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Morphology of lignin structures on fiber surfaces after organosolv pretreatment

Prajin Joseph[1] | Vegar Ottesen[1,2] | Mihaela Tanase Opedal[3] | Størker T. Moe[1]

[1]Department of Chemical Engineering, Norwegian University of Science and Technology (NTNU), Trondheim, Norway

[2]Innlandet Fylkeskommune, Hamar, Norway

[3]RISE PFI, Trondheim, Norway

Correspondence
Prajin Joseph, Department of Chemical Engineering, Norwegian University of Science and Technology (NTNU), Trondheim 7491, Norway.
Email: joseph.prajin@ntnu.no

Abstract

The redeposition of lignin to the fiber surface after organosolv pretreatment was studied using two different reactor types. Results from the conventional autoclave reactor suggest that redeposition occurs during the cooling down stage. Redeposited particles appeared to be spherical in shape. The size and population density of the particles depends on the concentration of organosolv lignin in the cooking liquor, which is consistent with the hypothesis that reprecipitation of lignin occurs when the system is cooled down. The use of a displacement reactor showed that displacing the spent cooking liquor with fresh cooking liquor helps in reducing the redeposition and the inclusion of a washing stage with fresh cooking liquor reduced the reprecipitation of lignin, particularly on the outer fiber surfaces. Redeposition of lignin was still observed on regions that were less accessible to washing liquid, such as fiber lumens, suggesting that complete prevention of redeposition was not achieved.

KEYWORDS
lignin morphology, lignin redeposition, organosolv pretreatment, SEM analysis of fiber surface

1 | INTRODUCTION

Pretreatment is an important step in the production of biofuel (ethanol). The primary aim of pretreatment is to make the cellulose accessible to enzymes during enzymatic hydrolysis.[1] There are several mechanical, chemical, and thermal pretreatment methods available to make the cellulose accessible by altering the structure or composition of biomass.[2] Lignin removal is one of the primary goals of many pretreatments. Since lignin acts as a protective layer, the removal of lignin improves the enzymatic hydrolysis.[3]

Lignin is an amorphous, phenolic polymer, whose primary purpose is to strengthen the cell wall and to protect the plant from microbial attack.[4] The primary monomers of lignin are *p*-coumaryl, coniferyl, and sinapyl alcohol, as shown in Figure 1. Softwood typically contains guaiacyl lignin made of coniferyl alcohol. Several linkages are present in lignin such as β-O-4, α-O-4, β-5, and β-β'.[5] Lignin–carbohydrate complexes (LCC) can also be present in the cell wall.[6]

During pretreatment, lignin is assumed to go through a phase transition from solid to liquid and back to solid via a complex mechanism.[7,8] Phenomena such as phase transition, reaction, and solubilization may occur during the pretreatment.[2] Researchers have noticed the formation of spherical structures on the biomass surface after pulping processes used for paper production.[9] Similar structures were also reported in pretreatment processes such as dilute acid pretreatment and hot water pretreatment.[10–13] These formations are proven to be consisting mostly of lignin,[14] but the formation of pseudo-lignin from carbohydrate degradation products has also been reported.[15,16] Researchers have tried to explain the mechanism behind the droplet formation. One plausible explanation, especially in the case of pretreatment with water,

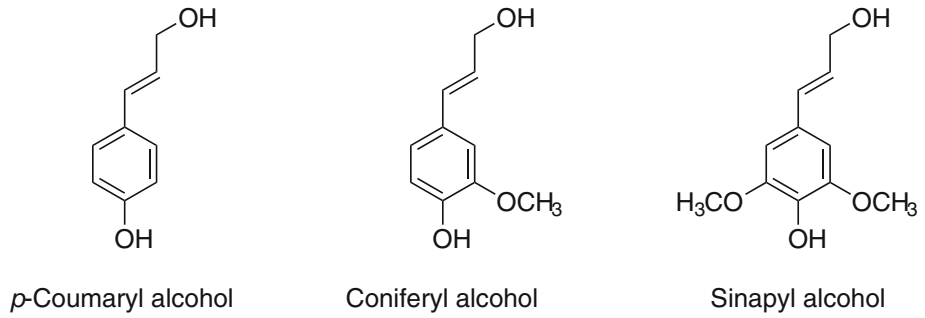

FIGURE 1 The structure of the primary monomers of lignin

is that temperature exceeding the glass transition temperature of lignin allows lignin to become mobile and move within the cell wall. When in contact with an aqueous medium, lignin tries to reduce the surface area in contact with water due to its hydrophobic nature. This will cause the lignin to form spherical structures and upon cooling these droplets get deposited on the biomass surface.[14] The presence of similar droplets in organosolv pulping or pretreatment[17] suggests that another mechanism is possible for the lignin redeposition other that the hydrophobicity of lignin. In such cases, the redeposition could be due to the reduction in solvent concentration during reaction or due to a reduction in temperature causing the solubility of lignin in the solvent to decrease. Both of these can occur during organosolv pretreatment.

