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Surface Characterization of Dilute Acid Pretreated *Populus deltoides* by ToF-SIMS

Seokwon Jung,^{†,§} Marcus Foston,^{†,§} M. Cameron Sullards,^{†,§} and Art J. Ragauskas*,^{†,‡,§}

[†]BioEnergy Science Center, [‡]Institute of Paper Science and Technology, and [§]School of Chemistry and Biochemistry, Georgia Institute of Technology, 500 10th St., Atlanta, Georgia 30332, USA

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Time-of-flight secondary ion mass spectrometry (ToF-SIMS) was used to analyze the molecular constituents on cross sections of juvenile poplar (*Populus deltoides*) stems before and after dilute acid pretreatment (DAP). Bulk analysis of milled and 50 μm thick cross sections of poplar before and after DAP was shown to be chemically equivalent by FT-IR and carbohydrate analysis. ToF-SIMS analysis of dilute acid pretreated material indicated significant changes in relative contents of cellulose, xylan, and lignin occurred upon pretreatment. The relative content of xylan after DAP increased by 30% on the surface of the poplar stem by ToF-SIMS, while bulk carbohydrate analysis showed that the relative concentration of xylose decreased 10-fold in comparison with untreated poplar wood. The relative content of cellulose and G-lignin units doubled on the surface of the poplar stem sections, while the bulk glucose concentration and Klason lignin increased 40% and 5%, respectively, as determined by bulk carbohydrate and Klason lignin analysis. The spatial distributions of the major lignocellulosic components on the surface of juvenile poplar stem before and after DAP were examined by SIMS and this data was processed into mapping images. Scanning electron microscopy (SEM) was used to evaluate the morphological changes of the cell wall layers before and after DAP, which was also correlated with the results of ToF-SIMS analysis.

Introduction

Lignocellulosic materials are the most abundant biopolymers in nature and have been highlighted as a potential source of second-generation biofuel referred to as biomass-to-liquid technology, which relies on the conversion of cellulosic biomass. These renewable resources will be drawn from agro-energy crops (nonfood crops) and biomass residues originating from both forest and agricultural ecosystems. These bioresources are attractive biofuel feedstocks as they avoid “food or fuel” concerns and are generally low-cost and widely distributed.^{1,2} Lignocellulosic materials are composed primarily of cellulose, hemicelluloses, and lignin in plant cell walls. The very same properties that make lignocellulosics attractive as plant cell wall components contribute to its resistance to deconstruction to simple monosaccharides. This property is commonly referred to as the recalcitrance of biomass. This property contributes substantially to the current cost of biofuels and is the primary deterrent to the widespread commercialization of these types of alternative biofuels. To overcome recalcitrance and increase conversion of biomass to biofuels by enzymatic hydrolysis and subsequent fermentation, a pretreatment process is typically employed prior to biomass deconstruction.³ Many studies have reported dilute acid pretreatment (DAP) significantly improves cellulose enzymatic hydrolysis rate and glucose yields as DAP alters the biomass ultrastructure, chemistry and

increases enzyme accessibility and activity.⁴ A deeper understanding of the surface change, chemically or physically, on plant cell walls during DAP will provide fundamental insight into the pretreatment process and guide further improvement.

To investigate and establish the fundamental mechanisms of plant cell wall structure, various analytical techniques have been applied including nuclear magnetic resonance (NMR), infrared spectroscopy (IR), Raman spectroscopy, X-ray diffraction (XRD), and X-ray photoelectron spectroscopy (XPS).^{5–8} Liquid and solids NMR has been widely used to characterize the chemical structures of plant macromolecules but provide little topological information.⁹ Electron microscopy (EM) techniques, such as scanning electron microscopy (SEM), transmission electron microscopy (TEM), and atomic force microscopy (AFM), have also been used to study the surface morphology, although they do not provide detailed chemical information.^{10–14} Fluorescence imaging using monoclonal antibodies has been widely used to analyze the surface chemistry in plant cell walls, but their use is limited because of the small library of antibodies for specific components within the plant cell wall.

Time-of-flight secondary ion mass spectrometry (ToF-SIMS) is a powerful method for characterizing the surface on solid samples without any special treatment with high

*To whom correspondence should be addressed. E-mail: Art.Ragauskas@chemistry.gatech.edu. Phone: (404) 894-9701. Fax: (404) 894-4478.

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spatial resolution. Because of its unique capabilities of analyzing a solid surface, this technique has been used for the characterization of organic and polymeric materials in various fields.¹⁵ Recent studies have highlighted some applications using ToF-SIMS in plant tissue. For example, the cross sections of Hinoki cypress have been characterized semi-quantitatively for the distribution of plant extractives and these results were used to identify the interface between sapwood and heartwood.¹⁶ Tokareva et al. have also employed ToF-SIMS to investigate the distribution of lignin, carbohydrates, extractives, and metals across the cross sections of Norway spruce.¹⁷ Though examples exist of using ToF-SIMS to characterize plant tissue, the application of ToF-SIMS to characterize biomass resources during their conversion to second generation biofuels has not been reported yet. This is partly because of the ToF-SIMS matrix-effect, defined as various ion yields depending on the chemical environment, and is the major barrier for quantitative measurement. It is difficult to make direct comparisons of the same analytical component or species between chemically altered samples because of this matrix-effect. To counter this effect several methods have been employed in an effort to make SIMS quantitative including (1) using a relative sensitivity factor, (2) matrix isotope species ratio, or (3) utilizing a method of standard additions.¹⁵ In recent studies, normalization of peak intensity has been used for semiquantitative analysis of natural polymers on wood-based fiber surfaces.¹⁸

In this study, we examined the ToF-SIMS technique to characterize cell wall component changes occurring on the surface of poplar stem after dilute acid pretreatment (DAP). Cross sections of extractive-free and holocellulose poplar stem were also analyzed and used as a reference. The change in major cell wall components (i.e., cellulose, xylan and lignins) on the surface of dilute acid pretreated poplar was semiquantitatively compared using both relative contents and the spatial distributions of major components visualized by ToF-SIMS. To further elucidate the spatial redistribution of major cell wall components experienced during pretreatment, SIMS images were transformed to processed mapping images via a MATLAB platform. Finally, the chemical changes in the surface components after DAP were correlated with gross topological changes using electron microscopy (EM) images.

Experimental Methods

Materials. All chemicals used in this study were purchased either from VWR International or Aldrich and used as received. Juvenile poplar (*Populus deltoides*) stems were harvested between 2007 and 2008 by National Renewable Energy Laboratory (NREL). *P. deltoides* x nigra (DN34) clones were grown for six months in a greenhouse on Sun-Gro Sunshine Mix #4 soil with Miracle Gro 20-20-20 formulation and fertilized every two weeks. The plants were grown on a flood table and watered four times a day automatically. The greenhouse maintained 16 h of light per day with 30–60% humidity. The entire poplar stems were cut and stored at –20 °C. The experimental procedure employed in this study is shown schematically in Figure 1.

