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Topochemical Understanding of Lignin Distribution During Hydrothermal Flowthrough Pretreatment

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Changes in surface properties during biomass pretreatments are important parameters to understand and engineer biological biomass conversion processes. In particular, different influences on the surface of biomass are expected between flowthrough and batch pretreatments. For a better understanding of biomass surface changes by hydrothermal flowthrough pretreatment, the mechanism by which the surface of biomass is altered in terms of cellulose and lignin was investigated using time-of-flight secondary ion mass spectrometry (ToF-SIMS) and compared with the bulk chemical composition and the results from scanning electron microscope (SEM). ToF-SIMS analysis results provide semi-quantitative information of cellulose and lignin and support the other observation from SEM and bulk compositional analyses. In brief, more lignin was observed on the surface of biomass at the early stage hydrothermal pretreatment, while the lignin mainly located at the cell corners was reduced by extended pretreatment time. Unlike batch pretreatment, pseudo-lignin formation was not observed on the poplar surface during the flowthrough process.

Lignin has been implicated as a significant inhibitor during enzymatic cellulose hydrolysis, limiting the accessibility of cellulases to cellulose by acting as a physical barrier between cellulases and cellulose and promoting non-specific binding cellulases on non-cellulosic surfaces.^[1] For this reason, diverse pretreatment strategies have been introduced to remove/reduce lignin content of biomass with minimal carbohydrate loss.^[2] Native lignin distribution and lignin re-distribution during pretreatment are important biomass recalcitrance factors because cellulase binding and activity are surface mediated processes. Though many pretreatments remove lignin, they also relocate and redeposit the remaining lignin at the surface of biomass.^[3] Donohoe et al. found evidence of lignin extrusion from the cell wall, mostly at cell corners and middle lamella, and proposed a mechanism for lignin removal during thermochemical pretreatments.^[3b] When thermochemical pretreatments reach the lignin glass transition temperature, lignin possibly coalesces into larger molten bodies that migrate within and out of the cell wall, and can redeposit on the surface of plant cell walls. Trajano et al. found that depolymerization and condensation reactions are the primary mechanisms for lignin extraction and re-deposition.^[4] Sannigrahi et al. also reported that pseudo-lignin could be generated from and deposited on carbohydrates without significant contribution from lignin during dilute acid pretreatment, especially under high severity pretreatment conditions.^[5] However, Bhagia et al. reported that a flowthrough of dilute acid pretreatment did not have pseudo-lignin presumably due to the removal of the constituents of pseudo lignin from the reactor.^[6]

Hydrothermal pretreatment is an attractive biomass conversion technology, since it does not require additional chemical reagents. Hence, it does not cause corrosion to the reactor and generates low levels of biological inhibitors; therefore, it has been considered as one of leading biomass pretreatment technologies. A flowthrough pretreatment system offers a number of advantages including monitoring of the evolution of pretreatment products as a function of time, preventing unwanted side reactions, and removal of pretreatment by-products that minimize subsequent separation/purification steps compared to a batch reactor system. Bobleter et al. investigated degradation of cellulosic matter using flowthrough reactors with hot-water.^[7] Flowthrough pretreatment increased hemicellulose and lignin removal and produced solids that are readily hydrolyzed by enzymes.^[8] Trajano et al. also used a flowthrough reactor, as a tool, to examine the mechanisms of deconstruction for lignin and xylan during the

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pretreatment of poplar.^[9] Although the chemistry of this process have been extensively investigated, to-date, all studies have characterized bulk changes in biomass during and after hydrothermal pretreatment.

The changes on the biomass surface during pretreatments have been mostly observed with imaging techniques such as SEM, TEM and other microscopic techniques in the previous studies.^[5,10] Although these imaging techniques provided morphological information associated with lignin migration and formation of lignin droplets, quantitative structural information is not available with these methods. Recently, ToF-SIMS has been applied to analyze the changes on biomass surface after pretreatments.^[11] ToF-SIMS is an emerging technique providing chemical information directly from the surface of biomass without sample treatment such as matrix application or isotopic labeling.^[11d,12] Mass spectra of chemical fragments obtained over the biomass surface as a result of secondary ion emission reflect chemical species, specifically cellulose and lignin, at the surface. These features of ToF-SIMS allow semi-quantitative comparisons with significant components on the surface and mapping them into a two-dimensional (2D) molecular image at a sub-micron scale of resolution using the mass signals of these characteristic secondary ions or chemical fragments.

