Declining Carbohydrate Solubilization with Increasing Solids Loading During Fermentation of Cellulosic Feedstocks by *Clostridium thermocellum*: Documentation and Diagnostic Tests

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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 addresses 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 biofuels using CBP with cotreatment at high rates, titers and yield in combination with catalytic upgrading into drop-in hydrocarbon fuel blendstocks.

The cellulolytic thermophilic anaerobe *Clostridium thermocellum* is one of the most effective biocatalysts for solubilization of carbohydrate harbored in lignocellulose. Pursuant to major goal #2 for CBI (above), we aim to demonstrate values of key performance indicators supporting the economic viability of CBP for conversion of cellulosic feedstocks to small molecules – e.g. ethanol, butanol, or 2,3-butane diol (BDO) - that can be upgraded to sustainable aviation fuels. As part of this effort, we seek to document, diagnose, and remediate factors that limit biomass deconstruction at high solids. This study aims to document the solubilization performance of *Clostridium thermocellum* at increasing solids concentrations for two lignocellulosic feedstocks, corn stover and switchgrass, and explore potential effectors of solubilization performance. Diminishing fractional carbohydrate solubilization with increasing substrate loading was observed for *C. thermocellum* mediated-solubilization and fermentation of unpretreated lignocellulose feedstocks. Results of experiments involving spent broth addition do not support a major role for inhibitors present in the liquid phase. Mid-fermentation addition experiments of cells (fresh biocatalyst), cellulose (model insoluble substrate) and cellobiose (model soluble substrate) confirm that *C. thermocellum* and its enzymes remain capable of converting model substrates during the middle of high solids lignocellulose fermentation. An increase in fractional carbohydrate solubilization was made possible by 1) mid-fermentation solid loading dilutions and 2) coculturing *C. thermocellum* with *T. thermosaccharolyticum*, which ferments solubilized hemicellulose. Incomplete utilization of solubilized carbohydrates suggests that a small fraction of the carbohydrates is unaffected by the extracellular carbohydrate active enzymes present in the culture.

The Center for Bioenergy Innovation 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.