NREL NATIONAL RENEWABLE ENERGY LABORATORY

Quarterly Update

National Bioenergy Center | Biochemical Platform Integration Project

Fall 2010

BIOMASS

The Biochemical Process Integration Task focuses on integrating the processing steps 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 economically produce ethanol or other fuels, as well as commodity sugars and a variety of other chemical products, from 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 contents of this update, or for further information on the Biochemical Process Integration Task, contact Dan Schell at NREL, phone 303-384-6869, e-mail dan.schell@nrel.gov.

33rd Symposium on Biotechnology for Fuels and Chemicals

This Symposium will be held at the Sheraton Seattle in Seattle, Washington, May 2–5, 2011. Meeting information can be found at the following Web site: http://www.simhq.org/meetings/sbfc2011/index.asp. Abstract submission is open and closes December 17, 2010.

R&D Progress

Rapid Analysis Models for Intermediate Process Streams

Rapid analysis techniques for compositional analysis of biomass materials based on near-infrared (NIR) spectroscopy and multivariate calibration algorithms have been in use for many years at NREL. Models for predicting corn stover and dried pretreated corn stover composition are used regularly, and models for predicting the composition of pretreated corn stover liquor and the whole slurry are in development. We have recently begun extending the use of these techniques to measure soluble sugars, organic acids, and ethanol concentrations in enzymatic hydrolysates and fermentation broths.

These new models were built using compositional data from a set of enzymatic hydrolysate and fermentation broth samples and synthetic simulated samples prepared from fermentation broth. Synthetic samples were produced by removing ethanol from the fermentation broth by air stripping and then spiking the fermentation broth samples with various sugars at different concentrations. NIR spectra were collected using a cam-lock transflectance cell or a fiberoptic probe. A probe could be used for *in situ* measurements, but the models built from probe data were disappointing. More work is needed to understand why spectra collected with a probe produced inferior models to those produced from a transflectance cell. A model built from spectra obtained from the transflectance cell using actual and synthetic samples with glucose and ethanol concentrations ranging from 0–140 g/L and 0–100 g/L, respectively, predicted concentrations for these analytes better than models built using only synthetic samples or actual samples. The approximate minimum uncertainties in predicting glucose and ethanol concentrations were 4.4 g/L and 1.6 g/L, respectively, for the best model. Predictions of concentrations in samples that differ significantly from the calibration sample set will have higher uncertainties. It may be possible to decrease the uncertainty slightly by adding more samples into the calibration model, but substantial reductions are unlikely. The next step is to determine if these techniques can be used for real-time component concentration measurement during pretreatment, enzymatic hydrolysis, and fermentation.

New Engineered *Zymomonas mobilis* Strain Ferments Arabinose to Ethanol

Arabinose is the third most abundant sugar in corn stover following glucose and xylose. However, arabinose is not converted to ethanol by *Zymomonas mobilis* strains currently used at NREL. This year we successfully introduced arabinose catabolic genes into the glucose-xylose fermenting microorganism *Z. mobilis* 8b, creating the plasmid-bearing strain 8b/pZB206. In a pure sugar solution this strain consumed 85% of the available arabinose (see Figure 1). Because chromosomally-integrated strains are preferred for their superior stability, work is continuing to develop stable arabinose-xylose-glucose utilizing integrants. We typically observe better performance for newly transformed strains after selection/enrichment for growth on arabinose, suggesting that beneficial mutation(s) may have occurred during selection. Identification of these beneficial genetic mutation(s) either through comparative genomic sequencing or other systems biology tools will be critical for future construction of *Z. mobilis* strains with improved arabinose utilization characteristics.

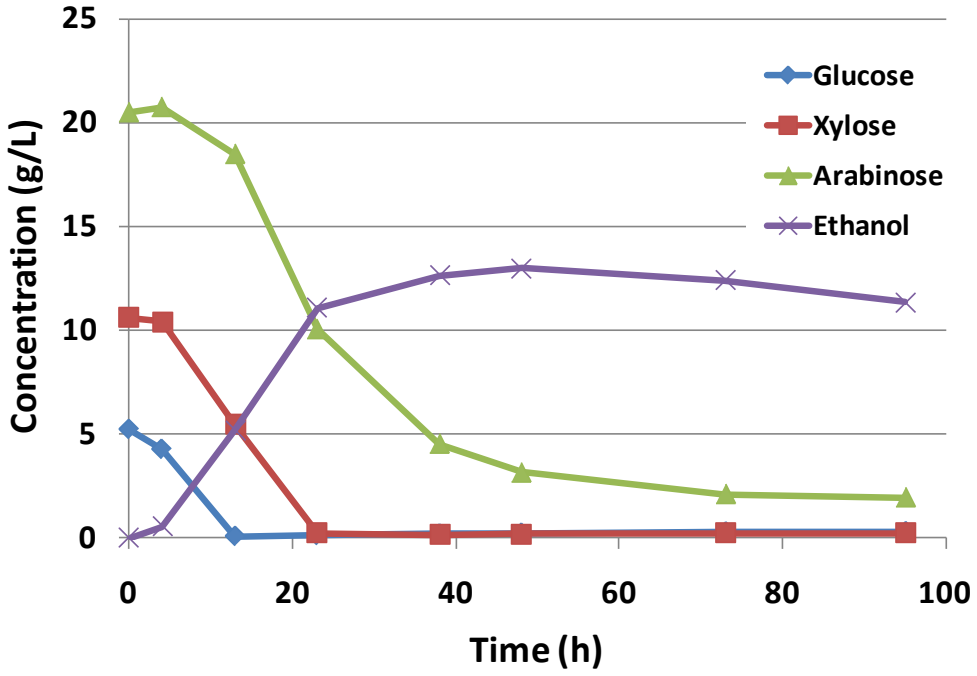

Figure 1. Sugar consumption and ethanol production profiles for the best plasmid-based arabinose-utilizing *Z. mobilis* strain

Biochemical Process Integration Task Information

Web-based information on the biochemical process integration project, including presentations made at past review meetings, is available at the following links: http://www.obpreview07.govtools.us/biochem/ and http://www.obpreview2009.govtools.us/biochem. A project review meeting is scheduled the week of February 14, 2011, in Denver, Colorado, but no further details are available at this time.

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