Some studies suggest that these droplets have a negative effect on enzymatic hydrolysis due to non-specific binding of enzymes to the lignin and steric hindrance.[12,18] These droplets increase the Klason lignin content of the pretreated biomass and hence the understanding of how different reactor setup affect the lignin redeposition could be a useful tool in designing the pretreatment process in the industry. In the current article, two batch reactor setups are compared, one an autoclave reactor where the biomass is cooled down with the liquor and a displacement reactor system where the spent liquor is displaced with fresh liquor before cooling down. The authors are comparing how these two methods affect the lignin redeposition and other factors influencing the droplet size and population.

Different reactor setups to improve the delignification process have been employed before. Flow-through processes have shown to improve the delignification in processes such as alkaline and organosolv pretreatment and to prevent the recondensation of dissolved lignin.[19–21] The reactor system used in this work is a design called Rapid Heating Displacement Pretreatment Reactor, designed and developed by RISE PFI.[22] The reactor has a solvent heating section which can heat up the solvent prior to mixing with the substrate enabling rapid heating (30 °C/min). The "High-pressure Rapid Heating Displacement Pretreatment Reactor" is a flow-through reactor, albeit with the possibility of very rapid heating of the cooking liquor. Due to heat transfer limitations in the rather large biomass particles, the rapid heating feature was not used. The key feature of the reactor system is to displace the spent liquor without reducing the process temperature or pressure. This key feature will help in understanding the effects the cooling stage has on the pretreatment process.

2 | MATERIALS AND METHODS

2.1 | Raw materials and chemicals

The wood chips used in this study were industrial Norway spruce (*Picea abies*) chips from Norske Skog Skogn, Norway. The chips were dried and fractionated to different particle sizes at RISE PFI. The −8/+7 mm fraction was stored at room temperature until further use. Absolute ethanol was obtained from VWR chemicals. Sulfuric acid (ACS reagent, 95%–98%), potassium permanganate (ACS reagent ≥99% pure), and mannitol (ACS reagent, ≥98%) were received from Sigma-Aldrich. All the chemicals were used as received.

2.2 | Pretreatment procedure

63 (w/w)% ethanol–water mixture was used as the solvent in all the reactions and the liquid to biomass ratio was 7.5:1. 1 wt% H_2SO_4 (oven-dried wood basis) was used as a catalyst. The choice of 63% ethanol in water was based on results obtained by Agnihotri *et al.* who obtained excellent delignification of Norway spruce (*Picea abies*) at this ethanol concentration, showing that Norway spruce required a somewhat higher ethanol concentration than the non-wood substrate sugarcane bagasse.[23] After the reaction, the chips were washed thoroughly, first with fresh solvent and then with water, and were stored in an airtight bag until further use.

2.3 | Conventional autoclave reactor

The reactor used in these experiments was a custom-made autoclave reactor system with six parallel autoclaves from TOP industries, France. Each autoclave has an internal volume of 1 L and the system uses electrical heating. The maximum operating pressure and temperature are 50 bar and 220 °C. The reactor cap is fitted with a thermocouple which is extended to the inside to measure the internal temperature. The reactor is seated in an inclined position and is agitated throughout the reaction for proper mixing and even heat distribution. After reaction, the

reactor was cooled down in a water bath before transferring the content.

2.4 | High-pressure rapid heating displacement pretreatment reactor

The displacement reactor is a state-of-the-art reactor setup with the provision of displacing the cooking liquor with fresh solvent without cooling down the reactor. It is a flow-through reactor with oil heating. The biomass is placed in the reactor and the cooking liquor is pumped to the reactor from the liquid storage tank. Even though the reactor is capable of rapid heating, a heating rate of 2 °C per minute was used to match the heating rate of the autoclave reactor and to reduce temperature gradients in the chips during heat up. A circulation of cooking liquor was employed throughout the reaction to assure proper mixing and an even temperature distribution. After the reaction, the cooking liquor was displaced by fresh liquid and the displaced effluent was collected for further analysis. The reactor is equipped with a provision to wash the pretreated biomass inside the reactor before cooling down. The washing liquid can be pumped from the reservoir tank and pass through the biomass the same way as the displacement is done.

