Deuteration Effects on Switchgrass Structure and Metabolism: Lignin Deposition Changes in Cell Walls of Deuterated Switchgrass

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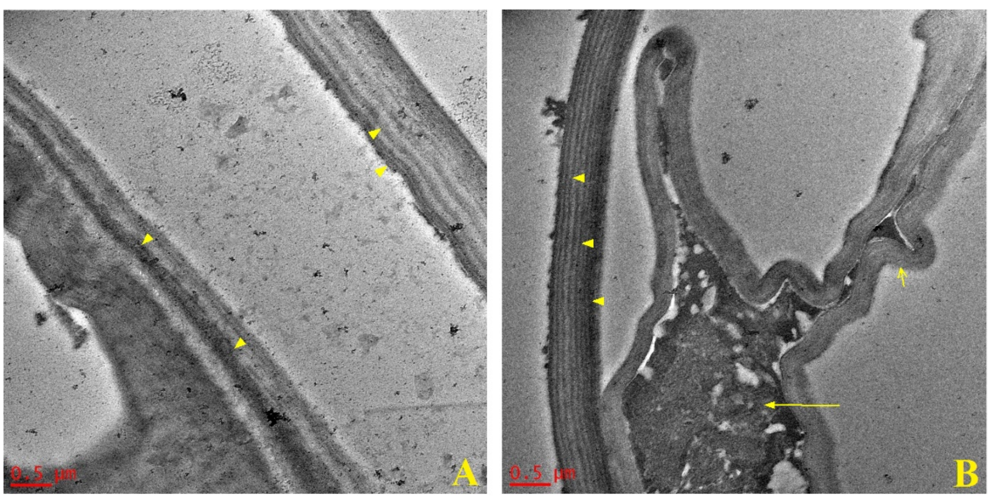
http://cmb.ornl.gov/research/neutron-scattering/lignocellulose-dynamics

Project Goals: Lignocellulosic biomass comprises the vast majority of biomass on Earth and has the potential to play a major role in generation of renewable biofuels if cost-effective conversion can be achieved. Largely composed of plant cell walls, it is a complex biological composite material that is recalcitrant to the structural deconstruction and enzymatic hydrolysis into sugars that are necessary for fermentation to bioethanol. The Scientific Focus Area in Biofuels is developing "Dynamic Visualization of Lignocellulose Degradation by Integration of Neutron Scattering Imaging and Computer Simulation" for multiple-length scale, real-time imaging of biomass during pretreatment and enzymatic hydrolysis. This is providing fundamental information about the structure and deconstruction of plant cell walls that is needed to drive improvements in the conversion of renewable lignocellulosic biomass to biofuels.



Differences in TEM image of protiated and deuterated switchgrass. Images **(A)** and **(B)** show protiated and deuterated switchgrass cell walls, respectively. Arrow heads show regions of higher lignification. Long arrow shows lignin-like deposit in image of deuterated switchgrass. Short arrow shows convoluted cell walls of deuterated switchgrass with reduced lignification compared to a cell wall with normal lignification on the left-side in the same image **(B)**. Scale bar is 0.5 µm.

Neutron scattering enables the study of structural and dynamic properties of lignocellulosic biomass at multiple length scales in a non-destructive manner. High levels of deuterium substitution are desired to enable contrast variation techniques which reduce background. However, plants grown in concentrations of D_2O greater than 30% typically exhibit slower growth, stunting, inhibition of root elongation, and delay or abolishment of flowering and seed set. To evaluate utility of deuterated lignocellulosic biomass for structural studies, any changes in lignocellulosic cell wall composition and molecular structure need to be determined. In contrast to the response of other plants including C_3 annual grasses *Triticum*, *Secale* and *Lolium*, the bioenergy crop switchgrass *Panicum virgatum*, a C_4 drought-resistant perennial prairie grass, has been shown to adapt to growth in 50% D_2O. Switchgrass grown hydroponically from tiller cuttings and transferred to 50% D_2O growth solution after rooting maintains growth rates, gross morphology, and cellular appearance, with 35% deuterium incorporation.[1] Deuterated switchgrass plants continue to grow for at least two years, grow new roots as well as tillers, and even produce reproductive tillers with normal appearing flower spikes.

However, enzymatic hydrolysis and microscopy identified ultrastructural changes in properties of deuterated switchgrass biomass with direct relevance to understanding recalcitrance and response to abiotic stressors.[2] In this study, enzymatic hydrolysis of bacterial cellulose, and cellulose and holocellulose isolated from switchgrass showed the expected kinetic isotope effect, with deuteration lowering glucose yields by 17, 18 and 4% of theoretical yield, respectively. However, the opposite trend was found for deuterated switchgrass, in which glucose yield was 5% higher than that obtained with protiated switchgrass at lower enzyme loading. These data indicated that alterations to lignin might be responsible for this novel inverse isotope effect. Lignin content of deuterated switchgrass was about 2% higher than that of protiated switchgrass, while nuclear magnetic resonance (NMR) spectroscopy indicated no significant compositional or structural differences in lignin, and Simons' staining found comparable cellulose surface area. However, extensive confocal fluorescence microscopy (CFM) and transmission electron microscopy (TEM) imaging showed that deuterated switchgrass had abnormal lignin distribution in some of its cell walls and many of them were collapsed possibly due to reduced rigidity, which would render them easier to deconstruct by cellulases (Fig. 1). The changes in morphology resembled those of drought stressed plants, consistent with abiotic stress due to growth in deuterated media. These protio/deutero investigations clearly illustrate the key role of component distribution in the multilamellar cell wall architecture on biomass recalcitrance.

References

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