Production and NMR Analysis of Deuterated Cellulose and Lignocellulosic Biomass and Its Utilization for Neutron Scattering Studies

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Project Goals:

Lignocellulosic biomass from plant cell walls has the potential to play a major role in generation of renewable biofuels if cost-effective conversion can be achieved. However, it is a complex composite material that shows significant recalcitrance towards the structural deconstruction necessary for production of bioethanol. A fundamental understanding of the structural changes and associations that occur between its component polymers at the molecular level during deconstruction is essential for enabling cost-effective lignocellulose-based fuels production. This Scientific Focus Area in Biofuels seeks to develop and demonstrate the "Dynamic Visualization of Lignocellulose Degradation by Integration of Neutron Scattering Imaging and Computer Simulation" to provide this fundamental information about the structure and deconstruction of plant cell walls by integration of neutron science and computational simulation with physical and chemical characterization by NMR techniques.

In support of neutron science experiments, NMR methods were developed and demonstrated for analysis of deuterium incorporation in cellulose and plant biomass produced by culture of target species under deuterating conditions. The methods provide crucial supporting information for visualization of lignocellulose structure by neutron techniques and computational simulation.

Small angle neutron scattering (SANS) provides useful tools for investigation of lignocellulosic structural complexity and its changes during pretreatment and hydrolysis to sugars. The use of deuterated materials in which the non-exchangeable hydrogen atoms are replaced with deuterium can greatly increase the value of these studies, as the scattering patterns of the different components can be separated by phase contrast, enabling simultaneous observations of each component. Partially deuterated biomass samples were produced by cultivation of duckweed (*Lemna minor*), and the grasses annual rye (*Secale cereale*) and switchgrass (*Panicum virgatum)* in D_2O/H_2O mixtures. Samples of cellulose with specific levels of deuterium incorporation were obtained by cultivating *Acetobacter xylinus* in a defined deuteration medium. Using these samples, NMR methods were developed for quantifying the degree of deuterium substitution in the complete and individual components of the cell wall. A second NMR methodology that requires no sample preparation and is non-destructive was specifically designed and demonstrated to analyze deuterium incorporation in whole biomass samples. It was possible to measure the ratio of the integrals resulting from the subsequent ^1H and ^2H spectra. These values were correlated with known molar ratios of protons to deuterons from a calibration curve that were generated by taking several calibration standards formed from mixtures of glucose/glucose-d_7. The first SANS experiments have been carried out using the deuterated cellulose and plant biomass materials. Combined with ^{13}C-NMR, X-ray diffraction (XRD), and gel permeation chromatography (GPC), the new NMR techniques provide crucial chemical and structural information needed to leverage the full potential of SANS and building of real-time computational models of biomass deconstruction.

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