2.5 | Biomass yield after pretreatment

The biomass yield after pretreatment was calculated gravimetrically. The mass of biomass after pretreatment was recorded. The moisture content of the sample was calculated gravimetrically. A pre-weighed amount of the sample was dried in an oven at 105 °C overnight and then weighed again. The weight and moisture content were used to calculate the dry mass of the pretreated sample. Dry mass was then used to calculate the % biomass yield after pretreatment as the percentage of biomass taken for pretreatment.

2.6 | Lignin analysis

Lignin analysis of pretreated biomass was carried out based on the NREL method for structural carbohydrate and lignin analysis in biomass.[24] The sample was air-dried to a moisture content less than 10%. The sample was then powdered and 300 ± 10 mg of the sample was transferred to a reaction tube. Three milliliters of 72% H_2SO_4 was added to the sample and mixed thoroughly with a Teflon rod. The tube was then placed in a Laboshake at 30 °C and incubated for 60 min with stirring the sample every 5–10 min. After incubation, the tube was removed and the contents of the tube were transferred to a 100 ml Pyrex glass bottle and diluted with 84 mL distilled water to make the acid concentration be 4%. The sample was then autoclaved at 121 °C for 1 h after which it was removed, cooled to room temperature, and filtered through a pre-weighed filter paper. The liquid was collected, neutralized using $Ca(OH)_2$, and analyzed using HPLC. The filter paper and the undissolved content were dried in an oven at 105 °C overnight and the Klason lignin was analyzed gravimetrically. The organosolv effluent lignin (OEL) was measured by gravimetry. Ten milliliters of effluent was taken and the lignin in the effluent was precipitated by adding three times the volume of water (30 ml). The precipitated lignin was then filtered using a pre-weighed filter paper and the filter cake with the filter paper was dried overnight. The dried filter cake was weighed to calculate the amount of lignin and the concentration of effluent lignin.

2.7 | Lignin staining and freeze-drying

The samples were stained with $KMnO_4$ as per the method of Gregersen *et al.*[25] The biomass samples were soaked in 4% $KMnO_4$ solution for approximately 5 min. After soaking, the sample was thoroughly washed with water to remove excess $KMnO_4$. The samples were frozen in a rotavap using liquid nitrogen and dried in a freeze drier under vacuum.

2.8 | Scanning electron microscopy

Samples were sputter coated with 20 nm gold or platinum using a Cressington 208 HR B sputter coater. Sputter-coated samples were then transferred to a Helios G4 UX FIB/SEM. Images were recorded using between 3 and 5 keV acceleration voltage, as indicated, with current and working distance as indicated. The detector used was an in-chamber Ion Conversion and Electron (ICE) detector, set to detect secondary electrons. For conventional electron microscopy, the sample was not tilted. For cross-section imaging/imaging of fiber lumen, the sample was tilted to 52°, and the sample was cut into using the instrument's Ga ion beam at high beam currents. Ion milling was performed using high currents. After milling, the milled area was imaged using the electron beam.

2.9 | Image analysis

The images from SEM were analyzed using ImageJ software. Images included in the manuscript have had brightness/contrast adjusted automatically using ImageJ's built-in algorithm. Lumen micrographs in Figure 6 were rotated to align the lumen horizontally in the image, then cropped. Droplets were identified by visual inspection, manually selected in ImageJ, and measured using the built-in algorithm. The size of the lignin droplets and the number of droplets per unit area were measured manually through area selection. Once selected, the particles were measured using ImageJ's built-in measure function. We assumed Feret's diameter was an accurate representation of the particle diameter. ImageJ reports the major Feret diameter, which for a non-circular bead is the largest end-to-end distance across the particle.[26]

3 | RESULTS AND DISCUSSION

Lignin analysis of all the pretreated samples is given in Table 1. Detailed reaction parameters of some of the autoclave reactor runs

