Lignin Removal Controls Kinetics of Biomass Deconstruction by Consolidated Bioprocessing

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Project Goals: The Center for Bioenergy Innovation (CBI) vision is *to accelerate domestication of bioenergy-relevant, non-model plants and microbes to enable high-impact innovations at multiple points in the bioenergy supply chain.* CBI will address strategic barriers to the current bioeconomy in the areas of: 1) high-yielding, robust feedstocks, 2) lower capital and processing costs via consolidated bioprocessing (CBP) to specialty biofuels, and 3) methods to create valuable byproducts from the lignin. CBI will identify and utilize key plant genes for growth, composition and sustainability phenotypes as a means of achieving lower feedstock costs, focusing on poplar and switchgrass. We will convert these feedstocks to specialty biofuels (C4 alcohols and C6 esters) using CBP at high rates, titers and yield in combination with cotreatment or pretreatment. CBI will maximize product value by *in planta* modifications and biological funneling of lignin to value-added chemicals.

Pretreatment or cotreatment is necessary for biological conversion of lignocellulosic biomass to realize high yields of sugars that can be converted to liquid fuels. Most effective pretreatments apply mechanical energy and/or chemicals to disrupt the cellulose/hemicellulose/lignin matrix in plant cell walls so enzymes can access polysaccharides for deconstruction to sugars. Current application of dilute acid retains most of the cellulose and lignin in the solids while hemicellulose is released into the liquid with high yields. Then, cellulase and accessory enzymes can saccharify cellulose and any other polysaccharides remaining in the solids into monomeric sugars. However, substantial negative impacts on enzymatic hydrolysis yields have been attributed to the lignin left in the solids, particularly at economically viable enzyme loadings. For dilute acid pretreatment, application of high temperatures and long times can effectively disrupt lignin, but enzyme loadings are still too high to be economically attractive. In addition, monomeric sugars released during pretreatment degrade if the severe combinations of temperature and time needed to improve glucose release at lower enzyme loadings are employed. Therefore, conventional pretreatment has suffered from balancing between application of sufficiently severe conditions to disrupt lignin while minimizing sugar degradation.

Co-solvent Enhanced Lignocellulosic Fractionation (CELF) is a chemical pretreatment that applies miscible THF-water mixtures as a co-solvent to reduce the temperature needed for dilute acid to be effective while also removing over 80% of lignin. In addition, CELF can also recover nearly 100% of the sugars from the combined operations of pretreatment and enzymatic hydrolysis even for application of enzyme loadings as low as 2 mg of protein /g glucan, albeit for times approaching one month for such low loadings. Additionally, CELF releases distinctive

lignin fractions from the solids that can be targeted for further processed to fuels, chemicals, and/or materials, thereby achieving more complete utilization of these 3 major biopolymers.

Combining enzyme production, saccharification, and fermentation in a single organism by a process known as CBP would reduce costs for biological conversion of biomass to fuels and chemicals. The thermophilic anaerobe *Clostridium thermocellum* has proven to be a particularly effective CBP organism that can achieve nearly complete breakdown of the polysaccharides left in solids from CELF pretreatment in only 2 days. Integration of CELF with C*lostridium thermocellum* therefore offers the potential to realize high yields from the polysaccharides in lignocellulosic biomass in short times while also recovering a large portion of the lignin for potential conversion to valuable products.

The CELF-CBP combination has been shown to be highly effective for virtually 100% sugar solubilization from switchgrass, corn stover, and poplar while removing over 80% of lignin. However, it is not yet clear what changes in CELF pretreated solids are responsible for CBP realizing such high yields in short times compared to enzymes. For instance, *C. thermocellum* releases more sugars than fungal enzymes from solids produced by CELF pretreatment at mild conditions that remove less lignin. Thus, *C. thermocellum,* appears to be able to bypass lignin in the solids to some extent despite not possessing lignolytic enzymes. In this study, CBP was applied to CELF solids with varying degrees of lignin removal to better understand the impact of the lignin on CBP and key differences that enhance deconstruction of CELF solids by CBP compared to enzymes. Solids were CELF pretreated at 150°C for 5, 15, and 25 minutes at 7.5 wt% solids loading in a 1:1 THF:water (w/w) co-solvent ratio that also contained 0.5 wt% sulfuric acid. Afterwards, the pretreated solids were subjected to CBP for 7 days with samples taken at 12 hours and every 24 hours thereafter. The results showed that despite applying CELF at 150°C for only 5 minutes, *Clostridium thermocellum* was still able to release 95% of the sugars in 3 days, while for solids pretreated at 15 and 25 minutes, sugar release reached 98% in 4 days and 100% in 3 days, respectively. Kinetic models were applied to predict polysaccharide deconstruction during CBP and understand the impact of removing different fractions from the biomass matrix on rate of the rate of breakdown. Comparison of the impact of xylan removal on the rate and extent of cellulose deconstruction by CBP to that by lignin release revealed that lignin had a greater effect on the effectiveness of biological deconstruction by both *C. thermocellum* and fungal enzymes. Overall, this work provides new insights into factors governing biomass deconstruction by CELF-CBP that can lead to more economical biofuels production.

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