Effect of Surfactant on Pseudo-Lignin Formation

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In this study, a nonionic surfactant (JFC-M) was used as an additive for hydrothermal pretreatment of crushed poplar wood. The pseudo-lignin extracted from holocellulose after hydrothermal pretreatment was characterized, and the composition of liquid and solid fractions obtained after pretreatment at different experimental conditions was analyzed. The results showed that the addition of JFC-M surfactant accelerated the dissolution of biomass cellulose and effectively inhibited the production of pseudo-lignin in hydrothermal processes, under the same hydrothermal pretreatment conditions. The pseudo-lignin yield for the control group was 14.2% (no JFC surfactant added), whereas when the JFC-M concentration was 2%, the pseudo-lignin yield was 9.8%.

DOI: 10.15376/biores.19.2.2714-2723

Keywords: Pseudo-lignin; JFC-M surfactant; Biomass; Hydrothermal pretreatment

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INTRODUCTION

As global energy demand continues to grow, lignocellulosic biomass is low-cost as a renewable energy source, and it is desirable to replace fossil fuels. The poplar used as a raw material in this study is a very low-cost energy crop due to its fast growth rate and drought resistance.

It is well known that hydrothermal pretreatment is currently one of the main technologies in the process of converting lignocellulosic biomass into ethanol. It can effectively dissolve hemicellulose and recombinant lignin under high temperature conditions, change the physical and chemical structure of biomass cell walls, remove stubborn obstacles, and increase the accessibility for enzymatic or microbial degradation (Zhao *et al.* 2012). Under certain conditions it can be used to avoid the degradation or loss of carbohydrates and the formation of inhibitory by-products (Sun and Cheng 2002). Usually hydrothermal pretreatment is carried out at 120 to 210 °C, with an acid concentration below 4%, and the residence time in the reactor ranges from a few seconds to two hours.

Many studies have focused on the behavior of lignin in acid pretreatment. Hemicellulose is almost completely removed under high temperature and dilute acid conditions (Meng *et al*. 2015; Zhang *et al*. 2015), and most of the pentosan can be recovered. However, the acid-insoluble Klason lignin content of the materials under dilute acid pretreatment is higher than that of the starting materials (Jung *et al*. 2010; Mao *et al*. 2010), and the lignin-like part was found to be there. The extra content of Klason lignin, *i.e.* the "pseudo-lignin," is formed by key intermediates such as 3,8-dihydroxy-2-methylchromone and 1,2,4-benzylglycerol, which are derived from furfural and 5-hydroxymethylfurfural through substitution reactions or acid-catalyzed condensation reactions on dehydrated polysaccharides (Li *et al*. 2005). It becomes deposited on the

surface of biomass residues or in filtrate in the form of liquids and microspheres, and its production is positively correlated with the strength of pretreatment (Sannigrahi *et al*. 2008). Pseudo-lignin it also plays a negative role in the conversion of biomass to biofuels: it produces unproductive binding with enzymes/microorganisms and blocks the surface binding sites of active cellulose, thereby hindering the biotransformation of pretreated biomass (Yang and Wyman 2006). Compared to lignin, pseudo-lignin has a greater inhibitory effect on cellulase hydrolysis, while furfural and hydroxymethylfurfural are formedIt also represents the loss of fermentable sugars, so it becomes especially important to avoid the formation of pseudo-lignin.

Surfactants have been gradually used in biomass pretreatment, enzymatic hydrolysis, and fermentation processes in recent years, which can maximize the efficiency and profitability of biorefineries. According to existing reports, surfactants can destroy the structure of lignocellulose, reduce the crystallinity of fibers, and increase porosity (Kaar and Holtzapple 1998; Qing *et al.* 2010), providing more accessible pores and higher specific surface area for enzymes, and improving cellulose saccharification efficiency. Surfactants can remove or dissolve substances that inhibit pretreatment efficiency (Mesquita *et al.* 2016; Muñoz et al. 2022). Likewise, surfactants improve enzyme hydrolysis yield by improving enzyme stability and enhancing enzyme-substrate interaction (Nargotra *et al.* 2019). However, there have been no reports on the effect of surfactants on pseudo-lignin. In this study, poplar was used as raw material to study the effect of surfactant JFC-M on pseudo-lignin yield under the condition of acetic acid + hydrothermal pretreatment. The pretreatment effect was evaluated from the recovery rate of pentosan in the liquid fraction, the recovery rate of cellulose in the solid fraction and the yield of pseudo-lignin after pretreatment.

EXPERIMENTAL

Materials

Poplar (8.8% moisture) was provided by Shandong Longli Company. The biomass raw material contained 42.7% glucan, 19.0% xylan, 24.9% acid-insoluble lignin, and 0.7% ash. The raw materials of poplar wood were crushed to a particle size of 0.05 to 0.2 cm by a plant grinder. Holocellulose was prepared by the sodium chlorite method and then dried at 45 °C. The holocellulose contained 59.8% cellulose and 26.0% hemicellulose. The penetrant was fatty alcohol polyoxyethylene ether-based nonionic surfactant JFC-M (R-$O(CH_2CH_2O)_5$-H, R=$C_{7\text{-}9}$). The reactor used for hydrothermal pretreatment was a magnetic electric heating jacket stirred high-pressure reactor (JRCL-DT; Shanghai Yushen Instrument Co., Ltd.). The total volume of the reactor was 100 mL, with an electric heater and magnetic stirring.