Ground Poplar Stem. Fresh poplar stems were predried in vacuum oven at 40 °C overnight prior to milling. Dried poplar

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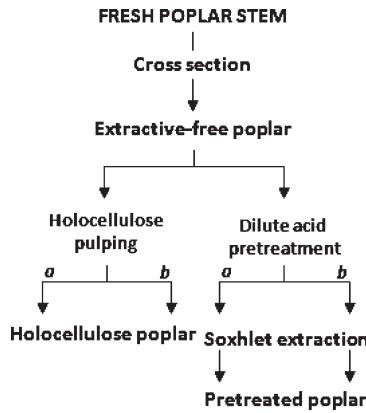


Figure 1. Flow diagram describing the sample preparation of poplar stem. (a) Regular experimental condition. (b) Severe experimental condition.

stems were ground using laboratory-scale Thomas-Wiley Mill machine and were simultaneously sieved with 20 mesh screen. The milled sample was stored at –20 °C for further treatment.

Cryotome Section of Poplar Stem. Cryotome section of poplar was accomplished by employing a slight modification of the literature method published by Tokareva et al.¹⁷ In brief, a piece of poplar stem less than 2 cm in diameter was sectioned to 50 μm thickness using a LEICA CM 3050S cryostat equipped with a disposable steel blade and embedding material (OCT compound, Tissue-TEK). Disposable steel blades were installed and used after removing the lubricant on the blade surface using dichloromethane and ethanol. To avoid any contamination from the embedding material, a piece of poplar stem was attached on the metal plate using a small amount of glue on the bottom edge instead of embedment. The chamber temperature for cryotome section was adjusted at –8 °C and cutting speed was manually controlled. Wood samples were cross sectioned to 50 μm thickness, which was the optimal thickness for pretreatment based on preliminary exploratory studies. The cross sections of poplar stem easily disintegrated during dilute acid pretreatment when the sectioned poplar was less than 30 μm thickness. In contrast, samples of +100 μm did not completely respond to DAP in a comparable manner to milled poplar. The cross sections of poplar stem were stored between glass slides at –20 °C in freezer to prevent rolling up and to maintain their native forms.

Extractive-Free, Holocellulose, and Cellulose Poplar Separation. Extractive-free samples of poplar were prepared according to TAPPI method T 204 cm-07.^{19,20} Extractives in both the cross sectioned and ground samples were removed by placing the samples (5.00 g of dry weight) into an extraction thimble in a Soxhlet apparatus. The extraction flask was filled with CH₂Cl₂ (300 mL) and then refluxed with boiling rate, which cycled the solvent 5 times/hour overnight. The extracted samples were then air-dried overnight. Holocellulose poplar was accomplished following literature protocols.^{21,22} The extractive-free samples were subjected to an oxidative treatment with NaClO₂ (1.30 g/g sample) in acetic acid (0.14 M, 375 mL) at 70 °C for 1 h in a sealed Kapak pack. This procedure was then repeated additional three times. For preparing a severe holocellulose pulped poplar, oxidative treatment under the same condition was repeated additional six times. Finally, the solid residue was

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filtered and washed thoroughly with deionized water. Cellulose-enriched poplar was acquired from the holocellulose samples (1.00 g of dry sample) by acid hydrolysis with 2.5 N HCl (100 mL) at 100 °C for 4 h according to a literature method.²³ The solid residue was filtered and washed with deionized water. Finally, the cellulose enriched sample was air-dried overnight and stored in desiccators over P₂O₅ prior to analysis.

Dilute Acid Pretreatment (DAP) and Severe DAP. Two kinds of dilute acid pretreated samples were prepared using different sulfuric acid concentration and temperature. For dilute acid pretreatment (DAP), both cross sectioned and ground extractive-free poplar (2.00 g dry weight) were presoaked in 1 vol % dilute sulfuric (v/v, 200 mL) acid solution for 4 h with stirring at room temperature.^{24,25} The presoaked samples were then filtered, washed with deionized water, and then transferred to a 4560 mini-Parr reactor (300 mL) and added to 1 vol. % dilute sulfuric acid solution (% dry solids content). The vessel was heated to 160 °C over ~30 min (at 5 °C/min). The reactor was held at 160 ± 2 °C (6.4 atm) for 10 min and then quenched in an ice bath. The pretreated samples were filtered and washed with deionized water and paramagnetic impurities were removed by washing the solids with a dilute aqueous solution of ethylenediaminetetraacetic acid (EDTA) and deionized water. The pre-treated samples were air-dried and subsequently extracted in a Soxhlet apparatus with CH₂Cl₂, and the extracted residue was then air-dried overnight. A severe dilute acid pretreatment (sDAP) was performed in the same manner as DAP procedure with 2 vol % dilute sulfuric acid solution at 175 °C for 10 min.

ToF-SIMS Analysis. Sample analysis was accomplished by employing cross sections of native and treated poplar. ToF-SIMS spectra and images were obtained by a PHI TRIFT III spectrometer (Physical Electronics Inc., U.S.A.) using a gallium liquid metal ion gun (LMIG, ⁶⁹Ga) as the primary ion source. The instrument was operated in positive mode (22 kV) with 600 pA of primary ion current. The instrument was operated in an ion microprobe mode in which the bunched and pulsed primary ion beam was rastered across the surface of sample. Three data points were acquired from the samples to reduce site specificity. A raster size of 200 × 200 μm was used for all data acquisition from the samples. ToF-SIMS image of individual components was obtained by mapping selected positive ions. MATLAB platform was used for mapping process from ToF-SIMS images. Only the selected pixels over middle brightness on SIMS image were filtered and transformed by MATLAB, version 7.2.

Carbohydrate and Acid-Insoluble Lignin (Klason Lignin) Analysis. Samples for carbohydrate constituents and acid-insoluble lignin (Klason lignin) analysis of both sectioned and ground poplar were prepared using a two-stage acid hydrolysis protocol based on TAPPI methods T-222 om-88 with a slight modification. The first stage utilizes a severe pH and a low reaction temperature (72 vol % H₂SO₄ at 30 °C for 1 h). The second stage is performed at much lower acid concentration and higher temperature (3 vol % H₂SO₄ at 121 °C for 1 h) in an autoclave. The resulting solution was cooled to room temperature and filtered using G8 glass fiber filter (Fisher Scientific, U.S.A.). The remaining residue which is considered as Klason lignin was oven-dried and weighed to obtain the Klason lignin content. The filtered solution was analyzed for carbohydrate constituents of the hydrolyzed poplar samples determined by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) using Dionex ICS-3000 (Dionex Corp., U.S.A.).²⁶ Error analysis was conducted by

performing carbohydrate and acid-insoluble lignin analysis at least three times on the sectioned and ground samples. The plotted data in Figure 3 represent the average and the error bars are one standard deviation.

