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Topochemistry of the Delignification of Japanese Beech (*Fagus crenata*) Wood by Supercritical Methanol Treatment

Masatsugu Takada,* Eiji Minami, and Haruo Kawamoto

Cite This: *ACS Omega* 2021, 6, 20924−20930

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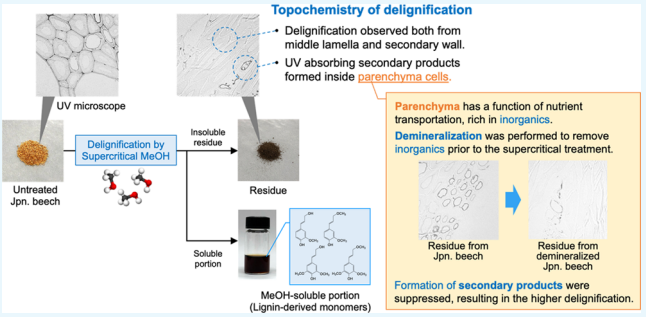
ABSTRACT: The topochemistry of Japanese beech (*Fagus crenata*) wood delignification was evaluated in this study following a supercritical methanol treatment (270 °C, 27 MPa). Ultraviolet microscopic analysis of the insoluble residue revealed that the lignin in the secondary wall was easily decomposed and removed because of the preferential cleavage of ether-type linkages. In contrast, the middle lamella lignin was initially resistant to supercritical methanol but eventually decomposed and was removed. In addition, UV-absorbing secondary products formed selectively inside the parenchyma cells. Results from the supercritical methanol treatment of demineralized beech wood indicated that inorganic substances in the lumen of parenchyma affected the formation of these secondary products, thus leading to an overestimation of the residual lignin. Therefore, the topochemistry of delignification was more precisely evaluated when using demineralized beech wood.



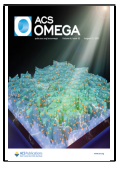
INTRODUCTION

Lignin is the second most abundant polymers in terrestrial plants, after cellulose, and it represents a potential source of energy and value-added chemicals. There are several degradation methods that enable further lignin utilization, including super- and subcritical fluid technologies, which comprise promising strategies for converting lignocellulosic biomass into chemicals. Under super- and subcritical conditions, the properties of a fluid (e.g., ionic products and dielectric constant), which are important parameters in a reaction, can be controlled by changing the temperature and pressure. For example, in supercritical water, the dielectric constant decreases and the ionic products increase at higher severity.[1,2] Lower dielectric constants can enhance the solubility of hydrophobic compounds in super- and subcritical water, which can promote lignin decomposition.[3−6] For example, when Japanese beech (*Fagus crenata*) was treated with subcritical water (230−270 °C) in a semiflow reactor, approximately 80% of the lignin was decomposed and removed.[7]

The super- and subcritical treatment of lignocellulosics had been investigated in a variety of solvents. Mishra treated Japanese beech with phenolic reagents, including phenol, catechol, and *m*-cresol, under super- and subcritical conditions and observed high delignification.[8] Following subcritical treatment with phenol (270 °C, 12 MPa), lignin was removed selectively, leaving most of the cellulose and hemicellulose. Yamazaki et al. treated Japanese beech with several straight-chain alcohols under supercritical conditions, and they determined that supercritical methanol achieved the highest delignification.[9] Regarding the methanol-soluble portion, when Japanese cedar and beech was treated by supercritical methanol (270 °C/27 MPa and 350 °C/43 MPa), lignin precursor compounds, such as coniferyl alcohol and γ-methoxy-coniferyl alcohol (produced via methanolysis), were retrieved.[10] These monomers were obtained in high yields, suggesting that supercritical methanol treatment is a promising method for lignin utilization and valorization.

From a topochemical perspective, lignin is not uniformly distributed within the wood cell wall, and its concentration, phenylpropane units, and interlinkages differ from one morphological region to another.[11−13] As a result, delignification does not occur uniformly during some pulping processes.[12,14] Our group previously used microscopy to study the topochemical changes during the delignification of Japanese beech in subcritical water and phenol.[15] In subcritical water, delignification occurred preferentially in the secondary wall (SW) fibers, relative to the middle lamella (ML) portion; the lignin in the ML demonstrated strong resistance to subcritical water. Following subcritical phenol treatment,

Received: May 5, 2021
Accepted: June 30, 2021
Published: August 2, 2021



preferential delignification from the SW was still observed; however, the ML portion also experienced significant delignification after 30 min of treatment. To our knowledge, the topochemistry of Japanese beech wood delignification during super- and subcritical methanol treatments has not yet been elucidated.

