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Morphological changes in the cellulose and lignin components of biomass occur at different stages during steam pretreatment

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Abstract Morphological changes to the different components of lignocellulosic biomass were observed as they occurred during steam pretreatment by placing a pressure reaction cell in a neutron beam and collecting time-resolved neutron scattering data. Changes to cellulose morphology occurred mainly in the heating phase, whereas changes in lignin morphology occurred mainly in the holding and cooling phases. During the heating stage, water is irreversibly expelled from cellulose microfibrils as the elementary fibrils coalesce. During the holding phase lignin aggregates begin to appear and they increase in size most noticeably during the cooling phase. This experiment demonstrates the unique information that in situ small angle neutron scattering studies of pretreatment can provide. This approach could be useful in optimizing the heating, holding and cooling stages of pretreatments to allow the exact size and nature of lignin aggregates to be controlled in order to enhance enzyme accessibility to cellulose and therefore the efficiency of biomass conversion.

Keywords Neutron scattering · Steam pretreatment · Cellulose · Lignin · Biomass · Biofuels

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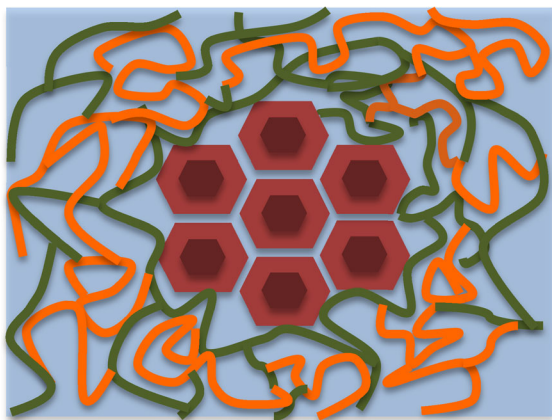Oak Ridge, TN 37831, USA

Introduction

We have been developing neutron scattering technologies to characterize the morphology of lignocellulosic biomass and how it evolves in response to industrial thermochemical pretreatments (Langan et al. 2012). Neutron scattering is sensitive, but differently to both hydrogen (H) and its isotope deuterium (D). This sensitivity to H and the ability to replace it by D to alter scattering levels provides distinct advantages for using small angle neutron scattering (SANS) techniques to highlight different components of complex biological systems over length scales from 1–1,000 nm. In particular, SANS can be used to study the morphology of the different components within biomass; lignin, hemicellulose, and cellulose (Pingali et al. 2010; Cheng et al. 2011; Li et al. 2011). This can be achieved by soaking samples in D_2O so that the scattering level of the water

Fig. 1 A schematic of native lignocellulosic biomass which consists of lignin (*orange*), hemicellulose (*green*) amorphous polymers on the peripheral and cellulose fibrils with the core being more crystalline (*darker shade* of *brown* indicates higher crystallinity). (Color figure online)

accessible matrix of lignin and hemicellulose polymers is raised compared to the water impenetrable elementary crystalline cellulose fibrils. Further, the ability of neutrons to penetrate through robust sample environments raises the possibility for studying pretreatment in situ, thereby providing a parametric picture of the morphological transitions taking place.

In previous studies we applied SANS in combination with molecular dynamics (MD) simulations to study morphological changes in biomass during steam explosion pretreatment (SEP) (Langan et al. 2014). Wood chips from poplar trees were chosen for study due to their potential as an abundant, low cost, feed stock (Sannigrahi et al. 2010). We found that these chips are composed mostly of cellulose in the form of elementary fibrils, with well-ordered cores and hydrated surfaces that are more disordered, which pack together tightly in microfibrillar bundles in some regions (a schematic is shown in Fig. 1). Within the bundles, and between the elementary fibers, a thin layer of water is trapped. These bundles are surrounded by a disordered hydrated matrix of covalently linked and entangled hemicellulose and lignin polymers. After the wood chips were steam pretreated in a pressure reaction vessel for about half an hour at 180 °C it was observed that the core water molecules originally trapped within the microfibrils had been released into the surrounding hydrated matrix, causing the fibrils to coalesce into bundles with more confined,

and therefore ordered, surface chains. We also found that the lignin-hemicellulose matrix had gone through a phase separation, with the more hydrophobic lignin aggregating into crumpled globules and with some loss of hemicellulose through autohydrolysis.

