## 17. Solid Residuals from Populus trichocarpa Demonstrate that Recalcitrance Persists During Consolidated Bioprocessing

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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). BESC researchers provide enabling technologies in characterization, 'omics, modeling and data management in order to (1) understand chemical and structural changes within biomass and (2) to provide insights into biomass formation and conversion.

We seek to determine the structural features of biomass that may influence the hydrolysis patterns of Clostridium thermocellum during the deconstruction of biomass. This study will provide a snapshot of features that have been associated with recalcitrance and their relationship to cellulose hydrolysis.

Consolidated bioprocessing (CBP) is a competitive alternative to current ethanol producing technologies for biomass, such as separation saccharification and fermentation whose cost has rendered them too costly for widespread industrial production. C. thermocellum is a CBP microorganism that localizes over 20 identified enzymes in its cellulosome, or multienzyme complex— a feature that starkly contrasts with conventional processing methods. Cellulases and hemicellulases are two groups of enzymes positioned in the cellulosome, and according to this study, display preferences towards the structural features of P. trichocarpa. Natural variants of autoclaved Populus trichocarpa were treated with C. thermocellum (ATCC 27405) at for five days at 58ºC and analyzed for structural properties that may influence enzymatic deconstruction. The remaining materials, or solid residuals, were compared against controls, or untreated natural variants, for properties including the degree of polymerization (DP) and crystallinity of cellulose (CrI), and the molecular weight of hemicellulose. The cellulases housed in C. thermocellum preferentially hydrolyze low DP (<4000) and higher CrI cellulose (~55%). Changes to hemicellulose structure were not definitive between the controls and the solid residuals.

These results may demonstrate that disruption of the hemicellulose structure precedes cellulose hydrolysis of short DP and higher CrI cellulose. These results suggest that the recalcitrance to complete biomass degradation exists in C. thermocellum as well as fungal cellulases.

*The BioEnergy Science Center is a U.S. Department of Energy Bioenergy Research Center supported by the Office of Biological and Environmental Research in the DOE Office of Science.*