Scanning Electron Microscopy. All cross-sectioned samples were mounted onto a stage and then coated with gold for 2 min by EM350 sputter. Images were acquired via a JEOL-1530 thermally-assisted field emission (TFE) scanning electron microscope (SEM) at 12 or 10 kV at various resolving powers.

Infrared Spectroscopy. IR spectra were measured on a Nicolet Magna 550 spectrometer with deuterated triglycine sulfate (DTGS) detector and OMNIC 6.0 software. Sixty-four scans at a resolution of 4 cm⁻¹ were averaged. Poplar samples (10 mg of dry weight) were uniformly ground and well mixed with KBr powder (500 mg packet, Thermo Electronic. Co., U.S.A.). The samples were then transferred to a pellet making die and FT-IR pellets were prepared applying ~11 Pa ram pressure (PHI Hydraulic laboratory press, U.S.A.) for 2 min under vacuum and FT-IR spectra was acquired under N₂.

Results and Discussion

Effects of Sample Preparation. Although the pretreatment of biomass, prior to enzymatic deconstruction, is typically accomplished on sawdust or milled wood for this study, we elected to examine cross sections of extractives-free and dilute acid pretreated poplar to facilitate ToF-SIMS analysis. To ensure that the 50-μm thick cross-sectioned poplar stem was undergoing comparable pretreatment chemistry as milled wood, 20-mesh ground poplar was also dilute acid pretreated and both materials were characterized for their sugars profiles and gross chemical constituents by FT-IR. The extractive-free, holocellulose, severe holocellulose, cellulose-enriched, and dilute acid pretreated poplar samples were prepared. The ground and cross-sectioned poplar from samples were analyzed by FT-IR in Figure 2. A major absorption band at 1740 cm⁻¹ was assigned to carboxylic-ester bond, which originates from acetyl groups of the noncellulosic fraction in the poplar cell wall.²⁷ This acetylated hemicellulose mainly exists as a form of 4-O-methyl-glucuronoxylan in poplar.²⁸ The absorption peaks around 1595 and 1510 cm⁻¹ were assigned to aromatic skeletal vibration from lignin. The absorbance at 1428 cm⁻¹ was also attributed to lignin, while other wavenumber ranges represented common absorption bands for example glycosidic linkages at 1150 cm⁻¹, β-(1→4) linkages at 890 cm⁻¹, and hydroxyl group at 3500 cm⁻¹.^{29,30} In ground poplar, acetylated hemicellulose absorbance at 1740 cm⁻¹ was observed in the extractive-free, holocellulose and severe holocellulose samples in Figure 2a. However, the hemicellulose absorbance peaks nearly disappeared in cellulose enriched and DAP poplar. This observation was consistent with carbohydrate composition of the ground samples, in which only trace amount of hemicellulose was detected in cellulose enriched and DAP poplar (Table 1). Two peaks corresponding to lignin (1510 and 1428 cm⁻¹) were clearly observed in extractive-free and DAP poplar, while an additional lignin peak (1595 cm⁻¹) was overlapping with the adjacent broad absorption (1650 cm⁻¹) originating from water. Moreover,

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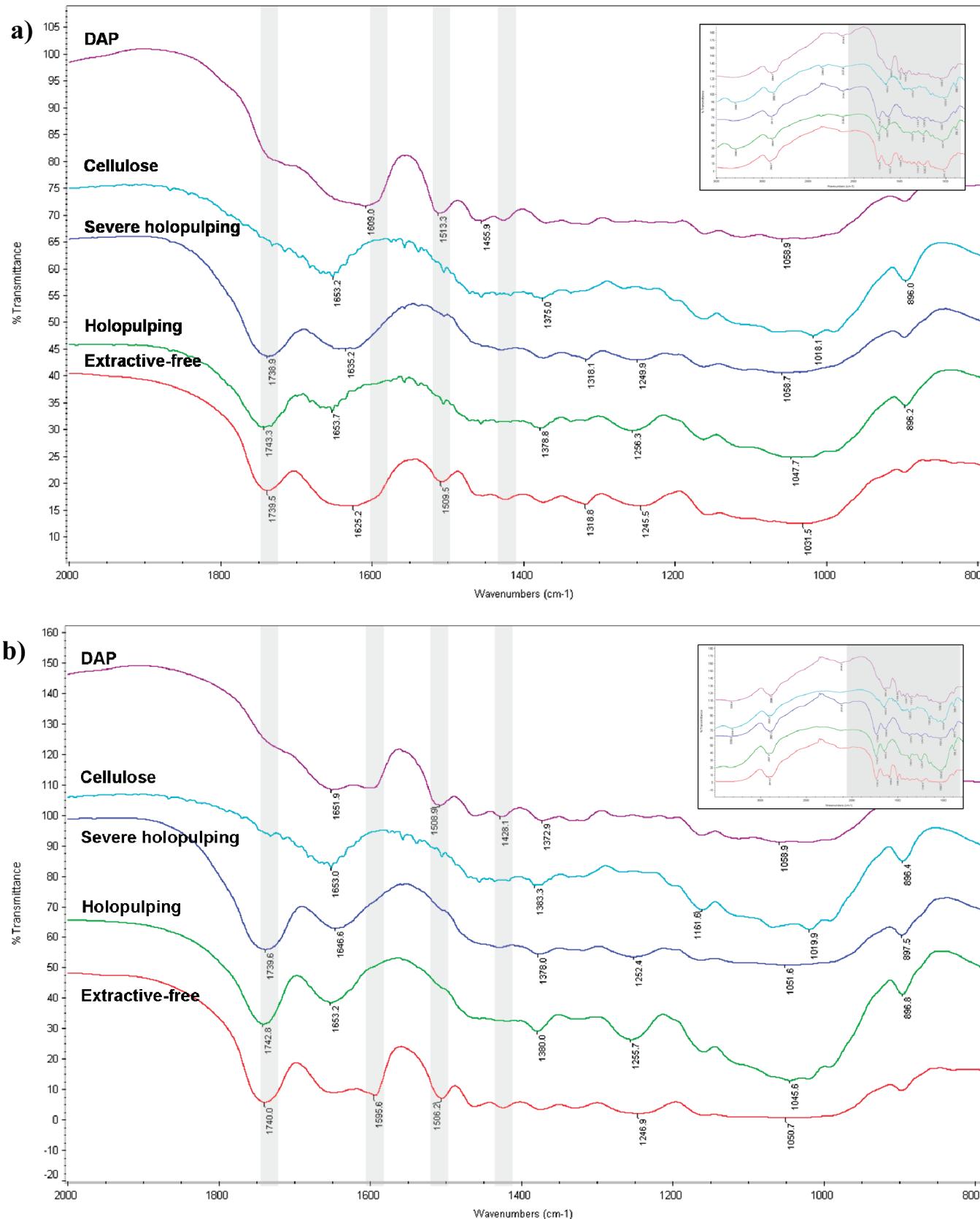