These experimental results were interpreted using MD simulations of two separate systems, one that modeled a hydrated microfibril and the other a hydrated matrix of lignin and hemicellulose. In simulations of the microfibril, enthalpic interactions between cellulose and water keep some (core) water trapped within the microfibril, but as the temperature is increased during SEP the entropic penalty dominates allowing and the core water molecules to be released to bulk solvent. In simulations of the matrix, lignin transitions to conformations that contain fewer kinks, thus leading to fewer lignin hemicellulose entanglements, and allowing the observed phase separation. The balance between the entropy and enthalpy of water is one of the key drivers of the morphological changes to both cellulose microfibrillar bundles and the matrix of lignin and hemicellulose within which they are encrusted.

A limitation of this earlier work is that the wood chips were examined outside of the pressure reaction vessel before and after SEP; there is no experimental information on what happens within the vessel during pretreatment. Therefore our interpretation of the morphological changes, informed by the MD simulations, as the emergence of lignin globules and the aggregation of cellulose fibers during the heating stage of the reaction has not been experimentally verified; it is possible that the morphological changes occur during the subsequent holding or cooling stage of the reaction. In order to address this knowledge gap we carried out an experiment to monitor morphological changes as they occur during pretreatment by placing a pressure reaction cell in a neutron beam and collecting time-resolved neutron scattering data.

Materials and methods

Biomass samples

Quaking aspen (*Populus tremuloides*) samples were obtained by Benchmark International in High Level, Alberta, Canada. Trees were destructively sampled to collect approximately 20–80 mm thick disks, or cross

sections, from individual trees at 0.3 m from the point of germination. All chips were taken from the outer portion of the section to minimize variability within a single chip. Chips had the following dimensions: 50.8 × 38.1 × 12.7 mm (2 × 1.5 × 0.5) in length, width, and thickness, respectively. The fiber axis ran along the length of the chip.

Bleaching

Lignin was removed to leave holocellulose by treating aspen chips in 1 % sodium chlorite solution at 70 °C for 2 days, at a pH below 5 by adding acetic acid.

Small angle neutron scattering (SANS) of biomass

SANS measurements on biomass were carried out on GP-SANS (CG-2) and Bio-SANS (CG-3) instruments at the high flux isotope reactor facility of Oak Ridge National Laboratory. The samples consisted of thin (~1 mm thick) circular wafers of 10 mm diameter tangential section cut from aspen chips. For the in situ measurements, the sample was soaked in D_2O for several hours and then, while still wet, placed in a stainless steel pressure reaction cell with 12.7 mm thick quartz windows (Melnichenko et al. 1999). A wavelength, λ, of 4.75 Å, a neutron spread $\Delta\lambda/\lambda$ of 0.14, and a sample-to-detector distance of 3.336 m were used to cover a Q range of 0.0125–0.375 Å^{-1}. The center of the area detector was offset to allow access to larger values of Q. The instrument resolution was defined using a circular aperture of 10 mm diameter. The detector images were normalized to incident beam monitor counts, and corrected for detector dark current, pixel sensitivity and scattering from backgrounds. The images were then transformed into polar reciprocal space coordinates Q and ϕ, where ϕ is the azimuthal angle around the beam direction (ϕ has values of $0°$ and $180°$ in the equatorial direction and $90°$ and $270°$ in the meridional direction).

Results

In situ SANS images were collected every 15 min as the temperature was ramped from 25 to 140 °C, held for 4 h at 140 °C, and then quenched to 25 °C (see Fig. 2). Data was collected in intervals of 15 min to
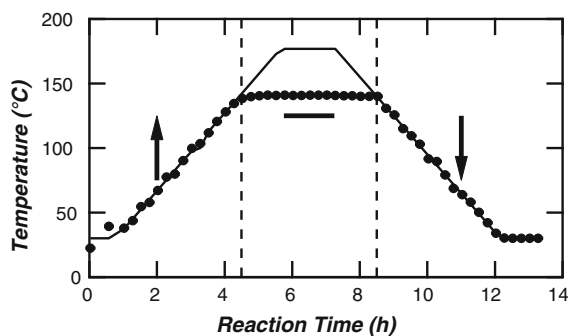

Fig. 2 The temperature series of the reaction vessel temperature (*black dots*) during the in situ neutron scattering experiment was increased from 40 to 140 °C (*upward arrow*), held at 140 °C (*flat line*) and reduced from 140 to 40 °C (*downward arrow*). The *black line* represents the set temperature

optimize two aspects: signal-to-noise ratio of the data and maintain a close resemblance to the protocols employed by the biofuel refineries.

