

The Effect of Lignin and Hemicellulose Removal on Switchgrass Deconstruction by *Clostridium thermocellum*

Ninad Kothari^{1,2*} (nkoth001@ucr.edu), Charles Cai,^{1,2} Rajeev Kumar,^{1,2} Charles E. Wyman,^{1,2} and Paul Gilna²

¹University of California, Riverside; ²BioEnergy Science Center, Oak Ridge National Laboratory, Oak Ridge, Tennessee

<http://bioenergycenter.org>

Project Goals: The BioEnergy Science Center (BESC) focuses on fundamental understanding and elimination of biomass recalcitrance. BESC's approach to improve accessibility to the sugars within biomass involves (1) improved plant cell walls for rapid deconstruction and (2) multi-talented microbes for converting plant biomass into biofuels in a single step [consolidated bioprocessing (CBP)]. Biomass research works with two potential bioenergy crops (switchgrass and *Populus*) to develop improved varieties and to understand cell wall biosynthesis pathways. We test large numbers of natural variants and generate specific modified plants samples. BESC's research in deconstruction and conversion targets CBP manipulating thermophilic anaerobes and their cellulolytic enzymes for improved conversion, yields, and titer. Enabling technologies in biomass characterization, 'omics, and modeling are used to understand chemical and structural changes within biomass and to provide insights into mechanisms.

When produced from lignocellulosic biomass, ethanol is a sustainable transportation fuel with very low net emissions of greenhouse gases and its use will reduce our heavy dependence on petroleum. Ethanol is a high-octane fuel and can be further upgraded to drop-in fuels such as butanol, jet fuel, gasoline, and diesel. Ethanol made from corn starch and cane sugar currently dominates the alternative fuels market. However, production from lignocellulosic biomass has the promise for larger-scale impact at a low cost, and commercial cellulosic ethanol projects are now starting operations. These conventional lignocellulosic biomass conversions to ethanol employ major operations for size reduction, pretreatment, enzyme production, enzymatic hydrolysis, fermentation, and product recovery. Unfortunately, the high doses of fungal cellulolytic enzymes required to achieve high sugar yields necessary for commercial success are too costly to support large expansion of this nascent industry.

Consolidated bioprocessing (CBP) eliminates this expensive separate enzyme production step and simplifies the process by combining enzyme production, enzymatic hydrolysis, and fermentation operations.

Clostridium thermocellum, a leading CBP microorganism, produces a complex cellulosome that hydrolyzes the polysaccharides in biomass and then ferments their breakdown products into ethanol and other metabolites. Although the *C. thermocellum* cellulosome can realize about 48% glucan conversion from milled switchgrass without pretreatment, these yields are still too low to be

economically attractive. Therefore, pretreatment may be necessary to enhance biological deconstruction by *C. thermocoellum*. A variety of pretreatment technologies can prepare lignocellulosic biomass for high yields from CBP by achieving distinctive changes in the solids' compositional and structural characteristics. However, because the influence of variation in the composition of pretreated biomass solids on deconstruction by *C. thermocoellum* is not understood—hydrothermal, dilute acid, dilute alkali, and co-solvent enhanced lignocellulosic fractionation (CELf) pretreatments were applied at varying conditions to milled switchgrass. Dilute acid and hydrothermal are considered to be leading pretreatments that reduce biomass recalcitrance by removing most of the hemicellulose and some of the Klason lignin (K-lignin). Dilute alkali pretreatments, on the other hand, remove a large part of K-lignin but only some of the hemicellulose. By comparison, the CELf invented at the University of California, Riverside employs tetrahydrofuran (THF) as a miscible co-solvent in combination with an aqueous dilute acid solution to realize high solubilization and recovery of hemicellulose sugars as well as greatly enhanced K-lignin removal from lignocellulosic biomass compared to dilute acid alone.

Each of these pretreatment technologies were optimized to maximize total sugar yields from pretreatment (Stage 1) combined with wild-type *C. thermocoellum* biological deconstruction (Stage 2). Biomass deconstruction by *C. thermocoellum* was further compared to that realized by application of various loadings of conventional fungal enzymes to the same pretreated solids.

The translation of sugar deconstruction by *C. thermocoellum* into metabolite production was also analyzed. The pretreatments were performed over a range of temperatures and times, and the resulting solids were washed thoroughly prior to CBP. *C. thermocoellum* fermentations were performed at a 5 g/L glucan loading in a 50 mL working volume incubated at 60°C with a 180 rpm shaking speed, and fungal enzymes mediated enzymatic hydrolysis was performed at 50°C and 150 rpm. The results showed that enzymatic hydrolysis at high and expensive enzyme loadings of ≥ 65 mg protein/g glucan was required to realize the high levels of polysaccharide deconstruction achieved by *C. thermocoellum* without added enzymes. This work also showed that physical removal of lignin from switchgrass had a greater impact on biological deconstruction by *C. thermocoellum* than lignin relocation and/or hemicellulose removal. Moreover, removal of lignin and hemicellulose simultaneously by CELf pretreatments resulted in 100% sugar release from switchgrass when combined with CBP. At the same time, metabolites production by *C. thermocoellum* followed the trend observed for deconstruction of the polysaccharides in the different pretreated solids.

The BioEnergy Science Center is a U.S. Department of Energy Bioenergy Research Center supported by the Office of Biological and Environmental Research in the DOE Office of Science