Effectiveness of Cotreatment during Fermentation on Various Feedstocks, Impact on Different Microbes, and Changes in Biomass Physical Properties

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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.

Milling during fermentation, also known as cotreatment[1], is being investigated as an alternative to thermochemical pretreatment for increasing the accessibility of lignocellulose to biological attack. Here we examine the effectiveness of cotreatment in combination with CBP fermentation by *Clostridium thermocellum* at low solids concentrations using switchgrass (with and without genetic modification aimed at decreasing recalcitrance), using more and less recalcitrant natural variants of *Populus*, and using corn stover and corn fiber. The ability to carry out fermentation in the presence of aggressive milling, previously assessed only for *Clostridium thermocellum,* is evaluated for *Thermoanaerobacterium saccharolyticum, Escherichia coli*, *Bacillus subtilis,* and *Zymomonas mobilis.* The substantial change in viscosity of corn stover slurries over the course of *Clostridium thermocellum* fermentation is evaluated with initial solids loading representative of an industrial process[2]. Results from screening various milling modalities for cotreatment effectiveness are also reported.

References

1. Balch *et al*. (2017), *Energy Environ. Sci.*, 10:1252-1261 doi:10.1039/C6EE03748H
2. Ghosh *et al*. (2018), *Biotechnol Biofuels*, 11:246. https://doi.org/10.1186/s13068-018-1248-z

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