The polar reciprocal space transformed data (Q vs. ϕ) were then separated into an isotropic background component and an anisotropic equatorial component using the following approach. At each value of Q the intensity distribution in ϕ was fitted, using least-squares refinement, by two Gaussian profiles

$$G = I_A \exp\left(-\ln 2\left(\frac{\phi - \phi_0}{\Delta\phi}\right)^2\right)$$

where the peak intensity I_A, peak position ϕ_0 and orientation fluctuation $\Delta\phi$ were refined together with an isotropic background, $B(Q)$ (which varies with Q but is constant with ϕ) as described by Nishiyama et al. (2013). The resulting fitted points for $I_A(Q)$ in the equatorial direction ($\phi_0 = 0°$) and $B(Q)$ over the course of the pretreatment are shown in Fig. 3. Further data over a more extensive Q range were collected from samples before and after SEP that had been bleached to remove lignin. The samples were sealed in quartz "Banjo" cuvettes (0.5 and 14 mm inner thickness and diameter) with D_2O. A wavelength, λ, of 4.75 Å, a neutron spread $\Delta\lambda/\lambda$ of 0.14, and three sample-to-detector distances of 1.089, 6.789 and 19.289 m were used to cover a Q range of 0.003–1 Å^{-1}. The center of the area detector was offset to allow access to larger values of Q. These data were collected to help interpret scattering features from cellulose and lignin in the in situ studies.
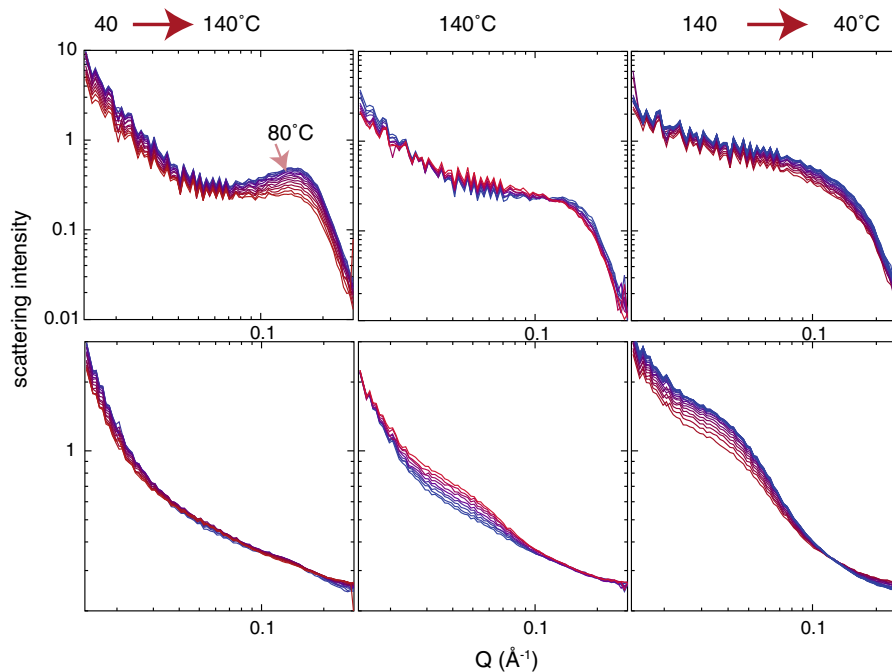
Fig. 3 Neutron scattering collected as the temperature in the reaction vessel was (*left column*) increased from 40 to 140 °C (*middle column*) held at 140 °C and (*right column*) reduced from 140 to 40 °C. *Top row* The anisotropic component of the neutron scattering intensity in the equatorial directions, $I_A(Q)$ *Bottom row* the isotropic component of the neutron scattering intensity, $B(Q)$. The intensity plots change color from *blue* to *red* as the temperature varies from 40 to 140 °C in the *left* and *right* columns. In the *middle column* the curves change from *blue* to *red* as the holding time increases. (Color figure online)

