A New Low-Cost Paradigm for Biological Conversion of Cellulosic Biomass: Evaluation of Economic Potential

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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 research in biomass deconstruction and conversion targets CBP by studying thermophilic anaerobes to understand novel strategies and enzyme complexes for biomass deconstruction and manipulating these microorganisms for improved conversion, yields, and biofuel titer.

Detailed comparative data has recently shown that *Clostridium thermocellum* achieves carbohydrate solubilization yields for several grasses with no pretreatment other than autoclaving that are $\geq$ 35% of theoretical, and higher than industry-standard fungal cellulase by 2 to 3-fold (Paye et al., 2016; Shao et al., 2011). Although mechanical milling has excessive energy requirements when used as a pretreatment for fungal cellulase, it is known that lignocellulose-containing slurries undergo a radical liquefaction at solubilization yields < 15%, as indicated by ~ 30-fold reductions in the two variables that determine milling energy: viscosity and particle strength. Noting that the yields achievable by *C. thermocellum* in the absence of pretreatment substantially excessed those needed to reduce milling energy requirements by over an order of magnitude, we are exploring whether non-biological disruption before biological processing (pretreatment) might be replaced by biological processing prior to non-biological disruption.

Recent data supporting this concept include:

- Two-fold greater enhancement of solubilization via milling partially-fermented solids as compared to unfermented-solids (Paye et al., 2016);
- Solubilization yields by cotreatment-enhanced *C. thermocellum* fermentation comparable to those obtainable using thermochemical pretreatment; and
- Milling at intensities sufficient to achieve high solubilization yields in the absence of thermochemical pretreatment has little or no effect on *C. thermocellum* whereas it results in complete cessation of yeast fermentation.

Here we report preliminary analysis of the potential economics of combining engineered thermophilic, cellulolytic microbes with cotreatment for conversion of corn stover to ethanol using the most recent National Renewable Energy Laboratory's design as a departure point. Our results, based on assumed success in several ongoing research directions, support potential for far lower processing costs as well as feasibility at much smaller scale compared to current technology.

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