Real-time Elucidation of Structure and Morphology of Native and Mutant Poplar During Dilute Acid and Alkali Pretreatments Using Neutron Scattering

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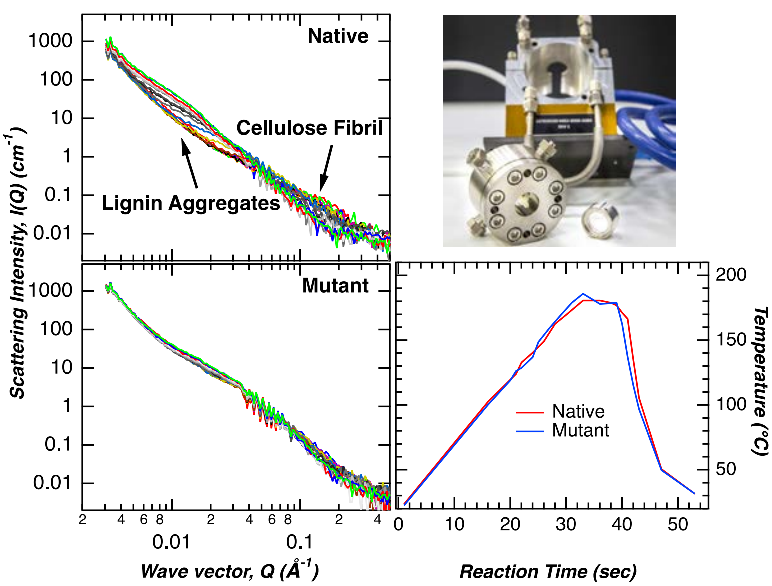
http://cmb.ornl.gov/research/neutron-scattering/lignocellulose-dynamics

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 is 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.



Dilute acid reactions of native and mutant poplar wood were examined by real-time small-angle neutron scattering. Time resolved curves for native (top left) and mutant (bottom left) poplar samples; real-time pressure reaction cell (right top); reaction temperature profiles (right bottom).

Biomass deconstruction by thermochemical pretreatments, a precursor to enzymatic hydrolysis, is intended to enhance enzymatic accessibility to cellulose. However, the complex nature of biomass causes initiation of several processes at different stages of the pretreatment regime. These processes cause structural rearrangements in cellulose and the matrix copolymers (lignin, hemicellulose, etc.,), as well as to mesoscale surface morphology. Knowledge of structural changes to biomass and the chronological order in which they appear during pretreatment will enable an informed approach to improving biomass conversion efficiencies.[1,2] Our recently published *ex-situ* results indicated that enzymatic hydrolysis of switchgrass produced higher yields despite dilute acid pretreatment causing higher degree of coalescence of neighboring cellulose microfibrils, increased cellulose crystallinity, and higher redistribution of lignin leading to larger aggregates.[2] Yet current conventional wisdom would suggest that such structural rearrangements should lead to reduced enzymatic yields. A plausible reason for such observation could be in our inability to observe structural changes occurring in-real time.

Consequently, we developed a pressure reaction cell to monitor morphological changes in biomass using small-angle neutron scattering (SANS) during thermochemical pretreatment in real-time.[3,4] This approach takes advantage of the non-destructive and high penetration properties of neutrons to perform *in-situ* studies. Most importantly, we have recently extended the capabilities of the Bio-SANS instrument at the High Flux Isotope Reactor at Oak Ridge National Laboratory by installation of an additional detector array; this upgrade makes it possible to capture neutrons scattered at higher angles simultaneously to the neutrons captured at smaller scattering angles by the main detector.[4] As a result, data collection times are dramatically reduced and it is possible to obtain structural information at shorter length scales than was previously possible. Here, we report on real-time SANS studies of native poplar and a transgenic variant that is deficient in lignin synthesis during dilute acid and alkali pretreatments. We observe significant differences in lignin aggregation patterns in the native and mutant poplar comparing the two pretreatment regimes. On the other hand, the scattering signature assigned to the cellulose microfibrils remains unchanged in dilute acid and alkali pretreatment. A quantitative analysis of the structural changes in the native and mutant plants will be presented. Using this approach, it is possible to obtain molecular level insights into structural rearrangements of biomass polymers during pretreatment to better understand the consequences of genetic mutations on the overall digestibility of biomass.

Reference

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2. S.V. Pingali et al., Understanding multiscale structural changes during dilute acid pretreatment of switchgrass and poplar. *ACS Sustainable Chemistry & Engineering* **2016**, (accepted) DOI:10.1021/acssuschemeng.6b01803.
3. S.V. Pingali et al., Morphological changes in the cellulose and lignin components of biomass occur at different stages during steam pretreatment. *Cellulose* **2014**, 21, 873-878.
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