U.S. DEPARTMENT OF ENERGY | Energy Efficiency & Renewable Energy

Conversion of Lignocellulose to Biofuel Precursors

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Background and Introduction

In the past 20 years the conversion of biomass to ethanol has been pursued as a cost effective approach to the generation of clean energy. However, to achieve competitiveness with traditional fossil fuels, further progress is needed especially in lowering costs associated with severe biomass pretreatments. One approach is to increase cellulose and hemicellulose oligomer saccharification via enzyme hydrolysis. Previous work at the National Renewable Energy Laboratory (NREL) has shown that addition of purified cellulase and hemicellulase enzymes can reduce the need for severe pretreatments. Recent studies at NREL have shown that enzyme cocktails can work synergistically in oligomer saccharification to increase overall glucose yields from biomass (Figure 1). However, since this finding, little work has been done to characterize these important interactions.

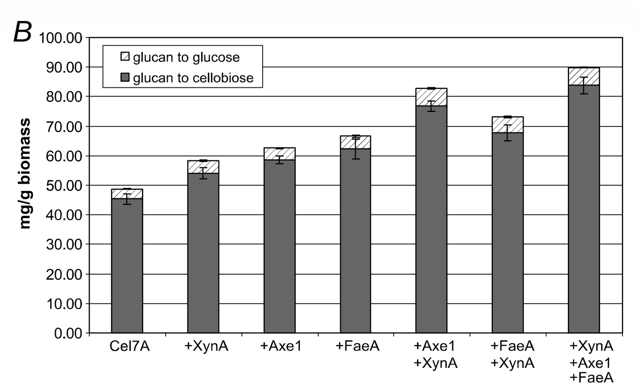
- Aim one of this fellowship is to increase the yields of glucose and xylose released from less severely pretreated corn stover to by characterizing various enzyme synergies and optimizing enzyme cocktails.

A

Enzyme (mg/g cellulose)	% CB	dev	% XB	dev
Single enzyme control digestions on pretreated corn stover				
XynA (2.5)	0.00	0.00	2.84	0.03
Axel (2.5)	0.00	0.00	0.00	0.00
FaeA (2.5)	0.00	0.00	0.00	0.00
Cel7A (50)	17.05	0.95	3.39	0.53
Cel7A (15)	6.15	0.22	2.93	0.03

Reported as the percent glucan converted to cellobiose and the percent xylan converted to xylobiose.

B



C

	Additive projection	Actual release	% synergistic improvement	% increase over Cel7A
(A) *Percent glucan converted to cellobiose*				
Cel7A 15 mg/g	6.24	6.24	NA	NA
+ XynA	6.24	7.42	18.93	18.93
+ Axe1	6.24	8.05	29.03	29.03
+ FaeA	6.24	8.57	37.24	37.24
+ XynA/ Axel	6.24	10.57	69.32	69.32
+ XynA/ FaeA	6.24	9.31	49.20	49.20
+ XynA/ Axe1/ FaeA	6.24	11.52	84.64	84.64

Figure 1. Demonstration of synergy between cellulase and hemicellulase enzymes. A) Glucan conversions from digestions of pretreated corn stover by individual hemicellulase, XynA, AxeI, or FaeA, and cellulase, Cel7A digestions on pretreated corn stover. B) Actual measurements of glucan conversion showing that the addition of hemicellulases increase the total amount of glucan conversion. C) Table showing the projected and actual contribution of added enzymes suggesting a synergistic effect. Selig et al. 2008. Bioresource Technology

While lignocellulosic ethanol is an important fuel in the immediate term, it is also important to consider advanced biofuels of the future. Recently, researchers at NREL have discovered how to create longer chain hydrocarbons, such as C8 to C16, from small chain carbons, such as polyhydroxybutyrate (PHB). *Saccharophagus degradans* 2-40 (strain 2-40) is a cellulolytic marine bacterium that is has been shown to produce PHB from carbon rich environments, however, research has not confirmed that strain 2-40 is able to create PHB directly from lignocellulose.

- Aim two of this fellowship will utilize the bacterium *S. degradans* 2-40 to convert lignocellulose into polyhydroxybutyrate (PHB) while increasing total PHB accumulation.

Approach

To maximize sugar yields from lignocellulosic biomass, the appropriate balance of enzyme concentrations and activities must be determined. This will be done by matching enzyme activities to the carbohydrate composition of pretreated corn stover (PCS) and in parallel with altering the total enzyme loadings. During "high solids" testing, meaning the substrate is ≥ 15% of the total volume, enzymes will be mixed in cocktails and added to the substrate in order to achieve the highest possible glucose yields. Smaller scale experiments will also be completed using novel enzymes in order to optimize enzyme cocktails.