In this study, ultraviolet (UV) microscopy methods were applied to study the topochemistry of Japanese beech delignification following treatment with supercritical methanol (270 °C, 27 MPa, 30 min). It is well established that inorganic species influence the pyrolysis of biomass and cell wall components.[16−19] Therefore, the wood flour was demineralized by washing with an acid solution, and the demineralized wood flour was treated with supercritical methanol to evaluate the impact of inorganic species on the delignification process.

MATERIALS AND METHODS

Samples and Chemicals. Japanese beech sapwood flour, which was harvested at Kyoto and milled using a Wiley mill, was passed through an 18-mesh screen and then extracted with acetone using a Soxhlet apparatus for 8 h. The obtained material was dried at 105 °C for 24 h before the experiments. All chemicals used in this study were of reagent grade, purchased from Nacalai Tesque, Inc. (Kyoto, Japan), and used as received, without further purification.

Supercritical Methanol Treatment and Analysis of Insoluble Residues. Approximately 4.9 mL of methanol and 150 mg of extractive-free beech wood flour were placed in a 5.0 mL reaction vessel, made of Inconel-625. The vessel was sealed and immersed in a preheated molten salt bath at 270 °C. After an adequate reaction time from 1 to 30 min, the reaction was rapidly quenched by placing the reaction vessel in a water bath. The resulting reaction mixture was filtered through a 0.45 μm membrane filter to isolate a methanol-insoluble residue and a methanol-soluble portion, both of which were oven-dried at 105 °C for 12 h to a constant weight.

The lignin-derived products in each fraction were examined as the combined yields of Klason lignin and acid-soluble lignin. The acid-soluble portion was analyzed using high-performance anion exchange chromatography for monosaccharide analysis. The cellulose and hemicellulose contents were calculated according to the quantities of glucose and other monosaccharides, respectively.

Scanning electron microscopy (SEM; SU-6600, Hitachi High-Technologies Corporation, Tokyo, Japan) was used to evaluate the surface structure of the insoluble residue. Samples were freeze-dried before being placed on a SEM stub and then gold-coated using a sputtering system. SEM analysis was performed using an accelerating voltage of 5 kV.

The distribution of residual lignin was observed using UV microscopy. The insoluble residues were embedded in an epoxy resin, and the embedded samples were cut into sections (thickness = 0.5 μm) with a diamond knife mounted on a Leica Reichert Supernova Microtome (Buffalo Grove, IL). The cut samples were placed on quartz slides, mounted with glycerin, and covered with a quartz coverslip before being analyzed with an MSP-800 microscope spectrometer (Carl Zeiss, Oberkochem, Germany) at 280 ± 5 nm. The morphological regions of the samples were analyzed via UV microspectrophotometry (UMSP) based on photometric point-by-point measurements (spot size = 1 × 1 μm^2). The changes in UV absorbance at 240 nm were recorded, and the measurements were repeated at least five times on each morphological region.

Demineralization of Japanese Beech Wood. To remove any inorganic species, the extractive-free Japanese beech wood flour was demineralized with 0.05 M HCl for 1 h at room temperature. The resulting mixtures were filtered with a membrane filter and washed with an excessive amount of water to obtain the demineralized flour. During the demineralization process, the chemical composition did not change, except for the fact that the inorganic content decreased from 0.6 to 0.05%. The content of inorganics was determined after incineration of the oven-dried samples at 600 °C for 4 h. The distribution of inorganic components was investigated using SEM with energy-dispersive X-ray spectroscopy (SEM-EDX; XFlash 5010, Bruker, Billerica, MA). The demineralized wood flour was treated in supercritical methanol, and the obtained insoluble residues were analyzed, as described above, via chemical composition determinations and UV microscopic observations.

RESULTS AND DISCUSSION

Delignification in Supercritical Methanol. The yields and chemical composition of the insoluble residues obtained throughout the supercritical methanol treatment (270 °C, 27 MPa) of Japanese beech wood flour are presented in Figure 1.

