Switchgrass Fermentation by Thermophilic Microbiomes

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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 study of lignocellulose-fermenting microbiomes can inform and enable development of industrial processes based on defined microbial cultures. Yet there have been few fundamental studies of lignocellulose fermentation under the conditions anticipated for industrial processes, including conversion of high solids (> 100 g/L). Motivated by this perspective, we have initiated study of efficient switchgrass fermentations by thermophilic anaerobic lignocellulolytic consortia of microbiomes.

Triplicate semi-continuous, anaerobic cultures were operated at 55°C on mid-season harvested switchgrass with no added organic nutrients for more than 19 months to obtain steady-states at solids concentrations from 30g/L to 150g/L and residence times (RT) from 20 to 3.3 days. Cultures were fed semi-continuously by replacing 1/10th of the fermentation broth at regular time intervals. Undiminished fractional carbohydrate solubilization was observed over a 5-fold range of feedstock loading. Stable methanogenesis with minimal accumulation of organic acids was observed under all conditions, which was something of a surprise at RT=3.3 days. Yet more surprising, initial experiments indicated that fractional carbohydrate solubilization by pure cultures of *Clostridium thermocellum* was at least as high as that obtained with lignocellulose-fermenting microbiomes at low solids loading. However, solubilization of higher substrate loadings was dependent on the cooperative action of mixed community microbiomes.

16S rDNA and metagenomics analysis was conducted to characterize the microbiome. At low (30 g/L) solids loading, Firmicutes dominated each of the cultures, representing between 54--96% of the sequence reads, and these populations increased with reduced residence time. Species present in the consortia were also matched to closely related cultured species. Sixty percent of the microbiome was shown to be potentially cellulolytic, with the most abundant matching to *Clostridium clariflavum.* Plate culture isolation further revealed that *C. clariflavum* was one of the major cellulolytic species existing in the mixed culture consortia.

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