11. Integrating Conventional and Novel Pretreatments with C. thermocellum CBP to Overcome Biomass Recalcitrance

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Project Goals: The BioEnergy Science Center (BESC) is focused on the fundamental understanding and elimination of biomass recalcitrance. BESC's approach to improve accessibility to the sugars within biomass involves (1) designing plant cell walls for rapid deconstruction and (2) developing multi-talented microbes or converting plant biomass into biofuels in a single step [consolidated bioprocessing (CBP)]. BESC researchers provide enabling technologies in characterization, 'omics, modeling and data management in order to (1) understand chemical and structural changes within biomass and (2) to provide insights into biomass formation and conversion.

The major barrier to low-cost utilization of plant biomass as a resource for renewable liquid fuels production is the high costs associated with overcoming plant cell wall recalcitrance, i.e., breaking down the plant cell wall into component sugars. This challenge is particularly great for conversion of plants with high lignin content such as hardwoods, and biomass pretreatment is generally needed to achieve the high yields vital to low costs. In order to cost effectively release and recover plant cell wall sugars, biomass pretreatment should maximize biological digestibility of the residual solid fraction while minimizing degradation of the more readily released sugars that pass into the liquid fraction. As such, pretreatment is a pivotal process step for successful biological production of fuels from biomass as it controls this trade-off between high sugar conversion (residual solids digestibility) and high sugar yields (sugar conservation).

In a typical process for biological conversion of cellulosic biomass to fuels mediated by externally secreted enzymes that are typically derived from the fungi Trichoderma reesei, biomass is subjected to either a physical or chemical pretreatment to open up the cell wall structure by removing and/or disrupting hemicellulose and lignin so that enzymes can access cellulose fibers and other polysaccharides. After pretreatment, biomass polysaccharides remaining in the solids are converted into individual sugars by enzymatic hydrolysis and then metabolized into biofuels, such as ethanol, by fermentation. However, the loadings of fungal cellulase and hemicellulase enzymes needed to realize high sugar yields from hydrolysis of the carbohydrates left in the solids produced by conventional pretreatments have been estimated to cost $0.68 to $1.47 per gallon of ethanol produced.1 CBP, on the other hand, has emerged as the most promising biological pathway to avoid this high cost of enzymes by utilizing a single organism for both enzyme production and fermentation, thus eliminating the need for added enzymes and streamlining multiple processing steps into a single unit operation. In particular, combining Clostridium thermocellum's powerful and extensive native enzyme system with appropriate pretreatment conditions has the potential to overcome biomass recalcitrance as the major economic barrier to large-scale production of fuels from cellulosic biomass.

The focus of this work was to understand the influence of pretreatment on C. thermocellum based CBP performance compared to that by fungal enzymes when applied to feedstocks with relatively low and high recalcitrance. Corn stover was chosen to represent the former because of its established greater susceptibility to breakdown into sugars and near term abundance. Poplus was employed as well because of its much greater resistance to sugar release and position as a leading hardwood for fuels production. Biological deconstruction of solids produced by hydrothermal pretreatment of natural poplar variants that had been previously identified to have lower recalcitrance as well as one line with high recalcitrance showed C. thermocellum to be more effective in releasing glucan and xylan from pretreated poplar solids than adding fungal enzymes even at very high loadings of up to 65 mg protein/g glucan for the latter. In addition, Co-solvent Enhanced Lignocellulosic Fractionation (CELF), a novel pretreatment technology recently invented by our team, and conventional dilute sulfuric acid (DSA) were used to pretreat corn stover and poplar followed by deconstruction of the resulting solids by both fungal enzymes and C. thermocellum. In this study, an aqueous mixture of 0.5 % w/w sulfuric acid and tetrahydrofuran at a 1:1 ratio that form a single phase was employed for CELF pretreatment at a 5-10 % w/w biomass solids loading and a temperature of 150-160 °C. Comparing the results demonstrated that combining CELF pretreatment with C. thermocellum fermentation resulted in nearly complete conversion (~100%) of the available glucan plus xylan for both poplar and corn stover within 48 hours while conversion was limited to approximately 71% and 85% for DSA pretreated poplar and corn stover, respectively, when followed by C. thermocellum fermentation for 7 days. While enzymes were able to achieve similar yields for CELF pretreated poplar and corn stover, either much higher enzyme loadings (>50 mg/g glucan) or very long incubation times (7 or more days) were required.

References

1. Klein-Marcuschamer, D., P. Oleskowicz-Popiel, B. A. Simmons, and H. W. Blanch, “The challenge of Enzyme Cost in the Production of Lignocellulosic Biofuels,” Biotechnology and Bioengineering 109(4), 2012, pp. 1083–7.

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