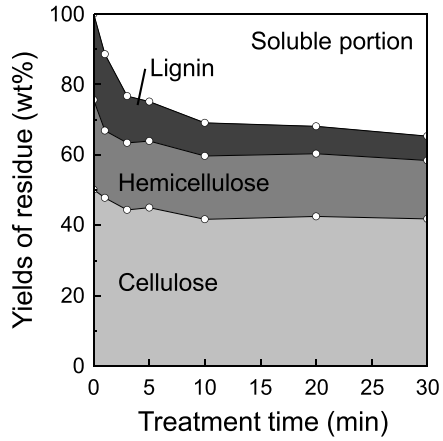


Figure 1. Yields of insoluble residues from Japanese beech wood treated with supercritical methanol (270 °C, 27 MPa).

Considering that the critical point of methanol occurs at 239 °C/8.1 MPa, the methanol medium in this study existed in its supercritical condition. As a result, the lignin gradually decomposed and eluted into the methanol such that, ultimately, more than 70% of the lignin had eluted into the methanol after 30 min. The hemicellulose partially decomposed in the first 5 min, but generally remained as an insoluble residue. The cellulose remained completely as an insoluble residue, indicating that the crystalline structure of cellulose is highly resistant to supercritical methanol under the applied conditions (270 °C, 27 MPa). Similar high resistance has been observed for crystalline cellulose in other sub- and supercritical solvents, such as subcritical water and phenol.[15]

Scanning Electron Microscopy Analysis of the Insoluble Residue. SEM was used to observe the surface morphology of the fibers in the insoluble residue after 30 min of treatment (Figure 2). The images revealed that the ML was peeled and removed, and the SW layers were exposed relative

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ACS Omega 2021, 6, 20924−20930

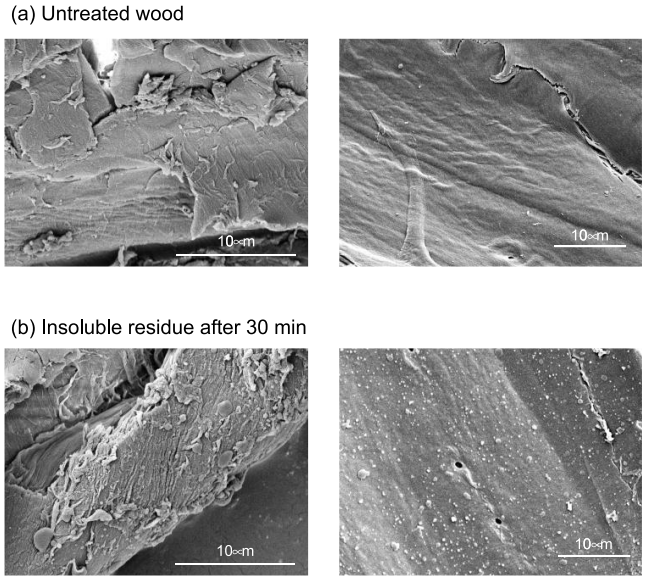

Figure 2. SEM micrographs of (a) untreated wood and (b) insoluble residue after the supercritical methanol treatment (270 °C, 27 MPa, 30 min).

to those in the untreated beech wood. In addition, numerous droplets formed on the surface of fiber lumens after the supercritical treatment. These droplets may have comprised lignin-derived compounds because we previously observed such droplets on the fiber surface that originated from the relocation of lignin after a steam pretreatment.[20] The extremely hydrophobic lignin-derived compounds, which could be dissolved in methanol under supercritical conditions, would reform as droplets on the surface of the cell wall, thus minimizing its surface area in an ambient methanol environment.

Ultraviolet Microscopy of the Insoluble Residue. UV micrographs of the insoluble residues are presented in Figure 3a. The UV absorbances corresponding to the SW of the fiber and vessel decreased as the treatment progressed, indicating that a significant delignification process was occurring. In contrast, the lignin in the ML portion remained after 3 min, exhibiting 45.5% delignification. After 10 min of treatment, the delignification reached 61.5%, and most of the lignin in the ML was eluted; in contrast, the lignin at the cell corners of the middle lamella (ML_{CC}) remained at this point but was ultimately eluted after 30 min of treatment. Furthermore, there were numerous substances inside the parenchyma cells that absorbed strongly in the UV region, and these are discussed in detail later in the text.

The changes in the UV spectra of each morphological region are shown in Figure 3b. In all morphological regions of the fiber SW, vessel SW, and ML_{CC}, the UV absorbances decreased after 30 min, indicating that delignification occurred in all of these locations.