Surface characteristics of different pretreatments including dilute acid, alkali, hydrothermal, organosolv and ionic liquid pretreatments using batch reactors have been analyzed by ToF-SIMS, while the changes after flowthrough pretreatments were not reported yet. Because of the aforementioned difference between batch and flowthrough pretreatment processes, it is attractive to investigate the biomass surface changes with flowthrough pretreatment. Herein, a series of hydrothermal flowthrough pretreatments were performed with poplar in order to delineate the spatial distribution of lignin on the surface after pretreatment. Cross sections of juvenile poplar stem were pretreated at a moderate temperature (i.e., 160 °C) to avoid disruption of the original plant cell wall ultrastructure. Pretreatment time was varied in order to observe the effect of pretreatment severity on lignin migration and re-deposition. The pretreated samples exhibited a range of lignin contents and were analyzed by ToF-SIMS. In this study, the lateral distribution of lignin on the surface of pretreated poplar stems was visualized as a function of hydrothermal flowthrough pretreatment and compared to its bulk composition.

Poplar stem cross-sections were subjected to hydrothermal flowthrough pretreatment at 160 °C for 10, 15, 120, and 150 min. Prior to the pretreatment, the samples were cross-sectioned, to facilitate intact samples for ToF-SIMS imaging.^[13] Cellulose/hemicelluloses recovery and delignification efficiency varied under different reaction conditions. The liquid hydrolysate, containing aqueous soluble, low-molecular weight carbohydrates and lignin, was collected and used to calculate total solids dissolution. As shown in Figure 1, from 10 min to 15 min, there was a 65% increase in total dissolution while there was a 43% increase in total dissolution from 15 min to 120 min. Increasing pretreatment time to 150 min did not show significant improvement in complete dissolution. The levelling-

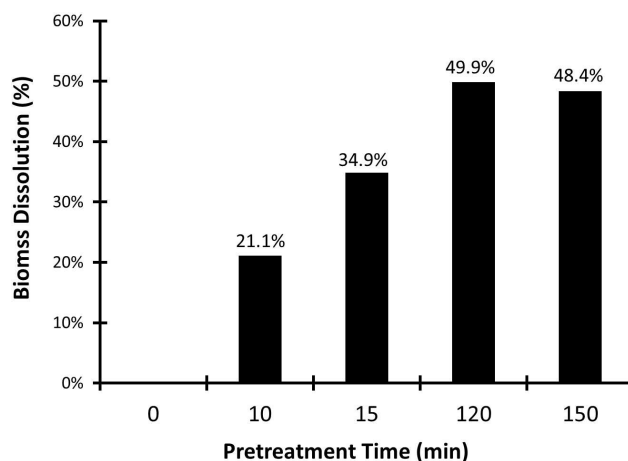


Figure 1. Solubilization yields of poplar after hydrothermal flowthrough pretreatment at 160 °C.

off of the total dissolution indicates there may be a limit to the amount of material that can be removed by hydrolysis at the applied temperature and flow conditions. This material most likely represents a more recalcitrant fraction of the pretreated biomass.

Bulk compositional changes of the structural carbohydrates and lignin in cross-sectioned samples were quantitatively analyzed using a traditional two-stage hydrolysis followed by a Klason lignin method and liquid chromatography to profile cell wall monosaccharides (Figures 2 and S1). Extractives-free

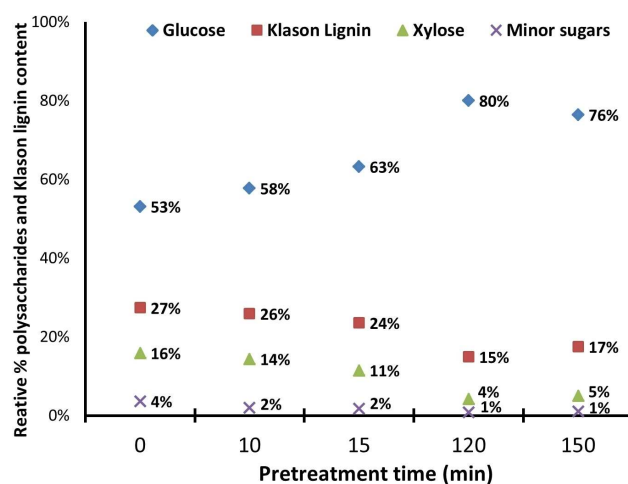


Figure 2. Bulk composition of poplar before and after hydrothermal flowthrough pretreatments as determined by liquid chromatography and normalized by the sum of all the measured components.

