5. Characterizing Cell Wall Crosslinking and/or Entanglement in Reduced Recalcitrance Feedstocks

Erica Gjersing[1*] (Erica.gjersing.nrel.gov), Bryon Donohoe,[2] William Rottmann,[3] Maud Hinchee,[3] Robert Sykes,[1] Geoffrey B. Turner,[2] Rui Katahira,[1] Angela Ziebell,[1] David Johnson,[2] Stephen Decker,[2] Mark Davis,[1] and Paul Gilna[4]

[1]National Bioenergy Center, National Renewable Energy Laboratory, Golden, Colorado; [2]Biosciences Center, National Renewable Energy Laboratory, Golden, Colorado; [3]ArborGen, Inc., Summerville, South Carolina; [4]BioEnergy Science Center, Oak Ridge National Laboratory, Oak Ridge, Tennessee

http://bioenergycenter.org

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, modelir in order to understand chemical and structural changes within biomass and (2) to provide insights into biomass formation and conversion.

The cell wall of plants is composed up of a variety of biopolymers including lignin, hemicellulose, pectin, and cellulose. When these polymers are deconstructed to monomeric sugars, they can be utilized to produce fuels and chemicals. However, this process of breaking down these polymeric components to their monomers, can be difficult due to the recalcitrant nature of lignocellulosic material. Understanding how the properties of the biopolymer matrix, such as crosslink density and/or entanglement, are affecting recalcitrance is vital to engineering plants for improved biofuels production and performance.
Lignin has been shown to play a major role in recalcitrance.1,2 Therefore, plant systems with changes in their lignin content and structure have been selected for these studies. Specifically, we have studied Eucalyptus urophylla x grandis antisense downregulated in p-coumarate 3'-hydroxylase (C3H) or cinnamate-4-hydroxylase (C4H). These transgenic lines have been identified with the properties of low recalcitrance, low lignin and improved biofuel processing efficiency. In addition, both the C3H and C4H downregulated lines were found to have significantly higher carbohydrate extractability when exposed to NaOH base extraction, indicating altered cell wall construction. The molecular weight of isolated lignin and lignin structure determined by HSQC NMR based lignin subunit analysis for control and the C3H and C4H downregulated lines were also examined. The slight reductions in weight average molecular weights of the lignins isolated from the transgenic lines does not appear to explain the difference in extractability while the HSQC NMR based lignin subunit analysis showed only slight differences in the lignin structure of the C3H and C4H downregulated lines when compared to the control.

To further investigate the role of the lowered lignin content on the reduced recalcitrance and increased extractability of the transgenic lines, we performed solvent swelling experiments and T2 NMR relaxation measurements. Swelling samples in water and measuring the difference between the wet and dry volume is an experiment that has been used to measure the dimensional stability of wood samples. For the eucalyptus samples, the control had a 14% volume change (ΔV), compared to the C4H transgenic lines with a 54% ΔV and the C3H line with 31%ΔV. These results indicate that the transgenic lines have increased mobility in their cell wall matrix that can expand to take up more water than the controls. To further investigate changes in the biopolymer matrix mobility, T2 NMR relaxation measurements were performed. These experiments are commonly used to measure mobility in synthetic polymer systems where an increase in T2 relaxation time indicates an increase in the mobility of the protons within the

polymer matrix and has been correlated to decreases in crosslink density and/or entanglement. For the C4H transgenic, there was a 68% increase in the T2 relaxation time while the C3H line displayed a 34% increase compared to the control. These experiments indicate that the reduced recalcitrance of these eucalyptus samples is correlated to the cell wall polymer mobility that is dependent on the crosslink density and/or entanglement of the biopolymer matrix.

References

1. Chen, F. and R. A. Dixon (2007), Nature Biotechnology, "Lignin Modification Improves Fermentable Sugar Yields for Biofuel Production," 25, 759−761.
2. Ziebell, A., K. Gracom, R. Katahira, F. Chen, Y. Q. Pu, A. Ragauskas, R. A. Dixon, and M. Davis (2010), The Journal of Biological Chemistry, "Increase in 4-Coumaryl Alcohol Units During Lignification in Alfalfa (Medicago sativa) Alters the Extractability and Molecular Weight of Lignin," 285(50), 38961−38968.

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.