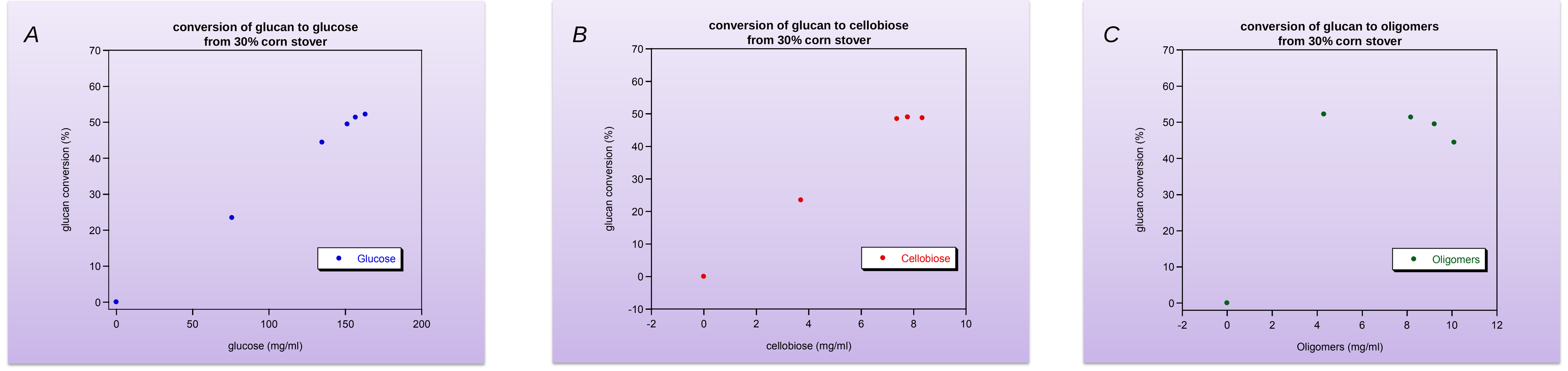


Figure2. Glucan conversion to A) glucose, B) cellobiose and C) unspecified oligomers. Graphs show that only ~50% of total glucan is being converted into glucose, or other cellulosic oligomers. Also, important is that glucan conversion seems to be inhibited by cellobiose or other oligomers. (unpublished data).

The ability of strain 2-40 to metabolize lignocellulose while accumulating PHB will be determined by inoculating corn stover, and other feedstocks, with 2-40. Initial experiments will be completed in shake flasks. PHB production will be assessed at various stages of growth as indicated by fluorescence intensity of Nile Red stained PHB granules. Once PHB production from lignocellulose has been determined cultures will be scaled up to fermenters and various culture conditions will be evaluated to determined optimal conditions for PHB production. After significant accumulation, PHB will be isolated from the culture, quantified and characterized for further processing into longer carbon chains by the chemical engineering group at NREL

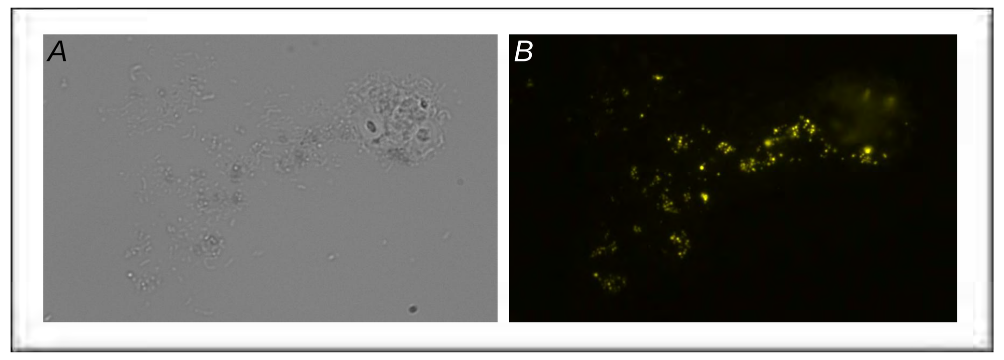


Figure 3. Image of 2-40 after 4 days of growth on non-pretreated corn stover. PHB granules stained with Nile Red. A) bright field B) fluorescence.

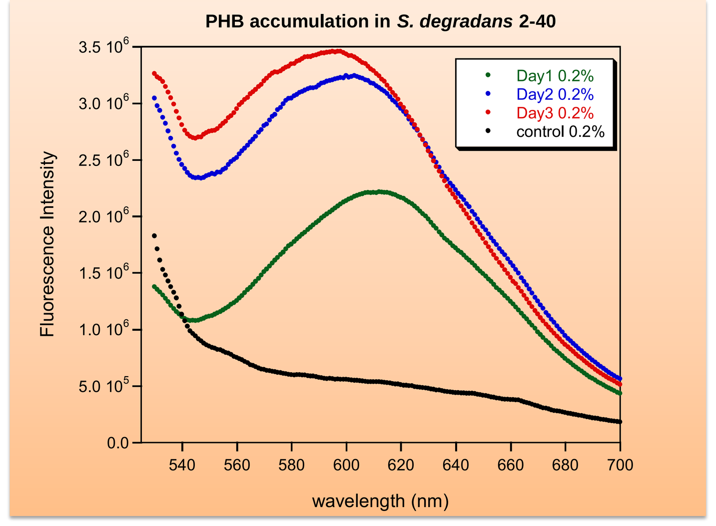


Figure 4. Graph demonstrating the increase in PHB accumulation in 2-40 over 3 Days of growth on 0.2% Solka-Floc, a commercially available lignocellulose like material. PHB granules are stained with a fluorescent dye, Nile Red and have optimal fluorescence between ~595 nm. Fluorescence intensity is indicates relative PHB accumulation. As 2-40 metabolizes Solka-Floc PHB accumulation increases, with the largest increase in accumulation between Days 1 and 2.

Impact

This project will further our understanding of enzyme synergy leading to the optimization of enzyme cocktail digestions of lignocellulosic biomass, as well as investigate future directions in advanced biofuel production. The results will have a significant impact on the DOE's mission to generate clean energy by lowering the costs that are currently associated with severe thermal chemical pretreatments of biomass and make progress in the production of new biofuels that will be a drop in replacement for current liquid hydrocarbon fuels.

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Developing a novel approach to the direct conversion of lignocelluosic biomass to next generation biofuel precursors.