Disc Milling of Fermented Corn Stover to Increase its Accessibility to Fermentation by *Clostridium thermocellum*

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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 specialty biofuels (C4 alcohols, C6 esters and hydrocarbons) using CBP at high rates, titers and yield in combination with cotreatment, pretreatment or catalytic upgrading. CBI will maximize product value by *in planta* modifications and biological funneling of lignin to value-added chemicals.

We are investigating mechanical disruption during fermentation (cotreatment) as an alternative to thermochemical pretreatment to increase the accessibility of lignocellulose (i.e., from poplar or switchgrass) to biological attack. Our overall goal is to explore and test the hypothesis that cotreatment can be an industrially feasible method to enhance carbohydrate solubilization by engineered thermophiles in a consolidated bioprocessing (CBP) configuration. The feasibility of such "C-CBP" requires that a) carbohydrate solubilization be meaningfully increased, b) microorganisms be able to actively ferment in the presence of milling, and c) energy requirements for milling to be sufficiently low

Mechanical disruption during fermentation, cotreatment[1], combined with consolidated bioprocessing offers documented potential for disruptive potential in the cost of lignocellulose conversion[2]. It has previously been shown that *Clostridium thermocellum* fermentation readily proceeds in the presence of continuous ball milling, and that such milling allows total carbohydrate solubilization in excess of 90% of theoretical to be achieved for both woody and herbaceous feedstocks without added enzymes and without thermochemical pretreatment beyond autoclaving[3,4]. Ferment-mill-ferment experiments are reported for fermentation of corn stover and switchgrass by *Clostridium thermocellum* augmented by disc milling. Process variables such as solids loading and disc rotation were investigated, and carbohydrate solubilization was documented. In this first report of the energy consumption for cotreatment, data is presented indicating that the energy requirements for cotreatment via disc milling may be comparable to those for thermochemical pretreatment, although more remains to be done in order to substantiate this conclusion. The energy requirements for ball milling, however, appear to be prohibitively high due to internal friction.

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References:

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