Title: Building a Molecular Understanding of Biomass Deconstruction

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Project Goals:

The goal of this project is to combine insights from solid-state nuclear magnetic resonance (ssNMR), x-ray crystallography, and enzymology to expand understanding of how biomass impacts and is impacted by both thermochemical pretreatment and enzymatic hydrolysis. Understanding biomass deconstruction on a molecular scale enables more detailed pretreatment and enzyme improvements and clearer, more specific identification and addressing of process bottlenecks.

Abstract Text:

Identifying bottlenecks in the deconstruction of plant biomass is an important aspect of developing increasingly effective deconstruction strategies. Because of the heterogeneity of biomass, both between plant species and within a single sample, the specificity of bottleneck identification has traditionally been a limitation. For example, it is well known that crystalline cellulose is less amenable to enzymatic hydrolysis than amorphous cellulose; and lignin is a potent inhibitor of cellulases, but correlations between these different molecular states and their impact on deconstruction is less clear.

We previously showed a correlation between enzymatic hydrolysis and the intensity of the split C4 resonance in bulk cellulose [1], which holds across plant species and co-solvents used in pretreatment. To better understand the reactivity of a model purified enzyme (*Rumiclostridum thermocellum* CelR) with cellulose produced via γ-valerolactone pretreatment (GVL cellulose), CelR variants consisting of the catalytic domain alone, the native enzyme (catalytic domain and a single carbohydrate binding module (CBM)), and an engineered native construct containing an additional CBM were constructed along with variants of each inactivated by mutation of the catalytic glutamate residue. Studies of the kinetics, binding affinities, and activity profiles of these variants on amorphous, crystalline, and GVL cellulose showed that the catalytic domain alone is nearly inactive; restoration of the native CBM improves specific activity by 10-fold and addition of the engineered CBM doubles again the specific activity.

In this work, we have extended our prior ssNMR studies by using spectral deconvolution and fitting to quantify contributions of different cellulose substructures within GVL cellulose and to correlate changes in the proportion of these substructures during thermochemical treatment and enzyme hydrolysis. Results show that the I_β form of crystalline cellulose accumulates during the GVL treatment and remains unhydrolyzed by CelR. I_β cellulose is therefore a major bottleneck for deconstruction by CelR and can be targeted to improve deconstruction overall at both the pretreatment and enzymatic hydrolysis levels. These findings provide a molecular-level understanding of the progress of biomass deconstruction, and insight into how to improve this essential process.

References/Publications

1. Walker TW, Kuch N, Vander Meulen KA, Clewett CFM, Huber GW, Fox BG, Dumesic JA. Solid-state NMR studies of solvent-mediated, acid-catalyzed woody biomass pre-treatment for enzymatic conversion of residual cellulose. *ACS Sustain Chem Eng*. 2020 Apr 27; 8(**16**): 6551-6563. doi: 10.1021/acssuschemeng.0c01538.

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