It is well known that lignin of hardwood is composed of both guaiacyl (G) and syringyl (S) propane units, and the ratio of these units varies from one morphological region to another.[13] The UV absorption coefficients of G and S lignin are different at 280 nm but relatively similar at 240 nm.[21,22] Therefore, the changes in UV absorbance at 240 nm were measured, as shown in Figure 3c. Assuming that the sample sections had the same thickness, the UV absorbances were proportional to the concentration of lignin, according to the Beer−Lambert law. Considering that the distribution of G and S lignin in beech wood is not uniform within an annual ring,[23] the latewood terminal zone was eliminated in this analysis.

The relative UV absorbance in the SW decreased much earlier than that in the ML_{CC}, indicating that the delignification treatment preferentially targeted the lignin in the SW, especially in the SW fibers. A similar tendency was previously

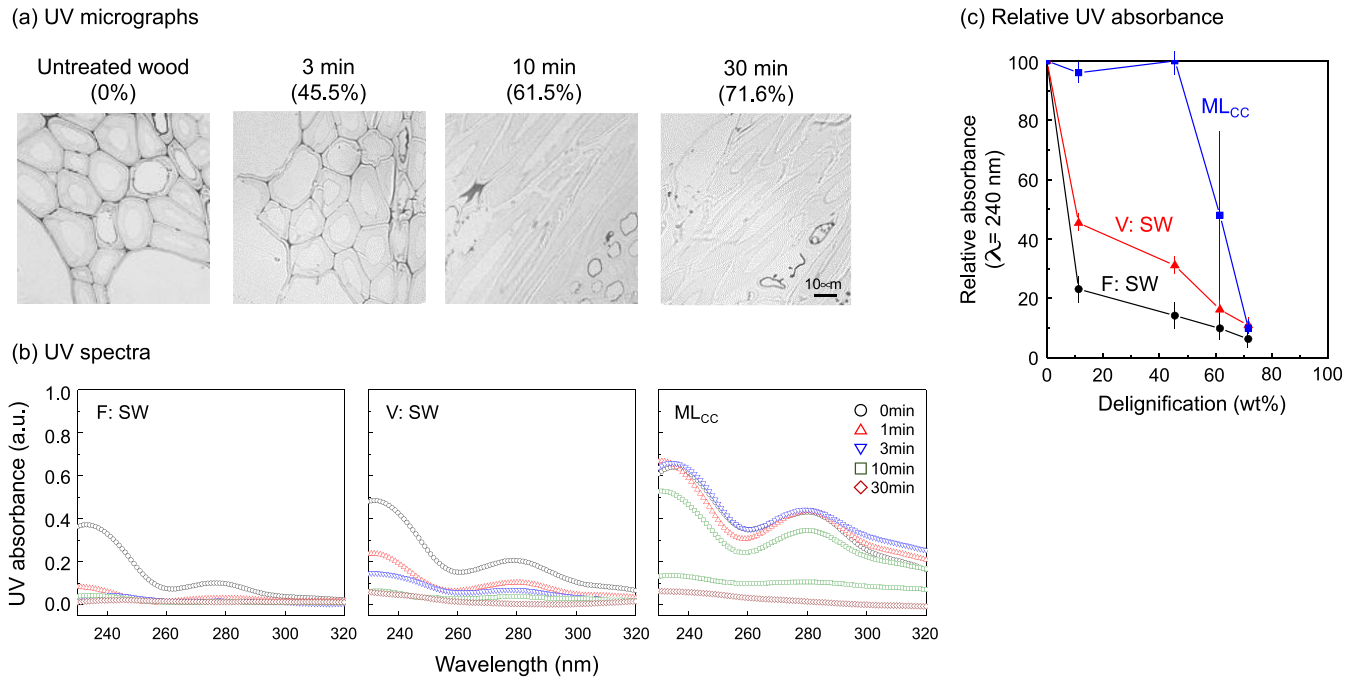

Figure 3. (a) UV micrographs of the insoluble residues from Japanese beech wood treated with supercritical methanol (270 °C, 27 MPa) at 280 nm, (b) UV absorbance spectra of each morphological region, and (c) relative UV absorbance of the three morphological regions of Japanese beech wood samples treated with supercritical methanol (270 °C, 27 MPa) at 240 nm.