TABLE 1 Lignin content of all the pretreated samples

Sample ID	Reactor type	Max. reaction temperature (°C)	Effluent lignin concentration (mg/ml)	Klason lignin
150A	Autoclave reactor	150	9.45	19.97
160A	Autoclave reactor	160	11.81	19.43
170A	Autoclave reactor	170	17.24	14.58
180A	Autoclave reactor	180	15,8	12.38
188A	Autoclave reactor	188	24.56	9.00
195A	Autoclave reactor	195	17.7	11.16
195B	Displacement reactor	195	-	13.64
195C	Displacement reactor	195	-	13.72
210A	Displacement reactor	210	-	15,36
210B	Displacement reactor	210	-	7.76
210C	Displacement reactor	210	-	9.73

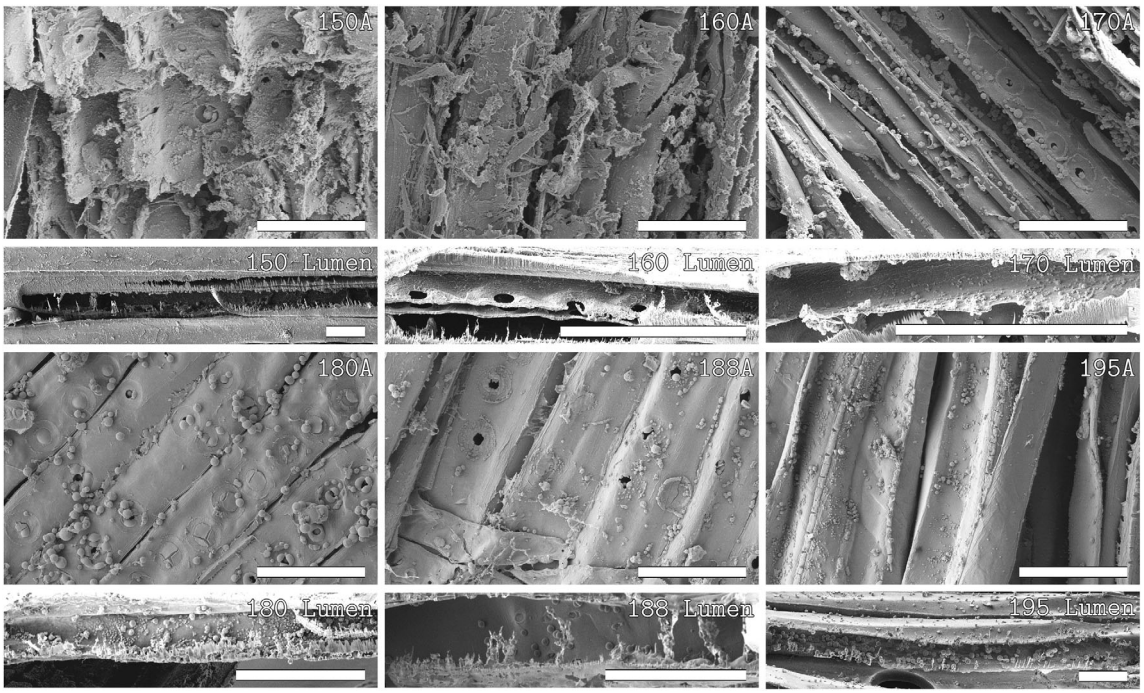

FIGURE 2 SEM images of different samples pretreated in an autoclave reactor. The top part is the surface of the fiber and the bottom part is the image of the lumen. Scale bars are 50 μm long

are reported elsewhere.[27] Figure 2 shows the SEM images of the samples from the autoclave reactor. No visible redeposition of lignin on the first two samples (150A and 160A) was observed. From sample 170A and up (A liquor lignin concentration of 15.8 g/L and higher), lignin redeposition was observed, and a plausible explanation for this is supersaturation. The redeposition of lignin happens in the cooling stage of pretreatment and when the temperature goes down, the solubility of lignin in the solvent reduces causing the lignin to precipitate. For samples 150A and 160A, it can be seen that the concentration of lignin in the effluent is low. It is assumed that even after cooling, the lignin does not reach supersaturation and there is no visible redeposition when the effluent lignin concentration is low. Once the pretreatment severity goes up, the concentration of lignin in the effluent increases. The high concentration of lignin in the effluent results in the solution reaching supersaturation while cooling causing the lignin to redeposit back onto the fiber surface. The surface of the wood fiber is more rough in the least pretreated samples compared to the other samples. It appears that the surface becomes smoother as lignin is removed.

