Evolution of Cellulose Structure throughout gamma-Valerolactone-Assisted and Enzymatic Biomass Deconstruction

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Project Goals: Our goals are to elucidate how cellulosic material is degraded by gamma-valerolacton assisted mild acidolysis and enzymatic hydrolysis with the engineered cellulase, CelR. Overall, we aim to understand how to increase yields of usable sugars from the degredation of different biomass sources.

Abstract text:

Biomass recalcitrance during deconstruction remains a key inhibitor to successful implementation of affordable biomass processing technologies. A clear connection between the cell wall structure and biomass deconstruction is necessary to understand how lignocellulosic material is broken down, and to identify defining features of residual cellulose. In this work, we have employed solid-state ^{13}C cross-polarization magic angle spinning (CP/MAS) nuclear magnetic resonance (NMR) spectroscopy to track domain changes in cellulose microfibrils throughout various chemical and enzymatic hydrolysis treatments. This work builds on previously demonstrated capabilities using NMR to predict enzymatic hydrolysis sugar yields of pretreated cellulose,[1] and moves toward a molecular understanding of how cellulose is degraded by GVL mild acidolysis. Raw biomass was pretreated in GVL-water co-solvents with mild sulfuric acid concentrations at increasing temperatures and subsequently hydrolyzed with an engineered cellulase. Residual solids from each step were characterized using ^{13}C CP/MAS NMR and spectra were fit with subpeaks corresponding to different cellulose microenvironments at the C-4 carbon center. The changes of the C-4 carbon subpeaks were tracked throughout each treatment to understand the physical changes occurring within different cellulose microfibril domains. We show that the xylan-cellulose and inaccessible fibril surface resonances decrease significantly upon hydrolysis with mild acidic GVL-water co-solvent pretreatment. Wide-angle X-ray scattering (WAXS) results support increasing Segal Crystallinity and tighetening of the lattice structure of residual cellulose samples with increasing GVL pretreatment. Enzymatic

hydrolysis leads to further depletion of inaccessible and paracrystalline peaks in NMR, but does not degrade I_β crystalline regions. This behavior can be interpreted as an opening of bound microfibril surfaces previously inaccessible to the solvent through GVL acidolysis and end-on degradation of the residual cellulose by enzymatic hydrolysis. The cleaving of xylan-cellulose linkages and opening of inaccessible fibril surfaces by acid co-solvent pretreatment primes the cellulose for enzymatic attack. This technique can monitor the evolution of structural changes to the cellulosic material and allows for comparison between different cellulose deconstruction methods. It guides larger questions of recalcitrance - mainly the need to hydrolyse unreacted I_β crystalline cellulose - and gives insight to needed improvement of subsequent deconstruction methods based on residual solids structures.

References/Publications

1. Walker, Theodore W., et al. "Solid-State NMR Studies of Solvent-Mediated, Acid-Catalyzed Woody Biomass Pretreatment for Enzymatic Conversion of Residual Cellulose." ACS Sustainable Chemistry & Engineering 8.16 (2020): 6551-6563.

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