

200. Alkaline Hydrogen Peroxide Pretreatment Differentially Affects Cell Wall Cross-Linking and Recalcitrance in Diverse Bioenergy Feedstocks

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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.

Conducting in-depth glycome analyses of plant biomass modified through pretreatment processes is a novel approach for studying/identifying cell wall components contributing to recalcitrance. Three taxonomically diverse bioenergy feedstocks [hybrid poplar (a woody dicot), goldenrod (a herbaceous dicot) and corn stover (a graminaceous monocot)] were subjected to alkaline hydrogen peroxide (AHP) pretreatment and subsequent enzymatic conversion studies in order to assess how they respond to mild alkaline oxidative pretreatment and to identify differing features of the cell wall matrix that contribute to their recalcitrance. After AHP pretreatment, these biomass types exhibited varied enzymatic conversion efficiencies with corn stover showing the highest sugar yield followed by golden rod and poplar. Glycome profiling was employed to screen various AHP-pretreated biomass samples along with untreated controls to determine changes in the composition and extractability of non-cellulosic cell wall glycans (Figure 1). The results obtained in this study demonstrate that distinct patterns of cell wall structural changes (and hence altered cell wall glycan extractability) occur among these biomass types when subjected to AHP pretreatment, causing varied enzymatic conversion efficiencies. Using glycome profiling, we found that hybrid poplar was relatively unaffected by AHP pretreatment in terms of composition, enzymatic digestibility, and the extractability of cell wall glycans, perhaps due to its higher lignin content and hence greater extent of base- and/or peroxide-stable cell wall crosslinking. In general, AHP-pretreated golden rod and corn stover biomasses showed an enhanced abundance of hemicellulose epitopes in mild cell wall extracts such as oxalate and carbonate, hinting at easier removal of a sub-class of hemicelluloses from pretreated biomass.

AHP pretreatment of goldenrod resulted in a decrease in all classes of alkali (1M KOH)- extractable glycans, notably xylans, xyloglucans, and pectic polysaccharides, indicating their solubilization during pretreatment. This was accompanied by an improvement in the subsequent digestibility of the remaining cell wall residue. AHP pretreatment of corn stover resulted in mild increases in the extractability of all classes of cell wall glycans, notably xylans, xyloglucans, pectic polysaccharides, and β -glucans, indicating overall weaker associations between cell wall polymers. In grasses, alkali-labile ester cross-links between cell wall matrix macromolecules and higher alkaline solubility of grass lignins are proposed to be important properties that are exploited by AHP pretreatment to solubilize lignin and "loosen" the

cell wall matrix, which in turn results in superior enzymatic conversion relative to goldenrod and poplar following AHP pretreatment.

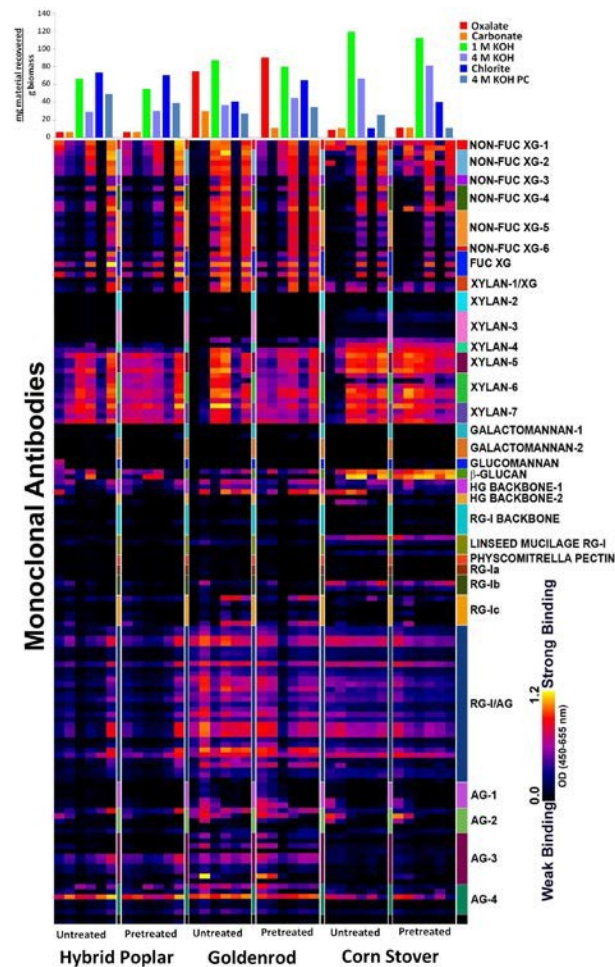


Figure 1. Glycome profiling of hybrid poplar, goldenrod and corn stover biomass samples before and after AHP pretreatment (12.5% H₂O₂ loading). Sequential cell wall extracts were made from untreated and pretreated biomass samples using increasingly harsh reagents. The extracts were ELISA screened using 155 mAbs directed against most major plant cell wall glycans. The resulting binding response data are represented as heatmaps with yellow-red- black scale indicating the strength of the ELISA signal (yellow, red and dark-blue colors depict strong, medium, and no binding, respectively). The mAbs are grouped based on the cell wall glycans they recognize as depicted in the panel at right hand side of the figure. The actual amounts of materials extracted out at each extraction condition are depicted as bar graphs at the top of heatmaps with color codes for reagents used for extraction.

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