poplar (i.e., untreated sample) has an initial glucose content of 53%, primarily from cellulose and a Klason lignin content of 27%. The remaining carbohydrate contained xylose and a few minor sugars, attributed to hemicellulose. After a 10 min

pretreatment, the relative glucose content increased, while hemicellulose and Klason lignin contents were reduced. Thereafter, the relative glucose content reaches a maximum after 120 min of pretreatment, decreasing upon further pretreatment. The relative content of hemicellulose related sugars decreased significantly after 15 min of pretreatment, leveling-off at a total content of 5%. The relative Klason lignin content reaches a minimum after 120 min of pretreatment, increasing upon further pretreatment. Though possible, due to the flowthrough arrangement, it is unlikely that pseudo-lignin formation or lignin solubilization/re-deposition occurs due to the reaction conditions employed. Most likely the cellulose content maximum and lignin content minimum observed at 120 min is due to a higher rate of cellulose dissolution than of lignin dissolution between 120 and 150 min. Suggesting after at least 120 min of pretreatment, the remaining lignin fraction is more recalcitrant than the cellulosic fraction. It has been proposed lignin condensation reactions can occur upon pretreatment of biomass under severe conditions.^[14]

The positive ion ToF-SIMS spectra of the pretreated samples were obtained from the surface of each sample (Figure S2). Then, the characteristic secondary ions emitted from lignin were assigned at m/z 137 and 151 for guaiacyl (G) and at m/z 167 and 181 for syringyl (S) lignin monomer units. Cellulose characteristic secondary ions were at m/z 127 and 145 as assigned in the literature.^[15] Using a semi-quantitative approach, the changes in the relative intensities of characteristic ions were compared after normalization. Normalized ion intensity of lignin or cellulose was obtained from the summation of the characteristic ions and compared in Figure 3.

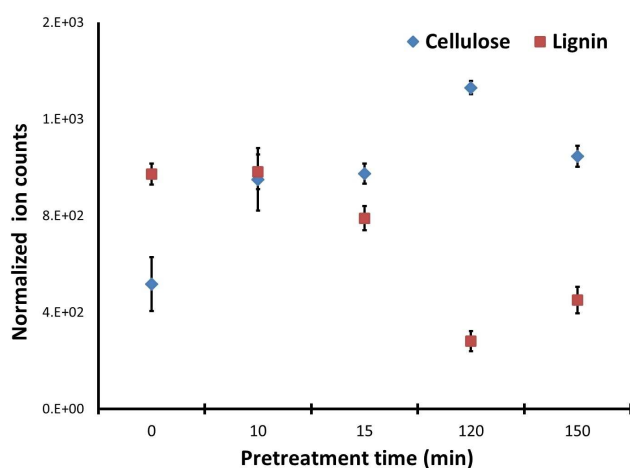


Figure 3. Normalized intensities of cellulose and lignin in cross-sections of the pretreated poplar by ToF-SIMS.

The normalized count of cellulose ions shows a maximum at 120 min, while the normalized count of lignin ions shows a minimum at 120 min, a similar trend to the bulk composition data. It is notable that after 10 min pretreatment the normalized cellulose ion count was almost double that of the untreated extractives-free poplar. This increase could have

resulted from increased exposure of cellulose at the surface of pretreated biomass. However, due to unknown ionization efficiencies and detector response factors, quantitative comparison of the increase in bulk composition to normalized counts of cellulose and lignin ions would be inappropriate. Nevertheless, the similar trend between the bulk chemical composition to normalized counts of cellulose and lignin ions as a function of hydrothermal flowthrough pretreatment suggest that the bulk and surface compositions change similarly.

Secondary ion images of the hydrothermal flowthrough pretreated samples illustrate the spatial distribution of lignin on the surface of cross-section poplar stems (Figure 4). Firstly, a total ion image was generated from each spectrum, representing the population of all released ions up to m/z 800 mass range. Thereafter, lignin ions pooled signals for m/z of 137, 151, 167, and 181 were overlaid on the total ion image using green dots.