The image analysis of the rest of the samples from the autoclave reactors showed an interesting trend; the size of the droplets is decreasing as the temperature or the severity of pretreatment is increasing. At the same time, the amount of beads per unit area

increased. This observation supports the hypothesis of supersaturation being the cause of redeposition. Supersaturation is the driving force for nucleation and crystal growth. As per supersaturation theory, both the nucleation rate and the growth are dependent on supersaturation. At lower supersaturation, the crystal growth rate will be faster than the nucleation rate, and hence, the crystals grow bigger. At higher supersaturation, the nucleation rate dominates leading to higher population density with smaller crystals.[28,29] It can be seen that at a liquor concentration of 15.8, the droplets are larger in size, but the population density is lower. As the lignin concentration goes up, the droplet size is reduced, but the number of droplets per unit area increases. Since the concentration of lignin in the effluent is higher at high severity, the cooling stage creates a higher number of nucleation sites. This could be the reason for the high droplet density at higher severities. Since the number of nucleation sites is high, the droplets will only grow a little. But at lower temperature or severity, the lignin concentration in the effluent is lower, and hence, the number of nucleation sites will be reduced relative to higher severity. This leads to more amount of lignin accumulating on to fewer nucleation sites causing the droplets to grow bigger. Figure 3 shows this effect.

Since the liquid lignin concentration is assumed to be the main parameter affecting the lignin reprecipitation, it is worth noticing that there appeared to have some anomalies in the effluent lignin calculation. For sample 180A, the lignin concentration is lower than that of sample 170A even though the Klason lignin content of 180A is lower.

A clear reduction in redeposition of lignin droplets was observed on samples pretreated in the displacement reactor as shown in Figure 4. This was an expected result since the washing stage is done before cooling down in the displacement reactor, in accordance with the redeposition hypothesis. Since most of the effluent containing lignin was displaced at a temperature close to the reaction temperature, the possibility of lignin redeposition to the fiber surface was eliminated. A comparison of Klason lignin content versus biomass yield after pretreatment on both reactors agrees with this observation. As shown in Figure 5, the Klason lignin content for the displacement reactor samples is lower compared to the ones from the autoclave reactor

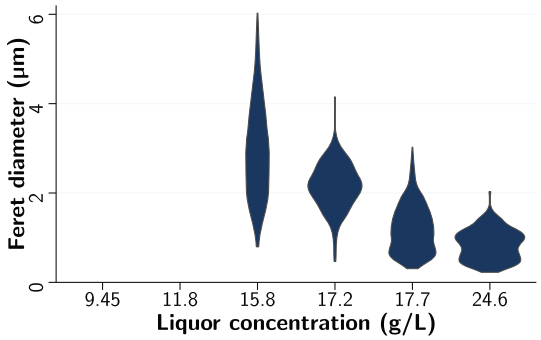

FIGURE 3 The size distribution of beads from different samples pretreated in an autoclave reactor plotted as a violin plot. The *x*-axis represents the lignin concentration in the liquid phase after pretreatment

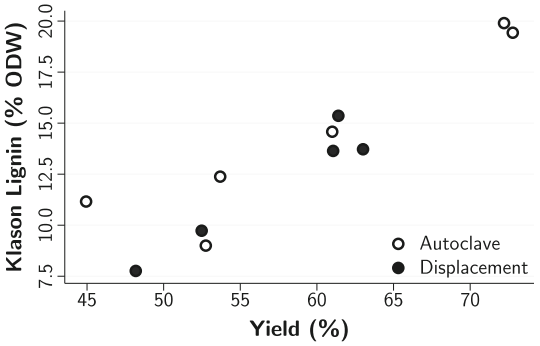

FIGURE 5 Graph of biomass yield after pretreatment versus the Klason lignin content for both the reactors

