210. Production and Characterization of Deuterated Switchgrass and Annual Grasses for Neutron Studies

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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, lignocellulosic biomass is a complex biological composite material that shows significant recalcitrance towards the structural deconstruction and enzymatic hydrolysis into sugars that is necessary for fermentation to bioethanol. 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" for multiple-length scale, real-time imaging of biomass during pretreatment and enzymatic hydrolysis. This will provide 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.

We have developed and demonstrated a suite of methods for production and characterization of highly deuterium-enriched herbaceous biomass with morphology and chemical composition similar to protiated controls. Three grass species, annual ryegrass (*Lolium multiflorum*), winter grain rye (*Secale cereale*), and switchgrass (*Panicum virgatum*) have successfully been grown in 50% and higher concentrations of D_2O for production of lignocellulosic biomass containing 35 – 45% deuterium substitution as determined by NMR and FTIR. Hydroponic cultivation of switchgrass from cuttings was developed and employed to enable multiple, periodic harvests of deuterated biomass from this perennial species. Standard characterization methods based on GPC, FTIR, and NMR were used to characterize the physical and chemical properties of the harvested lignocellulosic biomass from the plants cultivated in D_2O. To understand the characteristics related to enzymatic hydrolysis of deuterated biomass, we investigated the effects of deuterium substitution on the biomass composition, degree of polymerization (DP) and cellulose crystallinity for each species compared to protiated controls cultivated in H_2O. While the isolated hemicellulose molecular weight and cellulose crystallinity remained unaffected, deuterated annual ryegrass had cellulose weight average and number average molecular weights that were 80% of those of protiated controls. Deuterium incorporation into different biomass components maximizes the power of neutron scattering by making it possible to visualize individual components in native and pretreated states. The level of deuterium incorporation and structural characterization accomplished for these grass species support the effective use of such deuterated biomass for neutron scattering studies.

Oak Ridge National Laboratory is managed by UT-Battelle, LLC for the U.S. Department of Energy under contract no. DE-AC05-00OR22725. This program is supported by the Office of Biological and Environmental Research in the DOE Office of Science.