The extractives-free poplar image shows that lignin ions (green dots) were uniformly distributed across cell walls on the surface (Figure 4a). After 10 min pretreatment, more lignin ions were observed at cell corners (arrows) in Figure 4b. This increase on the surface of the pretreated poplar is possibly related to lignin migration mediated at pits, cell corners, delamination zones, and the middle lamella during pretreatment.^[3b] After 10 min pretreatment, lignin distribution pattern within the pretreated cell wall was changed (Figure 4b), while the level of the surface lignin ion counts was similar (Figure 3). It may be that as lignin is extruded via cell corner from the interior cell wall matrix area, while solvent flow partially dissolves and removes that lignin. The same pattern is observed that lignin ion intensity after 15 min pretreatment (Figure 4c) decreased at cell corners (arrow) compared to Figure 4b, while remaining at cell corner is greater than that of the extractives-free poplar (Figure 4a). However, overall surface lignin ion counts in extractive-free poplar is higher than that after 15 min pretreatment (Figure 3) due to non-disrupted and delocalized lignin distribution without pretreatment. After 120 min, Figure 3 showed that most lignin had been removed from the surface and this is also observed in Figure 4d. Interestingly, lignin ions intensity increased after further pretreatment for 150 min, mostly at cell corners (arrow), in Figure 4e. It could be caused by cellulose removal on the surface (Figure 3).

Topochemical changes after pretreatment were investigated by scanning electron microscope (SEM). Extractives-free poplar shows the multilayer cell wall: thick secondary cell wall, compound middle lamella (CML) and cell corner (CC) in Figure 5a. This multilayer cell wall after 10 min pretreatment began to split and the secondary cell walls became thinner. The crevice in the cell wall in Figure 5b is likely a path for lignin migration to the surface as abundant lignin ions were observed in the CML and CC in Figure 4b. After a 15 min pretreatment the cell wall lost most of outer cell wall layer, which can explain decreasing surface lignin count (Figure 3) and lignin localization at cell corner (Figure 4c). Further pretreatment (> 120 min) did not affect physical structure of cell wall (e.g. shape and volume), but maximum lignin loss occurred at both surface and

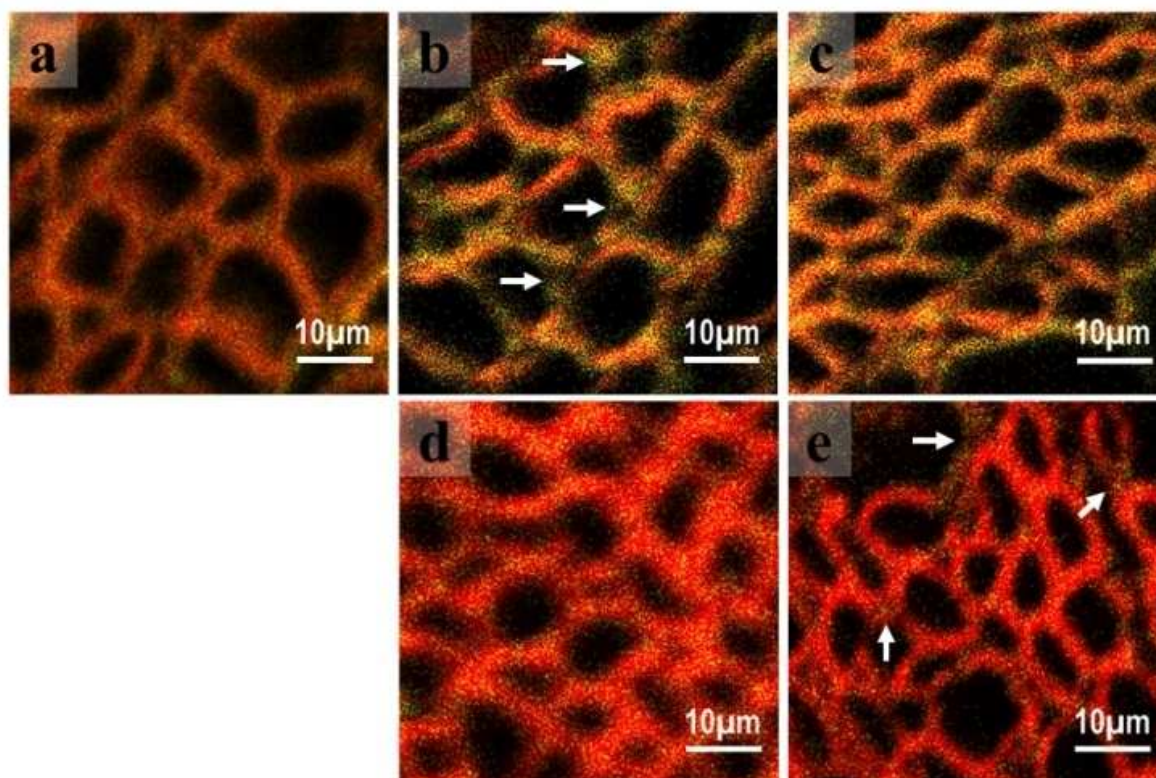


Figure 4. ToF-SIMS images of the cross-sectioned poplar before and after hydrothermal flowthrough pretreatment at 160 °C. Lignin ions (green dots, pooled signal for m/z 137, 151, 167, 181) were overlaid on total ion image (red). (a) Extractive-free poplar, (b) 10 min, (c) 15 min, (d) 120 min, and (e) 150 min pretreated poplars.