Figure 2. Part of FT-IR spectra of ground poplar (a) and sectioned poplar (b). Inner boxes represent full range spectra.

lignin absorption peaks were not observed in holocellulose and cellulose enriched poplar in Figure 2a. The spectra of cross-sectioned poplar presented identical spectral patterns with those of ground poplar in Figure 2b. Bulk carbohydrate

analysis of both ground and sectioned poplar exhibited nearly similar carbohydrate and lignin distribution values as summarized in Table 1. The results of these analyses suggest that under the DAP conditions ground

Table 1. Carbohydrate Compositions of Sectioned and Ground Poplar after Treatments

% ratio based on 100% ^a (STDEV) ^b	extractive-free poplar		holocellulose poplar		cellulose enriched poplar		DAP poplar	
	sectioned ^c	ground ^d	sectioned	ground	sectioned	ground	sectioned	ground
arabinose	0.6 (1.9)	1.7 (0.1)	< 1 (1.7)	0.9 (0.1)	< 1 (0.1)	< 1 (0.1)	< 1 (0.7)	< 1 (0.1)
galactose	2.0 (2.2)	2.3 (0.4)	1.3 (1.9)	1.6 (0.1)	< 1 (0.1)	< 1 (0.1)	< 1 (0.9)	< 1 (0.1)
glucose	72.9 (0.9)	71.7 (0.2)	73.3 (1.1)	72.2 (0.2)	96.6 (0.3)	96.9 (0.3)	98.4 (1.5)	98.0 (1.9)
xylene	20.2 (4.5)	21.1 (0.2)	21.5 (0.1)	22.2 (0.4)	1.4 (0.3)	1.3 (0.3)	1.0 (0.1)	0.6 (1.9)
mannose	4.4 (0.5)	3.1 (0.2)	3.6 (1.9)	3.1 (0.1)	1.9 (0.1)	1.8 (0.1)	< 1 (0.1)	< 1 (1.9)

^a Average of relative carbohydrate compositions based on total sugar units. ^b Standard deviation. ^c Cross sections of poplar stem (50 µm). ^d Ground poplar stem (20 mesh).

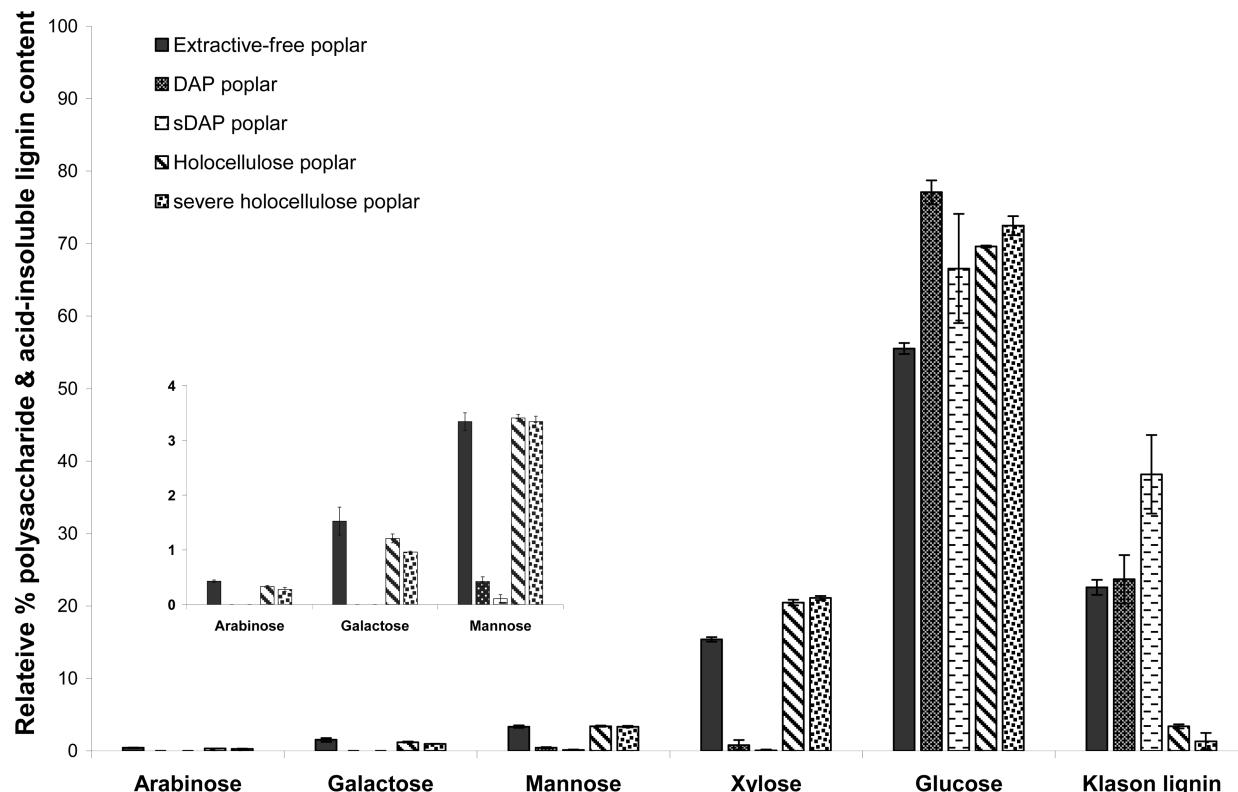


Figure 3. Carbohydrate and Klason lignin contents of cross sectioned poplar after treatment; Y scale is enlarged on inner graph to show the low contents of hemicellulose.

and cross-sectioned poplar are undergoing comparable chemical processes.

Carbohydrates and Klason Lignin Analysis. In an effort to quantitatively analyze the changes to the composition of the structural carbohydrates and lignin, which make up the bulk of the biomass samples, monosaccharide and Klason lignin analysis were performed on cross sections of poplar stems. An extractive-free sectioned poplar was used as a baseline material for comparison. Figure 3 summarizes the variation of bulk carbohydrate and Klason lignin distribution after various treatments. The majority of the hemicelluloses seen in the extractive-free poplar, characterized by the xylose, mannose, arabinose, and galactose contents, were hydrolyzed after DAP, while the relative glucose content increased by 40% after DAP. The relative Klason lignin content detected previously in extractive-free poplar slightly increased ~5% as a result of DAP. After severe dilute acid pretreatment (sDAP), the relative Klason lignin content was increased significantly by ~60%, while relative glucose content increased by ~20% as compared with that of extractive-free poplar. One possible explanation for this increase in Klason lignin content after sDAP is that with an increased

severity factor an increase in hydrolysis of cellulose and hemicellulose hydrolysis occurs, which would enrich the lignin component. In addition, at high temperatures and long residence times during pretreatment polysaccharides have been shown to undergo acid catalyzed dehydration to yield a lignin-like structure referred to as pseudolignin. This material is acid insoluble and therefore could contribute to the Klason lignin content.³¹ Li et al. estimated 25–40% of the residual lignin content in steam-exploded aspen wood (170 °C for 210 min) was actually pseudolignin. Holocellulose poplar was obtained from cross sections of extractive-free poplar. To further clarify the component distribution, especially for lignin at the poplar surface, an additional sample was obtained from a more severe holocellulose pulping process, which is defined here as severe holocellulose poplar. The relative Klason lignin content after holocellulose pulping process dramatically decreased over 85% as compared with that of extractive-free poplar. Interestingly, the severe holocellulose poplar still contained 1.5% Klason lignin,

