2. Biological Lignocellulose Solubilization: Comparative Evaluation of Feedstock-Biocatalyst Combinations and Enhancement via Co-Treatment by Intermediate Milling

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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 model organisms and thermophilic anaerobes to understand novel strategies and enzyme complexes for biomass deconstruction.

We conducted comparative studies under controlled, but not industrial, conditions to inform how choice of feedstock, biocatalyst, and fermentation conditions impacts the ability to overcome recalcitrance. Several anaerobic bacteria are able to achieve substantial solubilization of minimally pretreated switchgrass, with solubilization yields up to two times higher than commercial fungal cellulase. Performance of fungal enzymes was not significantly improved by addition of yeast, higher enzyme loadings, increased hydrolysis temperature, or lower substrate loadings. For both Clostridium thermocellum and fungal cellulase, conversion is twice as high for switchgrass harvested at mid-season as compared to late season.

However, solubilization of late-season switchgrass can be improved by mechanical disruption of the substrate after biological attack has begun (as done in rumen and by termites). Such "co-treatment" may be contrasted to pretreatment, for which the lignocellulose matrix is disrupted prior to biological attack. Brief (5 minute) ball milling of solids remaining after fermentation of senescent switchgrass by C. thermocellum increased carbohydrate solubilization upon reinoculation as compared to a control without milling (68±2% and 41±2%, respectively). When the same mechanical disruption was applied as a pretreatment before the first fermentation stage, carbohydrate solubilization was 55±2%. Residual solids were characterized in order to gain insight into the mechanisms underlying the effectiveness of milling. The main effect of milling observed was decreased particle size, with final d50 (volume-mean particle size) values of 411 ± 42 um for no milling, 89 ± 6 um milling before the first fermentation, and 29 ± 2 um milling between the first and second fermentations. Milling had little effect on substrate cellulose crystallinity but pore volume increased with milling and fermentation. The substantial impact of brief milling observed here supports the possibility of mechanical disruption in a side vessel much smaller than the fermenter.

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