High and Medium-Throughput Characterization of Biomass to Understand Causes of Recalcitrance in Plant Cell Walls

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Project Goals: BESC researchers in characterization, modeling, and data management areas are engaged in 1) applying advanced technologies to analyze chemical and structural changes within biomass and 2) storing, tracking, analyzing and integrating data and understanding across the Center. BESC characterizes samples in order to identify low recalcitrance lines of switchgrass and *Populus*. This collaboration with plant biologists provides understanding how and why plants low recalcitrance lines are easier to process.

The High-throughput (HTP) pipeline has analyzed tens of thousands of samples using the HTP pipeline which characterizes cell wall chemistry and sugar release on pretreatment and enzymatic saccharification (Sykes et al. 2008; Selig et al. 2011). In the last year, we have added two new capabilities, starch removal and rapid NMR analysis, and worked with plant biologists to interpret their data.

The characterization group works together with plant biologists to understand the chemical modifications occurring when plants are genetically modified and why they might cause changes in recalcitrance to pretreatment and saccharification. The characterization group also studies natural populations to understand how natural populations vary and what we might learn from natural diversity. Below (Figure 1) is an example of how the HTP data is used to study natural populations.

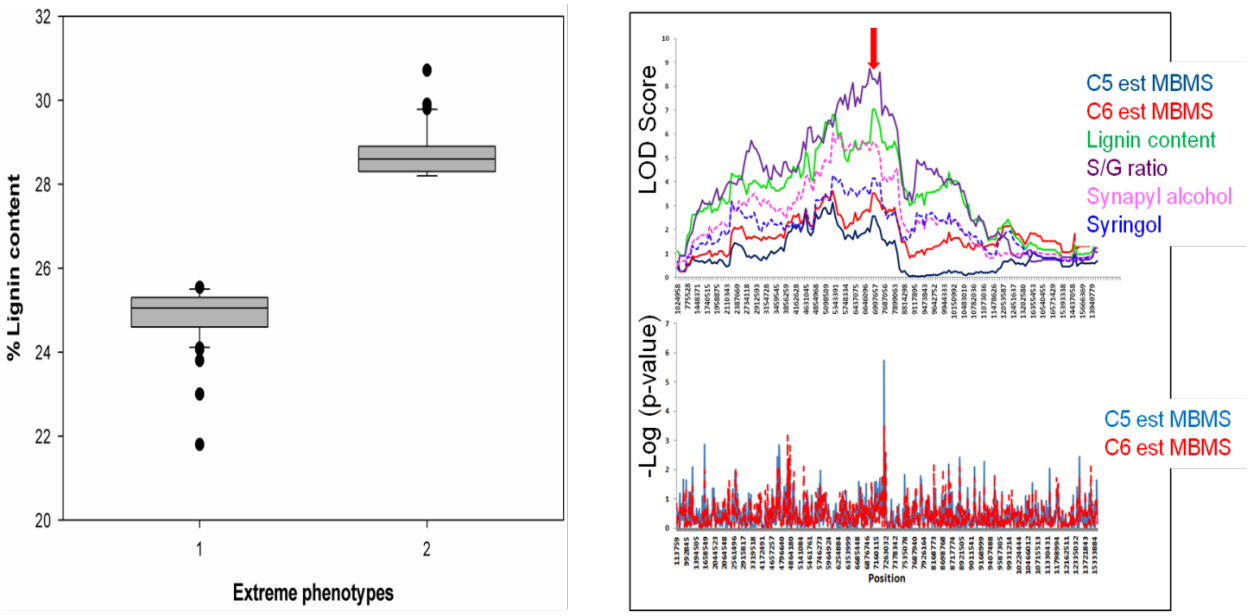

Figure 1: Data from a study of 800 naturally occurring *Populus* from the Pacific north-west. Left extreme lignin phenotypes, right genetic mapping finds results from both the cell wall chemistry and the sugar release assay coincide.

Plant cell wall sugar content is being determined by a HTP NMR (Nuclear Magnetic Resonance) adaptation to NREL's traditional wet chemistry analysis. This technique is also being used to assess the tendency of a plant to produce inhibitors e.g. furfural and HMF. The method uses proton NMR (Figure 2) and Partial Least Squares analysis in conjunction with a set of standards which have the full wet chemistry method. The current NMR facility houses a HTP NMR sample changer which can house >500 samples at a time and sample analysis time take 1-2 minutes. The original full wet chemistry method has also been down scaled to 1/300th by researchers at the University of California at Riverside and is available for high-throughput analysis (DeMartini et al. 2011).

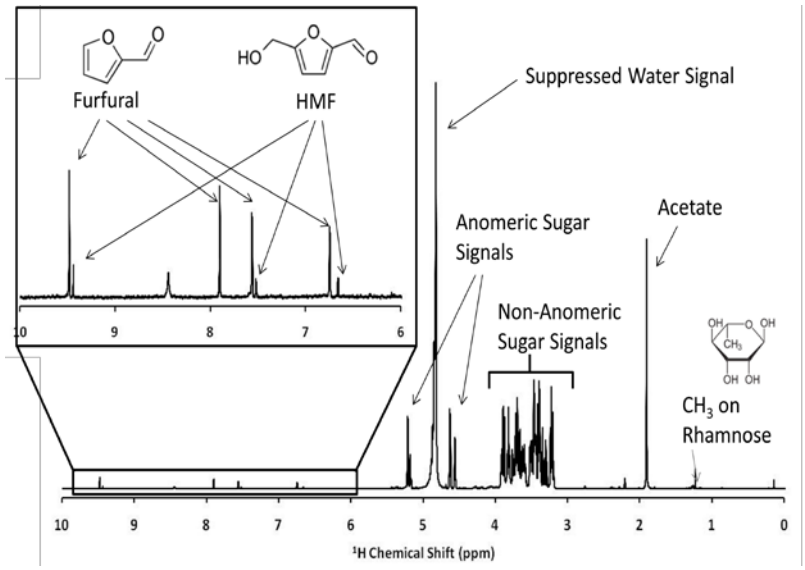

Figure 2: Example NMR spectrum from HTP sugar analysis.

References:

DeMartini, J. D. et al. (2011). "Small-scale and automatable high-throughput compositional analysis of biomass." *Biotechnol. Bioeng.* **108**(2): 306-312.

Selig, M. et al. (2011). "High-throughput determination of glucan and xylan fractions in lignocelluloses." *Biotechnol. Lett*: 1-7.

Sykes, R. et al. (2008). "Within tree variability of lignin composition in *Populus*." *Wood Sci. Technol*. **42**: 649-661.

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