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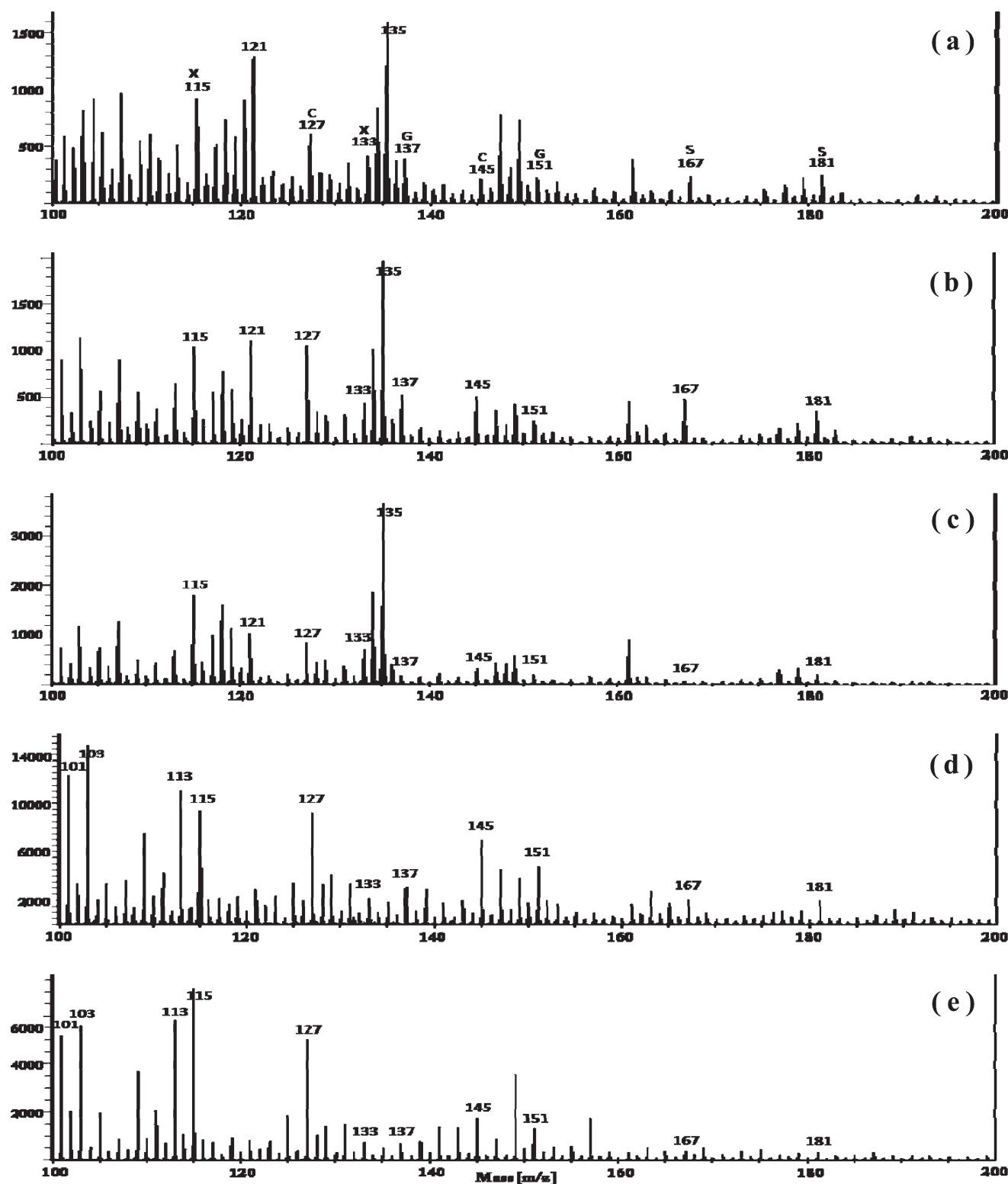


Figure 4. Part of positive ion ToF-SIMS spectra of cross sectioned poplar: Syringyl-lignin unit (S), guaiacyl-lignin unit (G), cellulose (C), xylan (X); extractive-free poplar (a), DAP poplar (b), regular holocellulose pulping poplar (c), sDAP poplar (d), severe holocellulose pulping poplar (e).

which was approximately half that of the holocellulose poplar.

Surface Analysis of Dilute Acid Pretreated Poplar. To investigate how the chemical constituents of the cell wall change during pretreatment, the cross sections of native and DAP poplar were analyzed by ToF-SIMS. Figure 4 shows positive ion mass spectra (m/z 100–200) obtained from

various treated poplar by ToF-SIMS. Characteristic ToF-SIMS ion fragments of the major components in poplar were identified according to published literature values.^{14,18,32,33}

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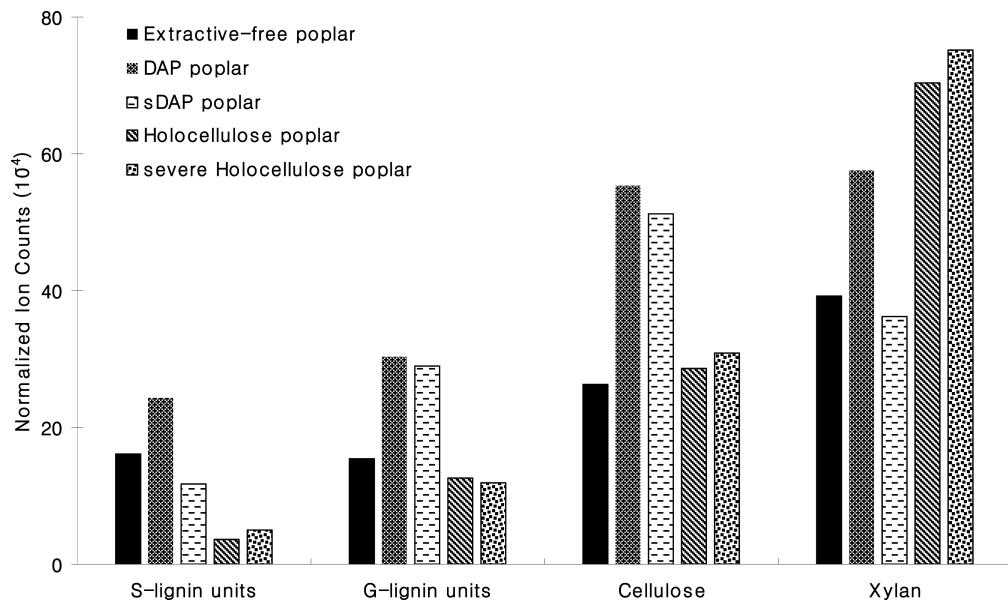


Figure 5. Relative intensities of each component in cross sections of different treated poplar by ToF-SIMS.

