206. Fundamentals of Aqueous Pretreatment Chemistry and Cell Wall Cellular Structures of Low Recalcitrance *Populus* Lines for Enhanced Performance

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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.

Lowering plant recalcitrance is a key to reducing pretreatment severity and enzyme doses that hinder large scale commercialization of cellulosic ethanol. Natural *Populus* lines with gene mutation in their lignin biosynthesis pathway were found to have reduced recalcitrance. High throughput pretreatment and co-hydrolysis (HTPH) was employed to screen 18 *Populus* lines along with 4 controls and BESC *Populus* standard to select those with the highest glucan and xylan release. At the same pretreatment severity, a temperature of 180°C greatly increased glucan and xylan release in several plant lines compared to 140°C and 160°C. This result likely indicates that some bonds have higher activation energies that require higher temperatures to overcome. We found several lines that gave 100% higher xylan yields in low severity hydrothermal batch pretreatment and 300% higher glucan yields in enzymatic hydrolysis at lower enzyme loadings relative to the BESC *Populus* standard. Correlations between glucan and xylan ranks for all lines indicated that higher glucan release led to higher xylan release and vice versa. ^{13}C-^{1}H NMR spectra of a low recalcitrant line showed a higher syringyl to guaiacyl lignin ratio and a higher p-hydroxybenzoate to guaiacyl ratio than for the *Populus* standard. This line was also found to contain a greater abundance of β-O-4 linkages and have a lower molecular weight than the standard. These variations in lignin are likely associated with enhanced sugar release.

Xylan yields were twice as high from selected lines than the *Populus* standard for hydrothermal batch pretreatment at low severities, while differences in xylan yields were less apparent for dilute acid batch pretreatment at low severities. These results suggest that changes in lignin architecture can lead to improved sugar release and several factors for better design of transgenic plants.

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