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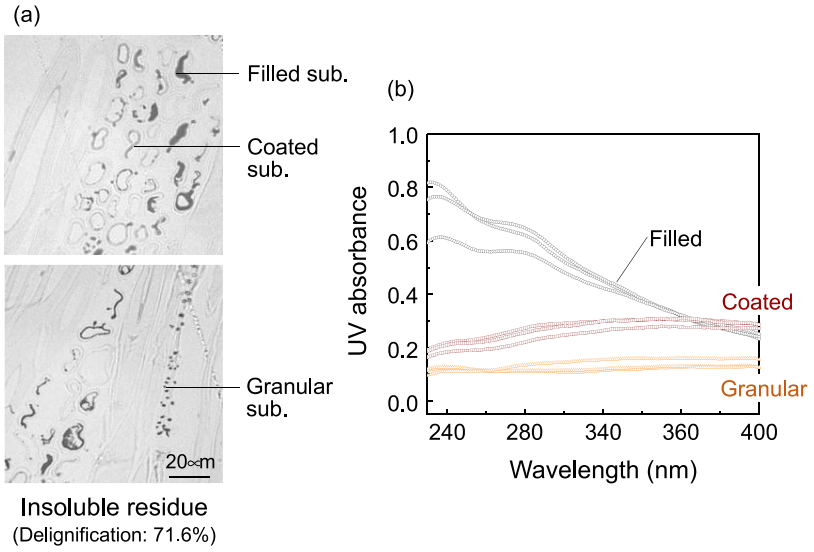

Figure 4. (a) UV micrographs of parenchyma cells and fibers in the insoluble residue from Japanese beech wood treated with supercritical methanol (270 °C, 27 MPa, 30 min) at 280 nm and (b) UV spectra of filled, coated, and granular substances.

