

Visualization of plant cell wall polymers using neutron scattering and deuterium labelling in *planta*

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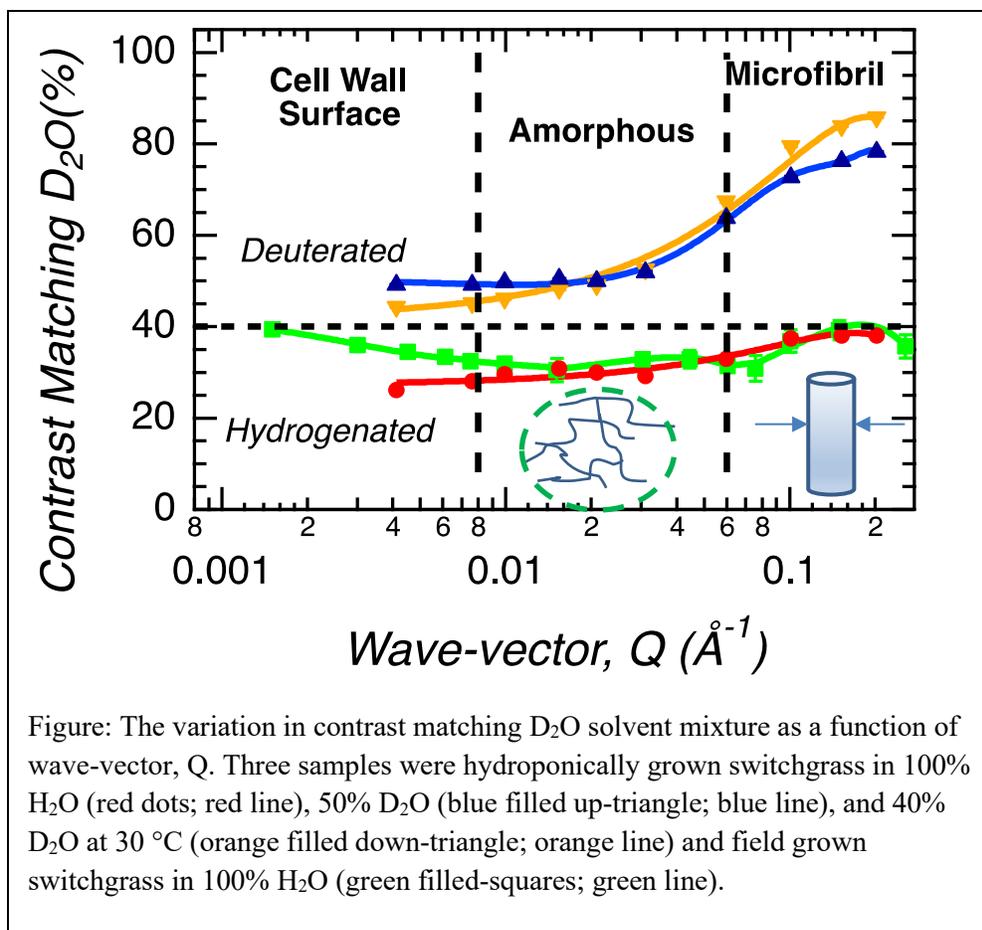
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<https://cmb.ornl.gov/dynamic-visualization-of-lignocellulose/>

Project Goals: The development of renewable biofuels is a key mission of the DOE Genomic Science program. Lignocellulosic biomass has the potential to be an abundant, renewable source material for production of biofuels and other bioproducts. The use of organic solvents to optimize biomass pretreatment has shown considerable promise, but their disruption of microbial membranes is key to toxic effects limiting fermentation titers. The Oak Ridge National Laboratory (ORNL) Scientific Focus Area (SFA) Biofuels Program utilizes multi-length scale imaging with neutron scattering complemented by high performance computer simulations, NMR, biochemistry and targeted deuteration to provide fundamental knowledge about the molecular forces that drive solvent disruption of the critical assemblies of biomolecules that comprise plant cell walls and microbial biomembranes.

The plant cell wall is a complex, multi-polymeric system that consists of primarily carbohydrates and lignin. Cellulose strands come together to form microfibrils while the amorphous polymers, hemicellulose and lignin form a network structure and fill in the interstitial space. Small-angle neutron scattering is ideally suited to study the complex hierarchical structure of biomass. However, neutron sensitivities of the different plant biopolymers are similar making structural association non-trivial and ambiguous. A promising approach is partial deuteration of the plants to increase the difference in the neutron sensitivity between the plant cell wall polymers. Here, we will present the results from three different partially deuterated plants: switchgrass (*Panicum virgatum*), kale (*Brassica oleracea*), and eucalyptus (*Eucalyptus camaldulensis*).

Partially deuterated switchgrass plants were obtained by hydroponic cultivation in media containing 50% D₂O to produce tiller biomass with 34% deuterium incorporation determined by NMR and contrast variation small-angle neutron scattering (CV-SANS).¹ The cell wall composition was found to be similar to the same cultivar grown hydroponically in H₂O, but with slightly increased lignin content and different lignin deposition pattern as determined by TEM and confirmed by SANS (see Figure). Surprisingly, these plants also had a lower recalcitrance to enzymatic hydrolysis. Partially deuterated kale was obtained by hydroponic cultivation in 31% deuterated media. Fourier-transform infrared spectroscopy (FTIR) results indicated that D/H substitution for carbohydrate is higher than for lignin. By combining CV-SANS and FTIR, it was determined that 50% of covalently bonded hydrogens were replaced with deuterium atoms in cellulose while only 10% for lignin. Similar results were observed for partially deuterated eucalyptus plants. These results open new avenues to visualize structural features of amorphous plant polymers in the plant cell wall from those of the well-organized cellulose microfibrils.



References:

1. Evans BR, Pingali SV, Bhagia S, O'Neill HM, Ragauskas AJ, "Structural Studies of Deuterium-Labeled Switchgrass Biomass", In Understanding Lignocellulose: Synergistic Computational and Analytic Methods, *ACS Symposium Series* 1338, ed. Micholas Dean Smith, Chapter 2, 17-32 (2019) DOI: 10.1021/bk-2019-1338.ch002

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