30°C for 30 minutes before adjusting the pH to 6 with sulfuric acid. Hydrolysate neutralized with ammonia hydroxide (pH adjustment to 6.8 at room temperature) also fermented well, achieving an overall ethanol yield of 80%; hydrolysates neutralized with lime or overlimed only achieved overall ethanol yields of 58%.

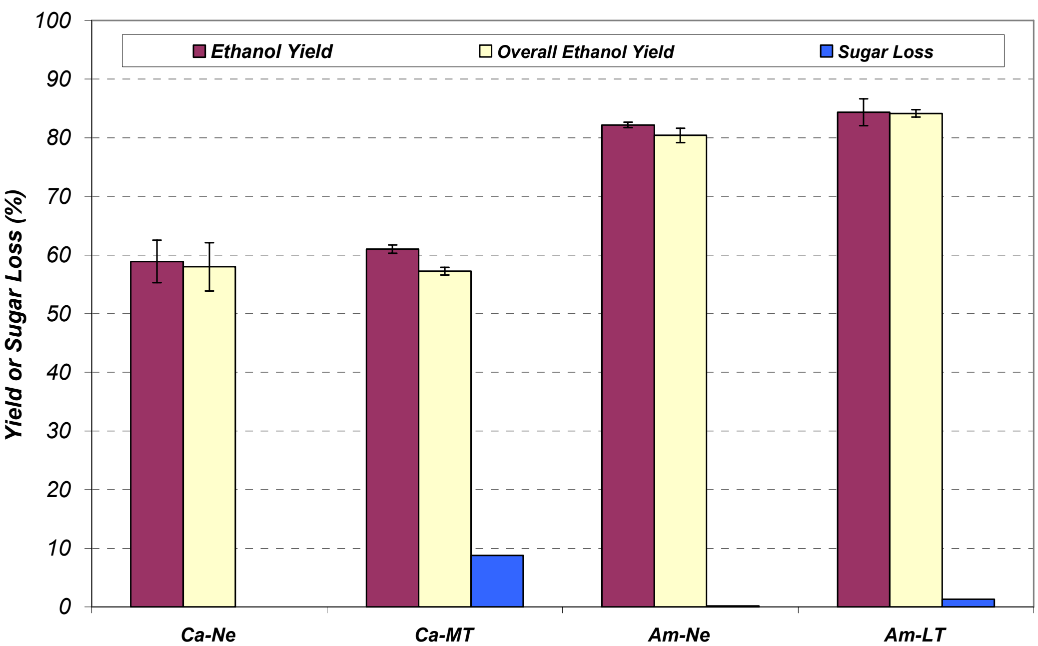

Figure 1. Ethanol and overall ethanol yields and sugar losses (combined glucose and xylose) during conditioning and fermentation of the liquor fraction of a dilute acid pretreated hydrolysate. Legend: Ca-Ne − neutralized with lime to pH 6.8; Ca-MT − overlimed to pH 10 and held at 50°C for 30 min, then pH adjusted to 6.8; Am-Ne – neutralized with ammonium hydroxide to pH 6.8; Am-LT − pH raised to 8.5 with ammonium hydroxide and held at 30°C for 30 min, then pH adjusted to 6.8.

Related Activities

NREL Research Group Publishes Perspectives Paper in the Special Issue "Sustainability and Energy" of the Journal *Science*. The paper by Himmel et al., entitled "Biomass Recalcitrance: Engineering Plants and Enzymes for Biofuels Production," is one of several articles in the February 9 issue that "examines one of the vital scientific and societal issues of our time – the need to move toward new sustainable sources of energy in the face of human-induced climate change, increasing worldwide energy demand, and dwindling supplies of fossil fuels." The article considers the natural resistance of plant cell walls to microbial and enzymatic deconstruction collectively known as "biomass recalcitrance." It is this property of plants that is largely responsible for the high cost of lignocellulose conversion we face today. To achieve sustainable energy production, it will be necessary to overcome the chemical and structural properties that have evolved in biomass to hinder its disassembly.

Reference: Himmel, Michael E., Shi-You Ding, David K. Johnson, William S. Adney, Mark R. Nimlos, John W. Brady, and Thomas D. Foust. "Biomass Recalcitrance: Engineering Plants and Enzymes for Biofuels Production." (9 February 2007) *Science* **315**: 804-807.

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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