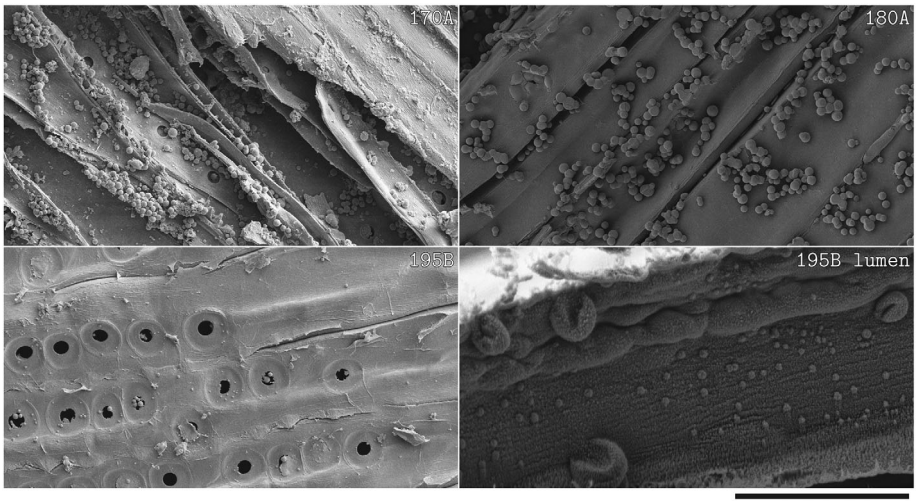

FIGURE 4 Comparison of the wood chips pretreated in autoclave reactor and in displacement reactor. The top two images are from the autoclave reactor, whereas 195B is from the displacement reactor. 195B lumen is the image of the lumen of the same sample. The scale bars are 50 μm long

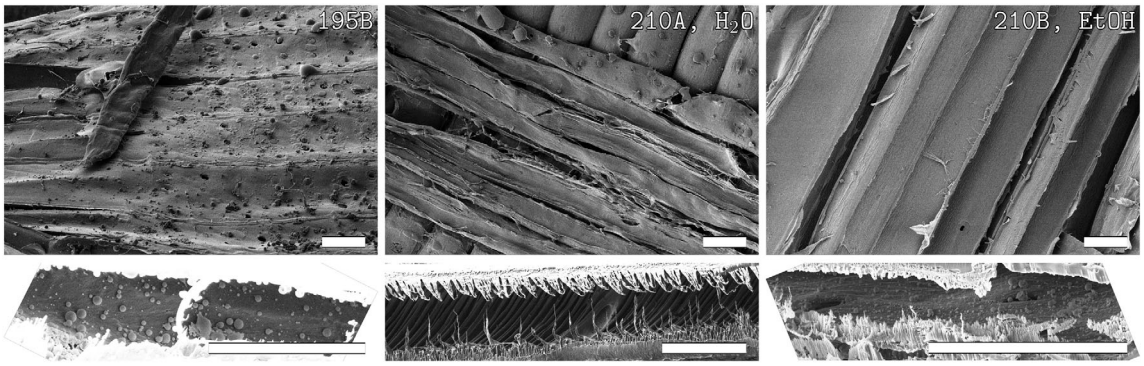

FIGURE 6 Comparison of water-washed and ethanol-washed samples from the displacement reactor. H_2O denotes the sample was washed with water after the displacement, whereas EtOH means that the first and second stage washes were done using ethanol–water mixture of the same concentration as the one used for pretreatment. The bottom part shows the lumen. The scale bars are 20 μm long

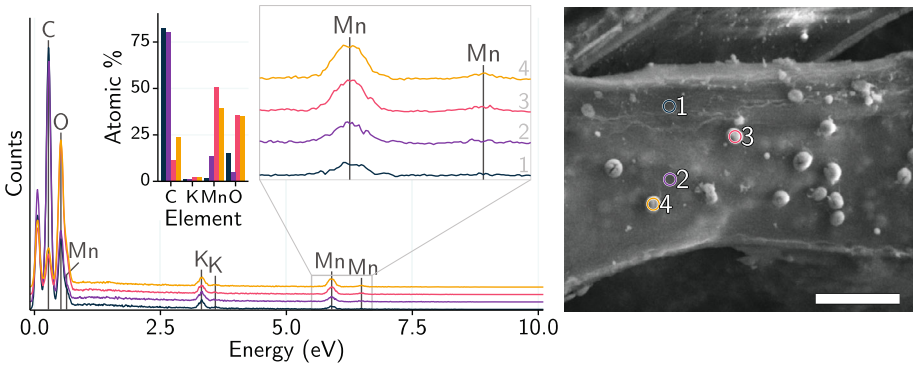

FIGURE 7 EDX data collected on a FEI Helios G4 UX FIB/SEM using Oxford Industries AZtec software running on Windows 10 Pro. The atomic percentage is as estimated by the AZtec software, without calibration standard. Electron micrograph collected using an in-chamber ETD detector set to secondary electrons. The scale bar is 10 μm long. The beam acceleration voltage was set to 10 keV. The EDX detector used was an EDX Oxford Xmax detector. Spectra are normalized using the area under curve from 0.8 keV to 3.1 keV. The area was calculated using a rolling mean function in R (rollmean function, Zoo v. 1.8, R base version 4.0.5 running on OS X 11.4.)