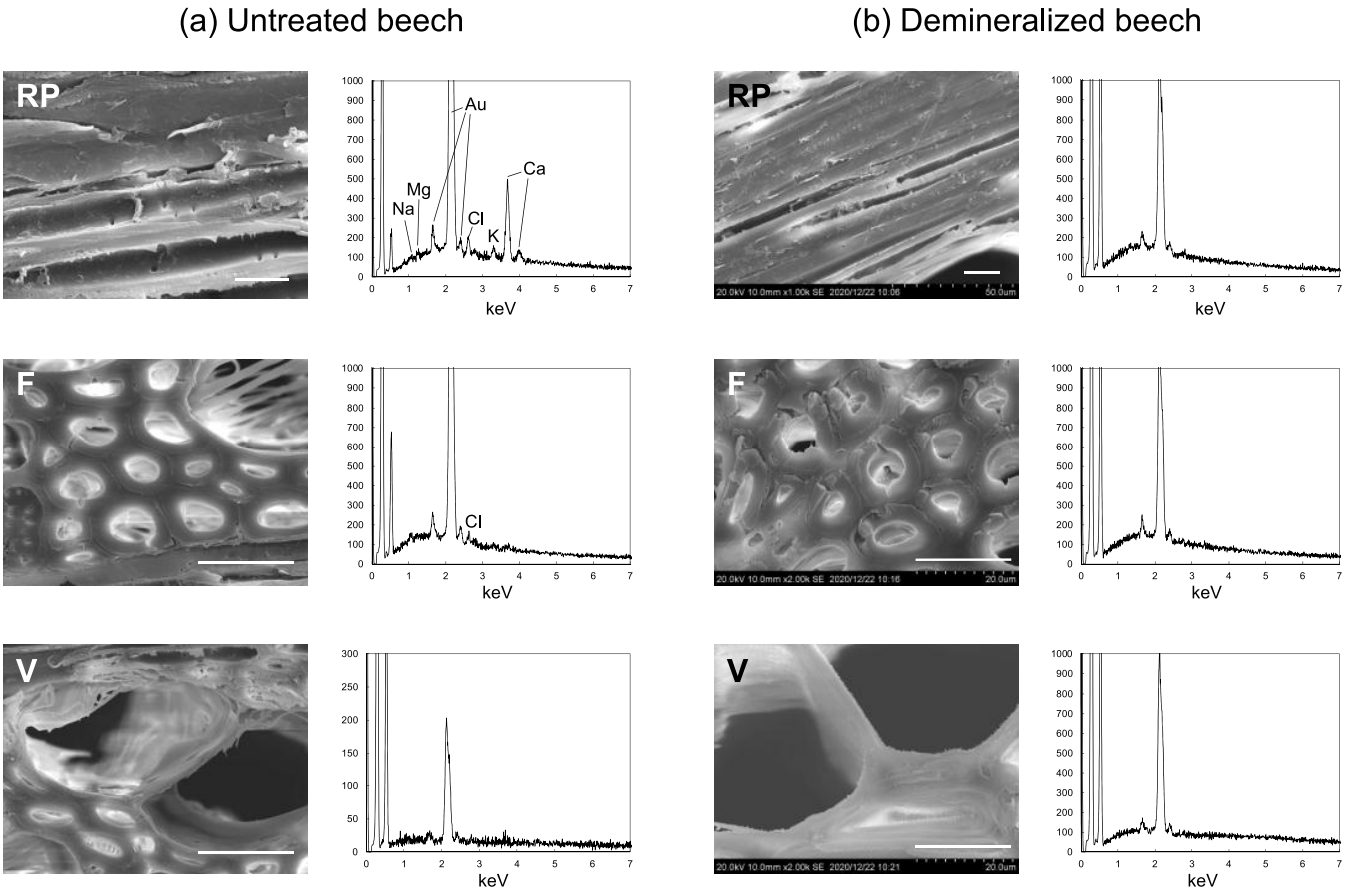

Figure 5. SEM micrographs and SEM-EDX spectra of each morphological region in (a) untreated beech and (b) demineralized beech wood. The scale bar corresponds to 20 μm. RP = ray parenchyma; F = fiber; V: vessel.

observed in the delignification processes during treatment with either subcritical water or phenol.[15] In the previous study, it was apparent that during the supercritical methanol treatment, ether-type linkages could be cleaved more easily than condensed-type linkages following the alkaline nitrobenzene oxidation of the insoluble residues.[10] Considering that the SW lignin was rich in ether-type linkages and the ML lignin primarily comprised a condensed-type structure, the ether-type lignin in SW would be cleaved and delignified preferentially, while the condensed-type lignin in ML would be resistant to supercritical methanol. Nevertheless, the residual ML_{CC} lignin was removed after 30 min of treatment; the removal of ML lignin was consistent with the SEM images (Figure 2b).

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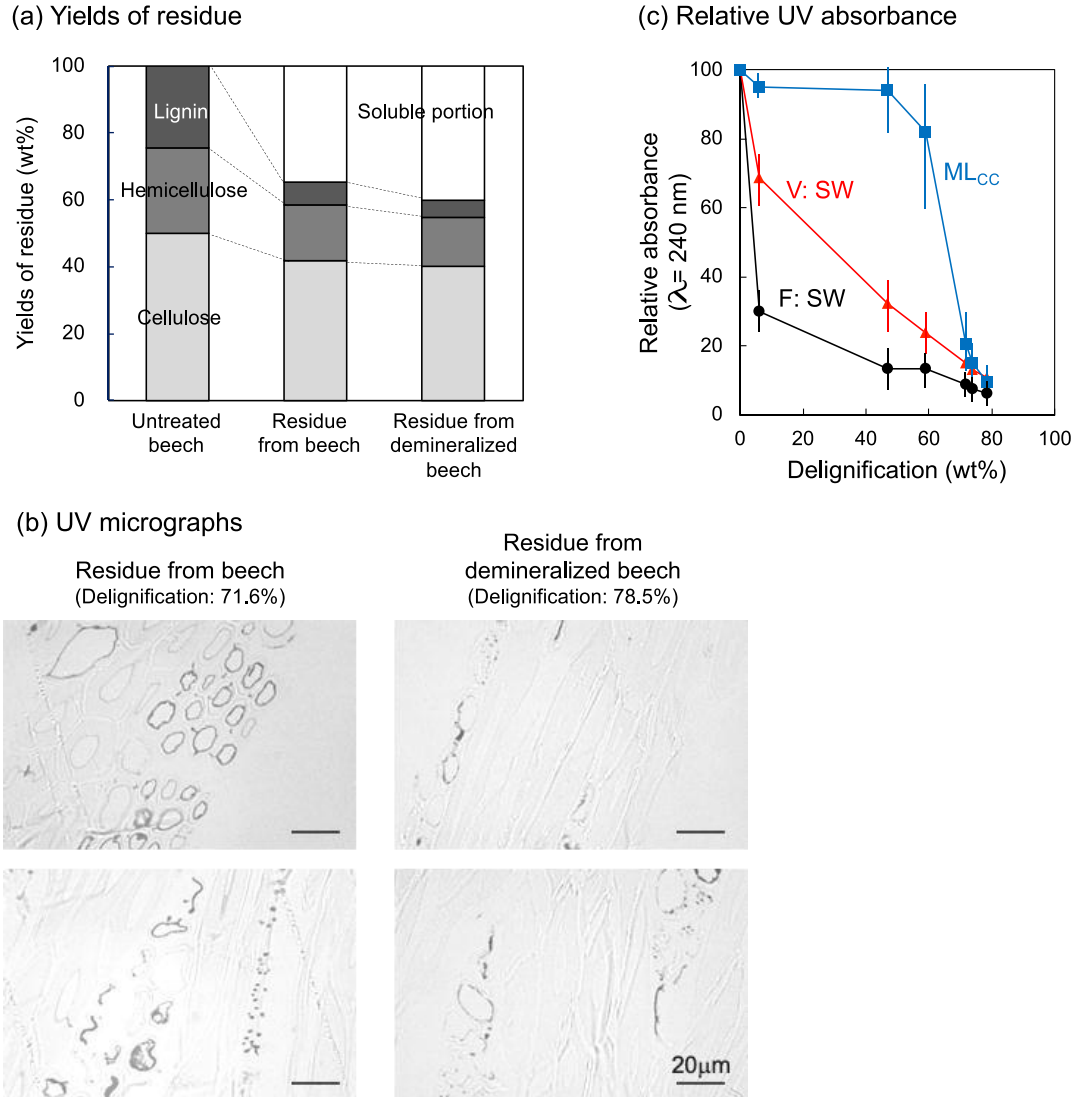

