Evaluation of Multiple Levers for Overcoming the Recalcitrance of Cellulosic Biomass

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Project Goals: The BioEnergy Science Center (BESC) focuses on fundamental understanding and elimination of biomass recalcitrance. BESC's approach to improve accessibility to the sugars within biomass involves (1) improved plant cell walls for rapid deconstruction and (2) multi-talented microbes for converting plant biomass into biofuels in a single step [consolidated bioprocessing (CBP)]. Biomass research works with two potential bioenergy crops (switchgrass and *Populus)* to develop improved varieties and to understand cell wall biosynthesis pathways. We test large numbers of natural variants and generate specific modified plants samples. BESC's research in deconstruction and conversion targets CBP manipulating thermophilic anaerobes and their cellulolytic enzymes for improved conversion, yields, and titer. Enabling technologies in biomass characterization, 'omics, and modeling are used to understand chemical and structural changes within biomass and to provide insights into mechanisms.

The primary barrier to economically competitive cellulosic biofuels is the resistance of plant cell walls to deconstruction – termed recalcitrance. Overcoming this barrier may be approached via recalcitrance "levers" drawn from three categories:

I. Start with nature's best with respect to feedstocks and biocatalysts;

II. Apply biotechnology to improve plants, enzymes, and microbes;

III. Augment biological capability with non-biological processing.

Here we evaluate the impact of multiple levers in each of these categories both individually and in combination, including:

- three engineered switchgrass plant lines and their parent lines;
- two natural variant *Populus* lines;
- solubilization mediated by either fungal cellulase in the presence of yeast (SSF), *Caldicellusiruptor bescii,* or *Clostridium thermocellum;* and
- augmentation of biologically-mediated solubilization using either cosolvent-enhanced liquid fractionation (CELF) or co-treatment (i.e., intermittent milling during fermentation).

Key observations include:

- The extent of increased solubilization observed for engineered/variant plant lines is highly dependent upon which biocatalyst is used. Thus, plant recalcitrance exhibits a strong dependence on the biocatalyst by which it is evaluated.
- The relative effectiveness of biocatalysts at mediating solubilization was *C. thermocellum*> *C. bescii* > fungal cellulose.
- The relative impact of various levers at enhancing solubilization was augmentation (co-treatment or CELF) > choice of biocatalyst > choice of feedstock and feedstock modification.
- Total carbohydrate solubilization in excess of 90% is observed for solubilization of all *Populus* and switchgrass lines tested using either co-treatment- or CELF-augmented *C. thermocellum* fermentation.
- Lignin-rich residues remaining after fermentation and co-treatment showed no evidence of chemical modification, in contrast to fermentation residues following thermochemical pretreatment, and appear promising for further processing into coproducts.
- We infer that *C. thermocellum* cultures are able to solubilize all major chemical linkages in switchgrass and *Populus* given physical access to them.
- Our results provide proof of concept for achieving high carbohydrate solubilization without thermochemical pretreatment and without added enzymes.

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