Discussion

During the heating phase of SEP a peak in the anisotropic equatorial scattering (at $\sim 0.17 \text{ Å}^{-1}$) begins to decrease in intensity after the temperature has reached ~ 80 °C. This diffraction peak has been reported before and has been shown to be due to side–side-packing of elementary cellulose fibrils in microfibrillar bundles; when soaked in 40 % D_2O to match out scattering from cellulose the diffraction peak disappears, confirming its origin in side-by-side packing of cellulose elementary fibrils into aligned bundles (Langan et al. 2014). We interpret this result as a gradual reduction in the population of microfibrillar bundles that contain core water layers between elementary fibrils and a coagulation of those fibrils as the temperature is increased above ~ 80 °C. There is little change in the isotropic background scattering which remains featureless.

During the holding phase the anisotropic equatorial scattering shows little further change except for a small shift in peak position to lower Q. However a scattering feature appears and increases in intensity in the isotropic scattering background which can be interpreted as a Guinier region. This feature, which has been reported before (Pingali et al. 2010), is considerably diminished in SANS from the wood chip that had been pretreated and then bleached and we therefore interpret it as being due to lignin (R_g is in the range of 20–30 nm). As the temperature is ramped down there is little change in the anisotropic equatorial scattering. However, there is a continued increase in the strength of the Guinier region in the isotropic scattering, which we interpret as the continued formation of lignin aggregates, and the Guinier shoulder moves to slightly lower Q, indicating that their sizes are increasing.

Changes to cellulose morphology occur only in the heating phase, whereas changes in lignin morphology occur in the holding and cooling phases. Figure 4 summarizes the process, during the heating stage water is expelled from cellulose microfibrils as the elementary fibers coalesce. After water has been expelled there is little further change in cellulose
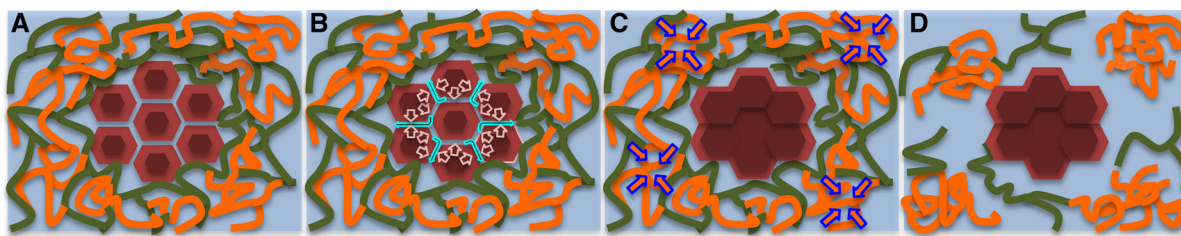
Fig. 4 A schematic summarizing the internal structural changes that biomass undergoes during SEP process. The *different panels* represents the different phases of the process: **a** native; **b** heating; **c** holding and cool down and **d** pretreated

during the holding and cooling phases, and this agrees with our previous MD simulations in which it was found that water expulsion is an irreversible process (Langan et al. 2014). On the other hand, there is little change to lignin during the heating phase, but aggregates begin to appear and increase in number during the holding phase. Lignin aggregates continue to appear and increase in size during the cooling phase. Our previous MD simulations suggest that lignin aggregation occurs during SEP as soon as lignin has phase separated from hemicellulose. The results presented here indicate that this phase separation starts at or above ∼140 °C during SEP.

This experiment is one of the first to directly probe morphological changes in biomass chips during SEP and it provides new insights into how the cellulose and lignin components change at different stages of the pretreatment. Having demonstrated the usefulness of this approach, we plan to carry out further studies to address several questions that arise from these initial results. In particular, we will extend the heating phase to higher temperatures (temperatures as high as 180 °C have been used for SEP) to verify that lignin aggregation starts during the heating phase after the temperature has reached 140 °C. We will examine dilute acid pretreatments, which involve more pronounced hydrolysis of lignin-hemicellulose bonds, to see if lignin aggregation starts during the heating phase at lower temperatures. We will also examine different types of biomass. Our hope is that this approach will allow us to optimize the heating, holding and cooling stages of pretreatments of different types of biomass to allow the exact size and nature of lignin aggregates to be controlled in order to enhance enzyme accessibility to cellulose and therefore the efficiency of biomass conversion.

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