Cellulose yields primary fragments of m/z 127 ($C_6H_7O_3^+$) and 145 ($C_6H_9O_4^+$), while xylan (major hemicellulose component) fragmented to m/z 115 ($C_5H_7O_3^+$) and 133 ($C_5H_9O_4^+$).^{14,18} Depolymerized fragments of lignin were also assigned employing the results by Saito et al. The signals at m/z 167 ($C_9H_{11}O_3^+$) and 181 ($C_9H_9O_4^+$) were assigned to syringyl (S) lignin units and m/z 137 ($C_8H_9O_2^+$) and 151 ($C_8H_7O_3^+$) to guaiacyl (G) lignin units.³²

All characteristic fragment ions of cellulose, hemicellulose, and lignin were detected with various intensities depending on the treatment conditions as illustrated in Figure 4. Although the bulk carbohydrate and Klason lignin analysis indicated a 95% drop in the relative amount of hemicellulose sugars after DAP, ToF-SIMS analysis detected the considerable presence of xylan fragments on the surface of the dilute acid pretreated poplar as shown in Figure 4b. Also the peak intensities of xylan did not change significantly even after severe dilute acid pretreatment (sDAP) as shown in Figure 4d. Ion fragments of G-lignin units in holocellulose poplar were still observed, while only trace levels of S-lignin units were detected in Figure 4c. A similar pattern of G and S-lignin units was detected even in severe holocellulose poplar (Figure 4e), which were in contrast to the results of bulk carbohydrate and Klason lignin analysis.

To observe quantitative cell wall component change on the surface of poplar, normalization of peak intensity from ToF-SIMS is required. Since secondary ion yields vary on the basis of chemical environment, the direct comparison of the absolute ion counts can not be employed to obtain quantitative information in SIMS.¹⁵ Therefore ion count normalization following the procedures described by Kleen was performed prior to comparison of relative intensities.^{34,35} The relative intensity of each species was calculated as the sum of its primary fragments in Figure 5.³⁴ The relative intensity of S-lignin units drama-

tically decreased on the surface of holocellulose poplar as compared with that of extractive-free poplar, but the intensity of G-lignin units only slightly decreased. However, the relative intensity of xylan increased approximately 2-fold, whereas the cellulose signal did not change significantly on the surface of holopulped poplar. Interestingly, after the severe holocellulose pulping treatment, the relative intensities of the major cell wall components did not change compared with those of regular holocellulose poplar, showing almost identical normalized ion counts. This result was contrary to bulk carbohydrate and Klason lignin analysis which indicated a ~50% reduction of relative lignin content after severe holocellulose pulping. ToF-SIMS alone would suggest that only a small proportion of the surface lignin is removed by the holopulping process. The observed increases in intensities of the characteristic cell wall components after DAP when compared with extractive-free poplar may result from two factors: matrix-effects or further exposure of characteristic components to the surface. Since DAP can break down the lignin-carbohydrate complex and disrupt the crystalline structure of cellulose, deformation of plant cell walls should be addressed.³⁶ This change in surface morphology and chemistry may permit higher yields of secondary ion fragments from the biomass surface. The relative intensities of cellulose and G-lignin units after DAP (1% H_2SO_4 at 160 °C for 10 min) were doubled as compared to those of extractive-free poplar. This most likely suggests more cellulose was exposed to the surface, which can be partially supported by bulk carbohydrate and Klason lignin analysis. The relative glucose content in bulk carbohydrate analysis was doubled after DAP as compared to extractive-free poplar while almost all hemicelluloses were removed upon DAP. Interestingly, the relative intensity of xylan after DAP increased by 30%, while the intensity of xylan after sDAP slightly decreased as compared to extractive-free poplar. This result suggests that DAP also

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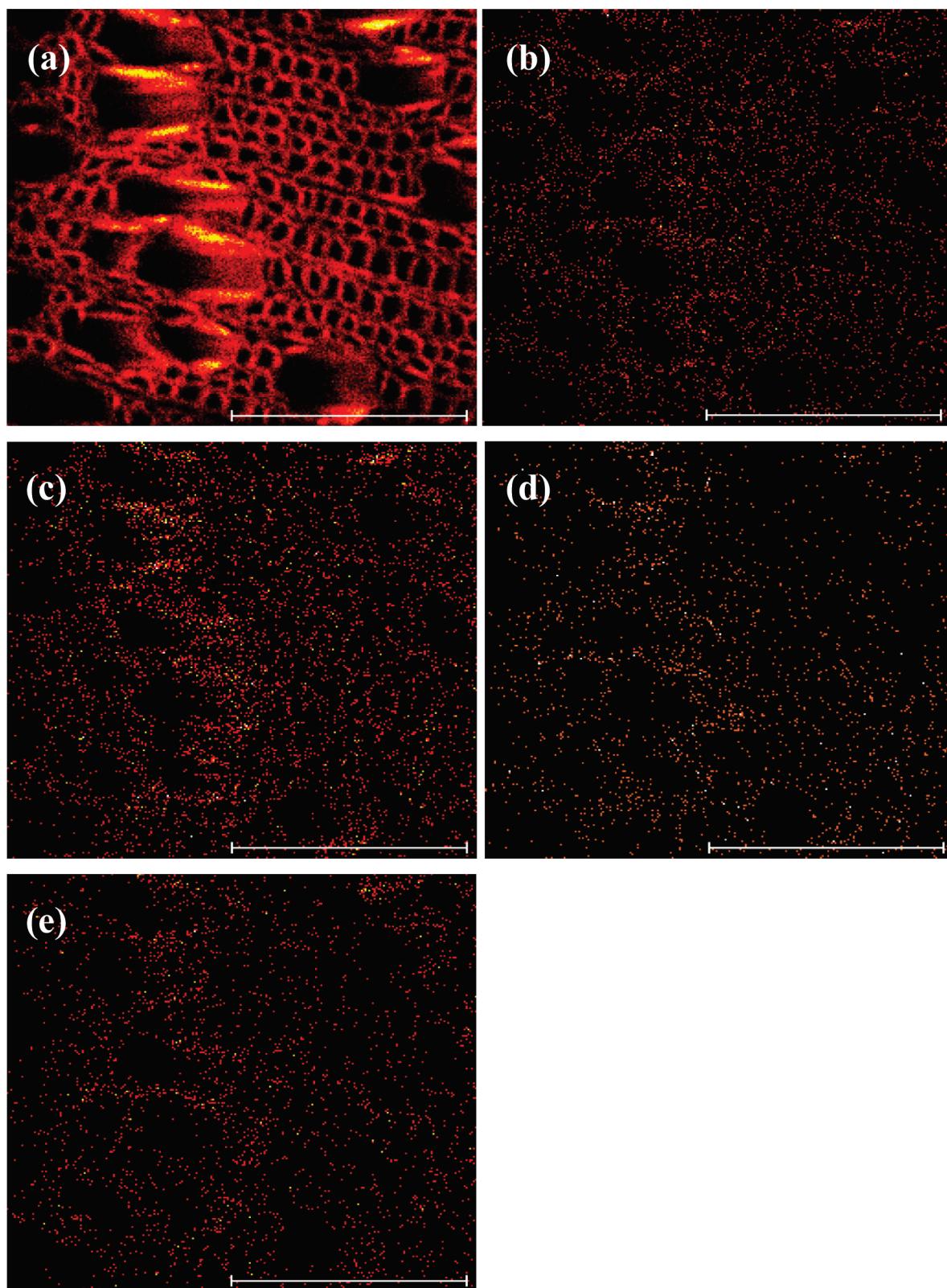