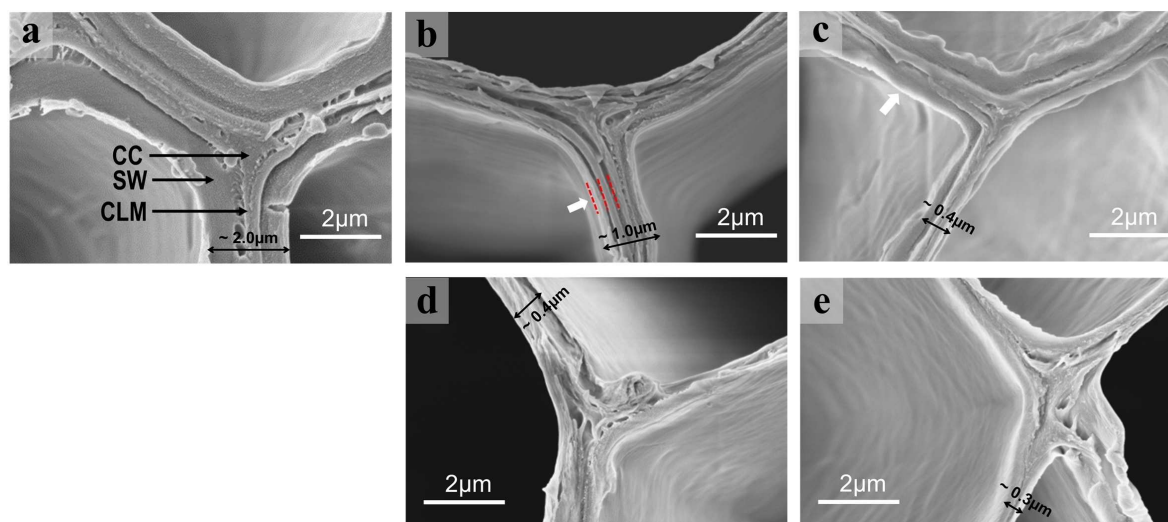


Figure 5. Electron microscopy of the cross-sectioned poplar before and after hydrothermal flowthrough pretreatment at 160 °C for extractive-free poplar (a), 10 min (b), 15 min (c), 120 min (d) and 150 min (e) pretreated poplar. Arrows and red dot lines indicate split cell walls. SW: secondary wall, CML: compound middle lamella, CC: cell corner.

bulk composition (Figures 2 and 3). This result agrees well with the migration of lignin out of cell wall via pit, cell corner and delamination area from inner cell wall matrix reported by Donohoe et al.^[3b] Lignin and/or pseudo lignin coalescence is

frequently observed as droplets on the surface of pretreated biomass in a batch reactor system. They have been reported as to inhibit biomass deconstruction and reducing pseudo lignin formation is also important key for biomass conversion

technology. Interestingly, such droplet was not observed in the pretreated samples in this study (Figures 5b–e). It is possible that flowthrough pretreatment prevents the formation and deposition of such droplets by rapidly removing lignin fragments from the reactor.

In summary, the removal and distribution of lignin during the hydrothermal flowthrough pretreatment under different conditions were demonstrated. The composition changes in the bulk and at the surface of the biomass followed similar trends during the pretreatment process. We also visualized the lignin locations and movements on the cell wall surface during pretreatment by ToF-SIMS imaging. The lignin migration during the pretreatment was mainly observed at the cell corner. Pseudo-lignin, which was observed after hydrothermal pretreatment using a batch reactor, but it was not detected from the poplar surface after the hydrothermal flowthrough pretreatment. This information can be used for the future in determining the spatial topochemical effects of pretreatment in biofuel production.

Supporting Information Summary

Details of sample preparation of cross-sectioned poplar, pretreatment and characterization methods were described in Supporting Information.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords: Flowthrough pretreatment · Surface characteristics · ToF-SIMS · Topochemical effect

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