for similar total biomass yield. The anomaly to this general observation can be seen as one of the displacement reactor samples has higher Klason lignin content at the same biomass yield. This sample was one of the displacement samples which was washed with water and not organosolv. The reason for the high lignin content is not clear to the authors at this point but assume that it might have happened due to a poor displacement caused by poor mass transfer or the introduction of water might have caused precipitation of lignin due to reduced solubility. Further investigation is required to confirm the reason for the observed anomaly. One of the autoclave samples also appeared to have similar Klason lignin content as the displacement reactor. The chip size used for this sample was different from the other samples, and the authors assume that it might have caused the anomaly. Since the main focus of this article is the redeposition of lignin due to effluent lignin concentration, the authors believe that even though the mass and heat transfer might have changed due to particle size, the sample is eligible for the comparison.

Even though the surface of the wood chips treated in the displacement reactor appears to be cleaner than the ones from the autoclave, there was still lignin redeposition inside the lumen. This observation suggests that the inner wall of the fiber is less accessible during the washing stage and the trapped cooking liquor in these regions could cause redeposition during cooling. These observed droplets on the surface pretreated in the displacement reactor, even though was much fewer compared to the autoclave samples, suggest that a one-stage displacement was inadequate to completely displace the cooking liquor. The remaining cooking liquor or the presence of lignin might have caused redeposition during the cooling stage when pure water was introduced.

In order to see the effect of displacement, two samples(210B and 210C) were washed with an ethanol–water mixture of the same concentration as the pretreatment liquor. Figure 6 shows a comparison of the water-washed and the ethanol-washed samples. The ethanol-washed samples appeared to have next to no redeposition on the surface, and the lumen was cleaner than for the water-washed samples. This shows that a second and third stage washing with the same solvent increased the displacement effect and prevented most of the lignin redeposition.

The observed correlation between the droplet size and population density and the effluent lignin content suggests that the redeposited droplets mainly contain lignin. Existing literature also suggests that the droplets are lignin redepositions. [14] An EDX-SEM analysis was done on the $KMnO_4$-stained samples where the spectrums were taken from different regions on the biomass surface (clean surface and droplets). The data are shown in Figure 7. The spectrum obtained from droplets shows higher amount of Mn than the clean surface. Since the $KMnO_4$ staining was targeted for lignin, this increase in the amount of Mn on droplets indicates that the droplets contain mostly lignin.

4 | CONCLUSION

The cooling stage of the pretreatment process causes part of the dissolved lignin to redeposit on to the fiber surface in the form of spherical beads. The size and population density of these beads depend on the concentration of lignin in the liquid phase. The use of a displacement reactor, where the cooking liquor is displaced with fresh liquor prior to the cooling down stage, was shown to prevent this redeposition of lignin to a great extent. The displacement stage was further improved by employing a two-stage washing process with the fresh cooking liquor prior to the cooling stage. Redeposition was still observed on regions which are less accessible to the displacing liquid.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

The authors confirm that the data supporting the findings of this study are available within the article.

ORCID

Prajin Joseph https://orcid.org/0000-0003-1825-8520
Vegar Ottesen https://orcid.org/0000-0003-1810-2710
Mihaela Tanase Opedal https://orcid.org/0000-0001-9515-8561
Størker T. Moe https://orcid.org/0000-0001-5100-574X