Figure 6. ToF-SIMS images of cross sections of DAP poplar: total ion image (a), cellulose ion image m/z 127 and 145 (b), xylan image m/z 115 and 133 (c), S-lignin units image m/z 167 and 181 (d), and G-lignin units m/z 137 and 151 (e) were taken using raster size of $200 \mu\text{m} \times 200 \mu\text{m}$ in positive mode. Scale bar = $100 \mu\text{m}$.

causes migration of xylan fragments to the surface of sectioned poplar prior to solubilization of xylan due to acid hydrolysis. As additional evidence of this migration, the severe pretreatment condition (2% H_2SO_4 at 175°C for

10 min) seems to release xylan from the biomass which has accumulated on the surface of the poplar during the pretreatment. Brunecky et al. observed similar migration of xylan in DAP corn stover by confocal laser microscope

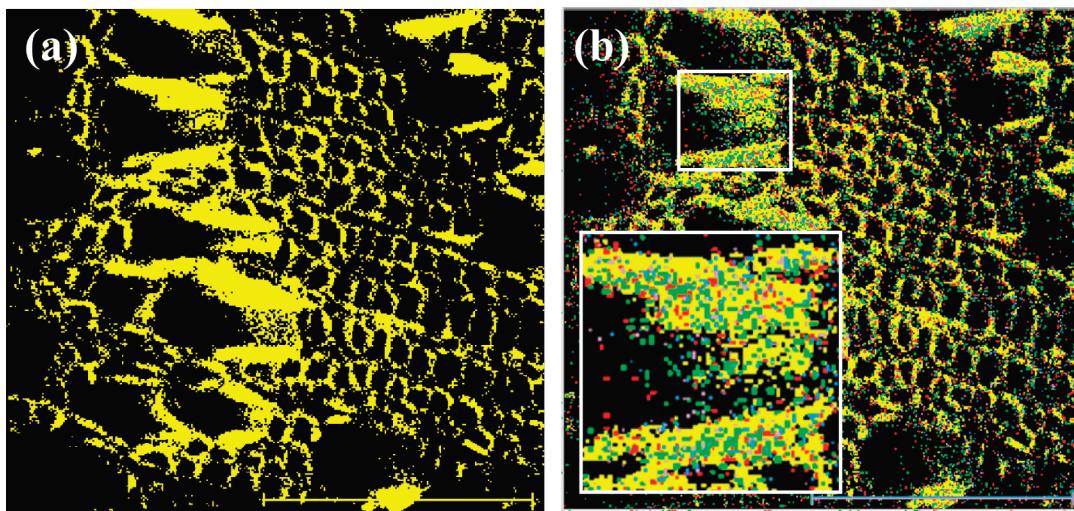


Figure 7. Images transformed by MATLAB platform using DAP ToF-SIMS images: total ion image (yellow) (a); cellulose (red), xylan (green), G and S-lignin units (blue and pale purple) (b). The white box was enlarged at selected area. Scale bar = 100 μm .

using fluorescent labeled antibody. They detected decreasing xylan signal as a function of increased DAP severity and suggested the migration of xylan from the central cell wall to the lumen and middle lamella during DAP.³⁷ Notably, the intensities of S-lignin units after sDAP dramatically decreased as well as xylan, whereas the intensities of cellulose and G-lignin units remained relatively similar to those of dilute acid pretreated poplar. This may indicate that S-lignin units are more reactive than G-lignin units and therefore easily removed upon pretreatment.

ToF-SIMS images were used to determine the spatial distribution of cellulose, xylan and lignin after DAP. Figure 6 shows ion count images of characteristic cell wall species on the surface of dilute acid pretreated poplar. Figure 6a represents a total ion image and the other images represent the integrated intensities of selected mass fragments as a function of pixel position (Figure 6b–e). Brighter colors on ToF-SIMS images correspond to higher intensities of the indicated species. According to Figure 6, the characteristic ions of the major components (cellulose, xylan, and G- and S-lignin units) were observed across the cell wall while the lumen were shown as black color on the surface of cross-sectioned poplar. The high number of low intensity signals often makes it difficult to identify the spatial distribution of a particular species in areas having overlapping ion fragments for various species and to determine how they relate to each other. To better understand where the characteristic ions are spatially located, ToF-SIMS images of dilute acid pretreated poplar were transformed by a MATLAB platform based on the pixel brightness in Figure 7. The transformed images only represent intense signals, as the MATLAB platform filters less intense spots from the original image, resulting in a visually clearer image with distinct areas of intensity. Overlaying the transformed images of individual cell wall components with one another or with the image of total ion count can permit the comparison of localized areas on cell wall surface. The image in the center area of Figure 7a can easily be cross-

correlated with the ToF-SIMS image in Figure 6a. Transformed images of all characteristic components were superimposed over the total ion count image as seen in Figure 7b. Red dots represent the localized positions of intense cellulose fragments, while xylan is shown as green dots. ToF-SIMS images of G- and S-lignin units were also transformed in the same manner, and depicted as blue and pale purple dots. Notably green dots, representing xylan, appeared more frequently and more widely dispersed along the cell wall than cellulose in Figure 7b even though both species exhibited similar levels of relative intensities in Figure 5. A potential factor contributing to the random appearance of xylan fragments could be due to hemicellulose reprecipitation during DAP. Another explanation is that a part of xylan may localized in the inner cell wall layer which was exposed on the surface as the cell wall morphology was changed during DAP.³⁸ Scanning electron microscopy (SEM) images partially supported the latter as morphological changes in the cell wall before and after DAP were observed in Figure 8. The middle lamella (arrowhead) was observed in the central area of the cell wall and the thick layers adjacent to the middle lamella include multilayer secondary cell wall as seen in Figure 8a.^{39,40} After DAP, the inner layers of the cell wall (i.e., part of secondary cell wall) were detached from the central area (i.e., middle lamella) and the detached inner layers became widely dispersed as seen in Figure 8c. Interestingly, the detached inner layer was not observed after sDAP in Figure 8d. The thin layer in Figure 8d could be the middle lamellar and the remaining secondary cell wall because border lines were observed at both side of thin layer (arrowhead). This observation also further supports the existence of xylan located preferentially in the inner cell wall layer, as reflected in the relative intensities of xylan after DAP and sDAP in Figure 5. Essentially, the relative intensity of xylan after sDAP dramatically decreased

