Reduced Lignification Enhances Cellulose Accessibility in Wood Cell Walls

Catherine I. Lacayo,[1] Shi-You Ding,[2] Mona Hwang,[1] and **Michael P. Thelen**[1]* (mthelen@llnl.gov)
[1]Lawrence Livermore National Laboratory; Livermore, Calif.; [2]National Renewable Energy Laboratory; Golden, Colo.

Project Goal: Effective deconstruction of plant cell wall lignocellulose is crucial to producing economical biofuels. The objective in this section of our SFA project (J. Pett-Ridge, Lead PI) is to dynamically monitor and quantitate the changes in lignin and polysaccharide content during various treatments of wood cells. We have focused in particular on investigating ways to reduce lignin content in walls, and how this influences enzyme accessibility to cellulose.

Most studies on cellulase enzymes use purified model substrates, usually either insoluble, crystalline cellulose, or soluble, chemically modified cellulose. While these substrates are good for assays such as measuring the release of glucose, they have dubious predictive value for the efficiency of chemical and enzymatic processing of plant feedstocks during biofuel production. Also, milled feedstock material such as switchgrass contain a heterogeneous mixture of cell types in different physical states, and therefore are difficult to use for statistical measurements in cell populations. A biologically relevant substrate for analyzing lignocellulose deconstruction is provided by cultured wood cells, which can be examined individually and in populations for the removal of lignin, cellulose, and hemicellulose. Toward this end, we established the *Zinnia* culture system for generating homogeneous suspensions of fully differentiated xylem cells, which are characterized by distinctly organized features found in all wood cell walls and are easily recognized by brightfield, fluorescence, and atomic force microscopy techniques.

Using this system, we characterized the topology of primary and secondary cell walls at nanoscale resolution, providing us with an architectural model of the *Zinnia* xylem cell (Lacayo et al, 2010, *Plant Physiol*). Lignin autofluorescence was diminished by about 90% in wood cells incubated for 6 hours in acidified sodium chlorite at 70 °C. By observing these oxidized cells in an environmental slide chamber under polarized light, we could track the intrinsic cellulose birefringence in secondary walls in real time during incubation with an endoglucanase. The results indicated that most of the cellulose was removed over a 3 hour period. This semi-quantitative analysis was confirmed by similar experiments in which fluorescence intensity was measured in cells probed with a GFP linked carbohydrate binding protein, *Ct*CBM3, which is specific for crystalline cellulose. When cellulose was enzymatically digested during the 3 hour incubation, GFP-*Ct*CBM3 binding decreased, leading to a statistically significant loss of fluorescence when compared to a control lacking cellulase. These studies provided the foundation for

further experiments designed to determine the accessibility of cellulase and xylanase enzymes in secondary walls.

The amount of lignin in wood cells was also decreased during xylem development by the use of specific inhibitors of lignin biosynthesis. Culture medium was amended with reduced glutathione (GSH) or diphenyleneiodonium (DPI), and secondary wall differentiation proceeded normally except that autofluorescence and phloroglucinol staining were significantly lower than in control cells. After the treated cells were incubated with cellulase, GFP-*Ct*CBM3 fluorescence measurements indicated a statistically significant correlation of cellulose removal with a decrease in secondary wall lignin. Although the inhibition of lignin synthesis during differentiation was not as pronounced as removing lignin post-differentiation by chemical oxidation, both resulted in increased secondary cell wall accessibility to cellulase. Our studies confirm observations of enhanced saccharification in cellulase treated *Arabidopsis* lignin biosynthetic mutants, and provide a quantitative approach to assessing enzymatic efficiencies in a different model system.

This work performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344.