Figure 6. Influence of demineralization on the delignification. (a) Yields of insoluble residues from demineralized Japanese beech wood treated with supercritical methanol (270 °C, 27 MPa, 30 min), (b) UV micrographs of the insoluble residues from demineralized Japanese beech wood treated with supercritical methanol (270 °C, 27 MPa, 30 min), and (c) relative UV absorbance of the three morphological regions of demineralized Japanese beech wood treated with supercritical methanol (270 °C, 27 MPa) at 240 nm.

The lignin in all three regions was successfully decomposed and eluted into methanol as the supercritical methanol treatment proceeded. However, the extent of delignification detected by the Klason method did not exceed 71.3%. Considering that numerous UV-absorbing secondary products were formed in the parenchyma cells, the leveling-off behavior of the delignification was likely caused by the formation of those secondary products.

Formation of Secondary Products in the Parenchyma. Figure 4a shows the UV micrographs of the insoluble residues after being treated with supercritical methanol for 30 min at 270 °C. There were numerous UV-absorbing substances observed specifically in the parenchyma cells (not in the fiber or vessel). In general, phenolic compounds exist inside the parenchyma of trunk wood;[24] however, the wood flour used in this study was extracted with acetone to fully remove the extractives before the supercritical methanol treatment, so those phenolic substances were not observed in the extractive-free wood flour. Accordingly, the UV-absorbing substances inside the parenchyma cells were most likely produced during the supercritical methanol treatment. Based on their shape, the substances could be classified into three types: (1) substances filling the lumen, (2) substances coating the lumen, and (3) granular substances. Their representative UV spectra are shown in Figure 4b. The filling substances had a small shoulder at 280 nm, and their overall spectral profile was different from that of lignin (Figure 3b). The UV spectra of the coating substances and granular substances were also clearly different from that of general lignin. The longer-wavelength bands seem to be derived from a carbonized compound; therefore, the UV-absorbing substances are different from lignin.

In this study, the delignification rate was calculated using the Klason method. However, upon applying this method, the secondary products generated selectively in the parenchyma cells would be considered as lignin, thus resulting in the overestimation of lignin content in the insoluble residue. Haas et al. also found that the pyrolysis of poplar wood formed char selectively in the ray parenchyma,[25] but the reason and the detailed mechanism are still not understood. The function of

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ACS Omega 2021, 6, 20924−20930

parenchyma cells involves nutrition storage, and therefore, the parenchyma is known to be rich in inorganics and protein.[26−28] In particular, the lumens of parenchyma cells are covered by an amorphous layer, which contains large quantities of inorganics.[29,30] In addition, several reports have discussed the influence of inorganic species on the pyrolysis of biomass and cell wall components, such as cellulose and hemicellulose.[16−19] For example, Raveendran et al. examined the effects of minerals on a pyrolysis reaction using various demineralized biomass samples, and in most cases, the demineralization reduced the char yields.[17] Additionally, Yang et al. suggested that the presence of K^+ and Ca^{2+} favored the formation of compounds containing carbonyl groups.[19] Therefore, we hypothesized that the presence of inorganic species would affect the formation of secondary products in parenchyma cells during the supercritical methanol treatment. Thus, in this study, the inorganic substances in the beech wood were removed by washing the samples with a dilute acid solution before the supercritical methanol treatment.