REFERENCES

[1] R. Kumar, C. Wyman, in *Bioalcohol Production*, Woodhead Publishing Series in Energy, Woodhead Publishing, Sawston, England **2010**, Ch. 3.
[2] H. L. Trajano, N. L. Engle, M. Foston, A. J. Ragauskas, T. J. Tschaplinski, C. E. Wyman, *Biotechnol Biofuels* **2013**, *6*, 110.
[3] V. J. Sewalt, W. Ni, H. G. Jung, R. A. Dixon, *J Agric Food Chem* **1997**, *45*, 1977.
[4] D. Fengel, G. Wegener, *Wood: Chemistry, Ultrastructure, Reactions; Walter 289 de Gruyter*, Walter de Gruyter, Berlin, New York **1984**.
[5] H. M. Chang, E. B. Cowling, W. Brown, *Holzforschung* **1975**, *29*, 153.
[6] G. Meshitsuka, Z. Z. Lee, J. Nakano, S. Eda, *J Wood Chem Technol* **1982**, *2*, 251.
[7] C. Liu, C. E. Wyman, *Ind Eng Chem Res* **2003**, *42*, 5409.
[8] H. L. McKenzie, *Tracking hemicellulose and lignin deconstruction during hydrothermal pretreatment of biomass*, University of California, Riverside, California **2012**.
[9] J. Simola, P. Malkavaara, R. Alen, J. Peltonen, *Polymer* **2000**, *41*, 2121.
[10] M. J. Selig, S. Viamajala, S. R. Decker, M. P. Tucker, M. E. Himmel, T. B. Vinzant, *Biotechnol Prog* **2007**, *23*, 1333.
[11] J. B. Kristensen, L. G. Thygesen, C. Felby, H. Jørgensen, T. Elder, *Biotechnol Biofuels* **2008**, *1*, 1.
[12] S. V. Pingali, V. S. Urban, W. T. Heller, J. McGaughey, H. O'Neill, M. Foston, D. A. Myles, A. Ragauskas, B. R. Evans, *Biomacromolecules* **2010**, *11*, 2329.
[13] L.-P. Xiao, Z.-J. Sun, Z.-J. Shi, F. Xu, R.-C. Sun, *BioResources* **2011**, *6*, 1576.
[14] B. S. Donohoe, S. R. Decker, M. P. Tucker, M. E. Himmel, T. B. Vinzant, *Biotechnol Bioeng* **2008**, *101*, 913.
[15] Y. Pu, F. Hu, F. Huang, B. H. Davison, A. J. Ragauskas, *Biotechnol Biofuels* **2013**, *6*, 1.
[16] R. Kumar, F. Hu, P. Sannigrahi, S. Jung, A. J. Ragauskas, C. E. Wyman, *Biotechnol Bioeng* **2013**, *110*, 737.
[17] Y. Xu, K. Li, M. Zhang, *Colloids Surf A: Physicochem Eng Asp* **2007**, *301*, 255.
[18] J. He, C. Huang, C. Lai, C. Huang, X. Li, Q. Yong, *Ind Crops Prod* **2018**, *113*, 368.
[19] V. Pihlajaniemi, M. H. Sipponen, O. Pastinen, A. Nyyssölä, S. Laakso, *Biotechnol. Bioeng* **2016**, *113*, 2605.
[20] K. L. Kadam, C. Y. Chin, L. W. Brown, *Environ Prog Sustain Energy* **2009**, *28*, 89.
[21] D. S. Zijlstra, C. A. Analbers, J. de Korte, E. Wilbers, P. J. Deuss, *Polymer* **1913**, *2019*, 11.
[22] K. Toven, K. Øyaas, M. T. Opedal, Ø. Eriksen, *Poster presented at 7th Nordic Wood Biorefinery Conference*, NWBC, Stockholm, Sweden **2017**.
[23] S. Agnihotri, I. A. Johnsen, M. S. Bøe, K. Øyaas, S. Moe, *Wood Sci Technol* **2015**, *49*, 881.
[24] A. Sluiter, B. Hames, R. Ruiz, C. Scarlata, J. Sluiter, D. Templeton, D. Crocker, et al., *Lab Anal procedure* **2008**, *1617*, 1.
[25] Ø. W. Gregersen, I. Skinnarland, P. Johnsen, T. Helle, *J Pulp Paper Sci* **1995**, *21*, J285.
[26] L. Feret, *La Grosseur des Grains des Matières Pulvérulentes. Eidgen*, Materialprüfungsanstalt ad Eidgen. Technischen Hochschule, Zürich **1930**.
[27] P. Joseph, M. T. Opedal, S. T. Moe, *Biomass Convers Biorefin* **2021**, 1.
[28] M. Çelikbilek, A. E. Ersundu, S. Aydın, *Advances in Crystallization Process* (Ed: Yitzhak Mastai), IntechOpen, London **2012**, 35347.
[29] D. A. Porter, K. E. Easterling, *Phase Transformations Metals and Alloys. Chapman Halls*, Van Nostrand Reinhold Co., London, England **1981**.

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