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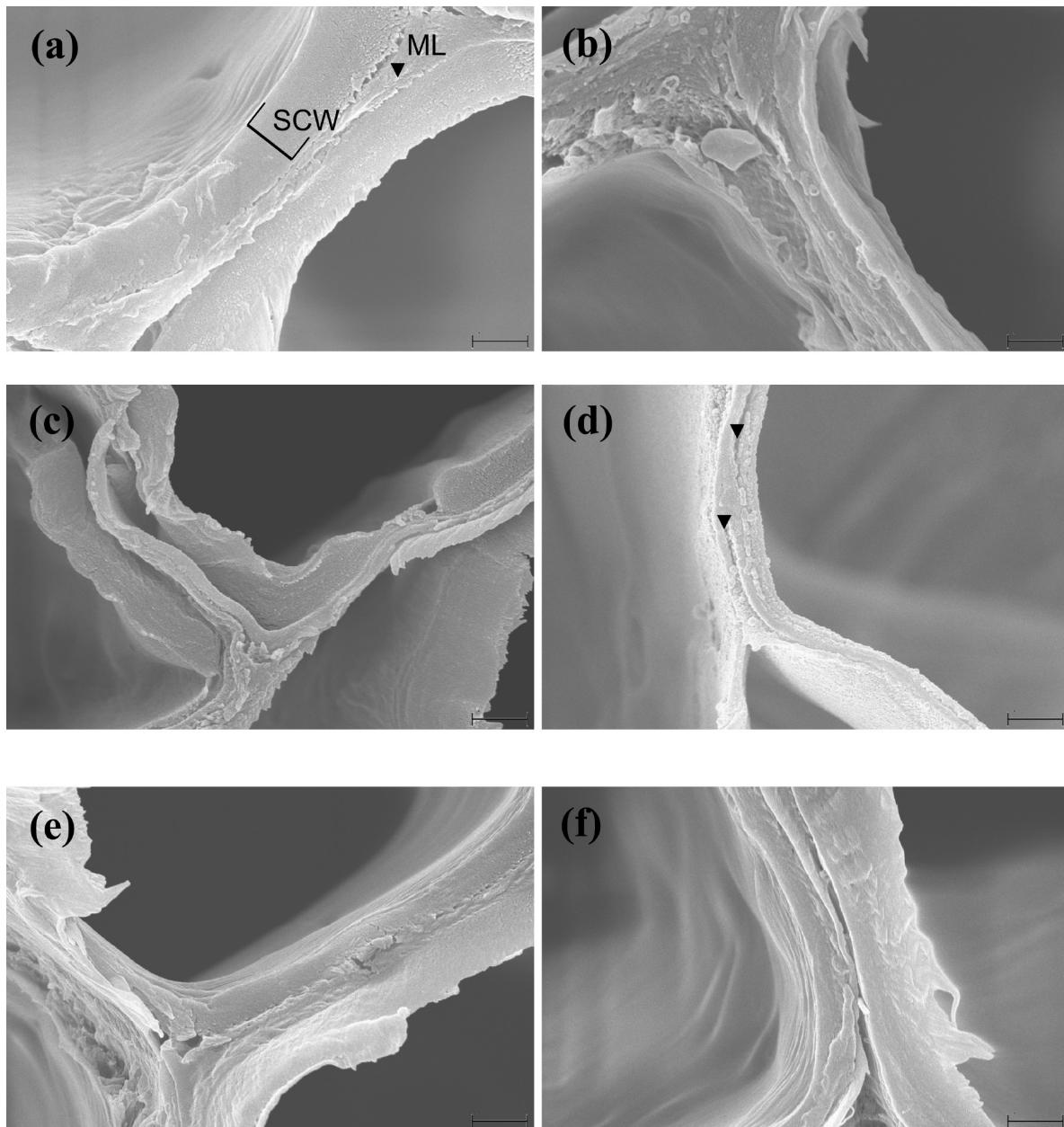


Figure 8. Electron micrograph of cross-sectioned poplar: untreated poplar (a), extractive-free poplar (b), DAP poplar (c), sDAP poplar (d), holocellulose poplar (e), and severe holocellulose poplar (f). ML—middle lamella, SCW—secondary cell wall, Scale bar = 1 μ m.

compared to that of DAP, but the intensity of cellulose did not change between DAP and sDAP in Figure 5. However, holocellulose pulping process did not affect the cell wall thickness much as shown in Figure 8e–f. The thickness of cell wall even after holocellulose pulping treatment was similar to that of untreated poplar and cell wall detachment was not observed.

Conclusions

The present work obtained a cryotome section of dilute acid pretreated and untreated poplar stem for the surface analysis. Both ground and cross-sectioned poplar underwent comparable chemical processes as a result of dilute acid pretreatment (DAP) as determined by FT-IR and carbohydrate analysis. Cross sections of dilute acid pretreated poplar stem were then analyzed for surface characteristics using ToF-SIMS and

SEM. The change of major cell wall components after DAP was semiquantitatively analyzed using the relative intensities obtained from ToF-SIMS. The relative content of xylan after DAP increased by 30% on the surface of the poplar stem by ToF-SIMS, while bulk carbohydrate analysis showed that the relative concentration of xylose decreased 10 times in comparison with untreated poplar wood. After severe dilute acid pretreatment (sDAP), the relative intensity of xylan by ToF-SIMS dramatically decreased and only thin cell wall layer was observed in SEM image. On the other hand, relative intensity of cellulose did not change after sDAP compared to DAP. Transformed image by MATLAB was introduced to investigate the spatial location of the major cell wall components. The results show the possibility of surface characterization on the native and treated stem by ToF-SIMS analysis.

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Nomenclature

DAP = dilute acid pretreatment

sDAP = dilute acid pretreatment under severe condition

S-lignin = syringyl lignin

G-lignin = guaiacyl lignin

ML = middle lamella

SCW = secondary cell wall