Supercritical Methanol Treatment of Demineralized Beech Wood. Demineralization was achieved by washing the extractive-free beech wood flour with a weak acid solution. During this treatment, the chemical composition of the samples did not change, except for the fact that the inorganic content decreased from 0.6 to 0.05%. Figure 5 shows the SEM micrographs of each morphological region of the extractive-free wood and the demineralized wood, with their corresponding EDX spectra. It is clear that the lumen of the parenchyma cells contained various inorganic components, such as Ca, K, Mg, and Na, and they generally had greater quantities of inorganics than other morphological regions. These inorganic components were removed by the demineralization treatment, which did not modify the cell wall structure. The obtained demineralized wood was then treated with supercritical methanol, as in the previous experiments.

Figure 6a shows the yields and composition of the insoluble residues from demineralized beech wood treated with supercritical methanol (270 °C, 27 MPa, 30 min). The yield of insoluble residue from demineralized beech was lower than that obtained from untreated wood flour. It was clear that the amount of residual lignin decreased and the extent of delignification reached 78.5% after 30 min.

Figure 6b shows the UV micrographs of the insoluble residue from the extractive-free and demineralized beech wood samples after the supercritical methanol treatment (270 °C, 27 MPa, 30 min). The formation of secondary products in the parenchyma cells was suppressed in the demineralized beech wood compared with the extractive-free beech wood, indicating that the presence of inorganic substances contributed to the formation of secondary products. The secondary products would be counted as lignin in the Klason method, resulting in the low extent of delignification shown in Figures 1 and 3c.

In contrast, the insoluble residue from the demineralized beech wood treated with supercritical methanol still contained some secondary products. In addition to the inorganics, proteins may impact the formation of secondary products and parenchyma cells contain relatively high amounts of protein.[27] There are several reports describing the degradation of protein in supercritical water,[31−33] but to our knowledge, the effect of protein on cell wall components, such as cellulose and hemicellulose, has not yet been investigated. However, Kruse et al. gasified protein-rich biomass and their gasification yields were lower than for protein-less biomass. They proposed that the Maillard reaction between glucose and protein-derived amino acids would inhibit gasification.[34,35] Although the detailed mechanism and influence of protein on the cell wall components have not yet been elucidated, it is reasonable to propose that protein may also affect the formation of secondary products.

Figure 6c shows the changes in the UV absorbance (at 240 nm) of the insoluble residue from demineralized beech wood treated with supercritical methanol, which enabled observations regarding the delignification in each morphological region. The delignification tendency in each morphological region was similar to that shown in Figure 3c. However, the presence of secondary products should suppress delignification, and therefore, Figure 6c is likely more appropriate than Figure 3 in terms of evaluating the topochemistry of delignification.

CONCLUSIONS

The topochemistry of Japanese beech wood delignification was evaluated following a supercritical methanol treatment. UV microscopy revealed that lignin in the SW was easily decomposed and removed, and lignin in the ML was initially resistant, although most ML lignin was removed eventually. Inorganic components in the lumen of parenchyma cells affected the selective formation of secondary products, resulting in the overestimation of residual lignin. Therefore, the use of demineralized beech wood enabled a more accurate evaluation of the delignification topochemistry. The influence of inorganics must be carefully considered to promote the effective utilization of high-ash biomass, such as herbaceous plants.

AUTHOR INFORMATION

Corresponding Author

Masatsugu Takada − *Graduate School of Energy Science, Kyoto University, Kyoto 606-850, Japan;* orcid.org/0000-0002-8322-3423; Phone: +81-75-753-9122; Email: takada.masatsugu.6n@kyoto-u.ac.jp

Authors

Eiji Minami − *Graduate School of Energy Science, Kyoto University, Kyoto 606-850, Japan*

Haruo Kawamoto − *Graduate School of Energy Science, Kyoto University, Kyoto 606-850, Japan*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.1c02345

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported by KAKENHI Grant Number (no. 20K22591), for which the authors are extremely grateful. The authors thank Dr. Shiro Saka for helpful discussions. They also thank Suzanne Adam, Ph.D., from Edanz Group (https://en-author-services.edanz.com/ac) for editing a draft of this manuscript.

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https://doi.org/10.1021/acsomega.1c02345
ACS Omega 2021, 6, 20924−20930