Hydrothermal Pretreatment

The holocellulose was crushed to a particle size of 0.05 to 0.1 cm. A total of 2 g was added to JFC-M + acetic acid mixed solution with a solid-liquid ratio of 1:20 (w/v), and the acetic acid concentration was 1.0% (w/v). The JFC-M concentration and reaction time were used as single factors for hydrothermal experiments. The volume of the hydrothermal reactor was 100 mL, the set temperature was 200 °C, the reactor was initially at room temperature, the magnetic stirring was set at 180 rpm, and the heating rate was 2.9 to 3.4 °C/min. The timer was started when the target temperature of 200 °C was reached.

At the end of the timer, the reactor remained sealed and magnetically stirred until the reactor cooled to 40 °C. Samples were taken, and the wet material in the reactor was filtered and washed. The content of glucose, xylose, and acid-insoluble lignin in the solid fraction was analyzed, and the carbohydrates, furfural, hydroxymethylfurfural and acetic acid in the filtrate were analyzed. All pretreatment experiments were performed in duplicate experiments, and variance statistical analysis was performed for each pretreatment data.

Analytical Methods

Carbohydrate analysis

The content of glucose, xylose, and acetic acid in the liquid fraction was determined by Shimadzu high-performance liquid chromatography (LC-16) using the following HPLC conditions: Aminex HPX-87H column (300 × 7.6 mm) at 45 °C, mobile phase 5 mmol/L H2SO4, flow rate 0.6 mL/min, injection volume of 20 μL, refractive index detector (RID-20A). At the same time, high performance liquid chromatography was used to analyze the content of furfural and 5-hydroxymethylfurfural under the following conditions: AlltimaTM C18 column, column temperature 30 °C, mobile phase acetonitrile: 0.2% acetic acid aqueous solution = 5:95, flow rate 0.5 mL/min, injection volume of 20 μL, ultraviolet-visible light 280 nm detection (SPD-16).

Pseudo-lignin determination

The solid fraction after the pretreatment of the holocellulose was extracted using a Soxhlet apparatus with 1,4-dioxane/water (9:1, v/v) under nitrogen conditions, concentrated in vacuum, then dissolved in deionized water to precipitate pseudo-lignin, and finally determined after vacuum drying at 40 °C.

Scanning electron microscopy (SEM)

The solid fraction sample was fixed on the SEM stage. The stage was placed into the SEM instrument, and vacuum was performed to remove moisture and gases from the air to reduce the interaction of the electron beam with the gas. The next steps were to adjust the accelerating voltage of the SEM instrument (5 kV beam acceleration voltage), working distance, and magnification, and then turn on the electron beam, start observing the sample and acquire SEM images of the pretreated cellulose and pseudo-lignin. Finally, according to the SEM images, the morphology, surface structure and particle size of the samples were analyzed.

FTIR spectroscopic analysis

Each sample was prepared into a homogeneous solid sample by uniform pressing of the sample into sheets, using a spring device to ensure good contact. For measurement using an FTIR instrument, the wavenumber range was 4500 to 500 cm^{-1} with a resolution of 4 cm^{-1} and an average of 64 scans. Baseline correction was performed using the appropriate software to eliminate the effects of depth and frequency variations. According to the FTIR spectrum, the position and intensity of the absorption peaks were observed to determine the functional groups present in the sample.

XRF analysis

XRF was used to analyze the inorganic components of hydrothermal pretreatment materials before and after adding JFC-M surfactant. The equipment model used for XRF

spectral analysis was a Japan ZSX Primus III+, Eleven major elements (in alphabetical order) Al, Si, S, Cl, K, Ca, Fe, Zn were determined.

RESULTS AND DISCUSSION

Liquid Hot Water Pretreatment

This study evaluated the effects of JFC-M surfactant concentration (based on OD holocellulose or based on the whole aqueous system) on the recovery of hemicellulose, cellulose recovery, and pseudo-lignin yield. The chemical composition of solids and liquids after hydrothermal pretreatment is listed in Table 1, in which the displayed data are averages of replicated experiments.

Table 1. Chemical Composition of Solid-Liquid Components of Holocellulose After Hydrothermal Pretreatment

JFC-M (%)	0	0.5	1	1.5	2	2.5
Solid recovery (%)	69.02	67.59	66.16	64.09	64.11	63.90
Solid fraction (%)						
Cellulose	80.37	82.86	84.39	85.70	88.36	88.65
Hemicellulose	0.99	0.90	0.51	0.56	0.47	0.53
Pseudo-Lignin	14.23	12.86	12.30	11.87	9.79	9.92
Liquid fraction (%)						
Glucose	3.67	4.20	4.32	4.32	4.36	4.43
Xylose	17.63	18.47	18.78	19.85	20.09	20.33
2-Furfura	1.91	1.76	1.63	1.19	1.18	0.94
5-HMF	0.49	0.44	0.42	0.31	0.33	0.21

With the increasing addition of JFC-M, the solid recovery rate (expressed as the remaining solid mass after pretreatment divided by 2 grams of absolute raw material) after hydrothermal pretreatment of holocellulose decreased from 69.0% to 63.9%, according to the change of pseudo-lignin yield, it can be considered that the addition of JFC-M inhibited the condensation of the degradation products of cellulose and hemicellulose, thereby reducing the yield of pseudo-lignin.

Under all experimental conditions, the cellulose content in the solids increased compared with the control group (without JFC-M), which was 82.9 to 88.6%, and the cellulose content increased with the increase of JFC-M concentration. Hemicellulose, the main component extracted from hydrothermal pretreatment, was almost completely dissolved, and 0.47 to 0.99% hemicellulose remained in the solid. Compared with the control group (without JFC-M) of 14.2%, when the concentration of JFC-M reached 2.0%, the yield of pseudo-lignin decreased to the lowest value (9.79%), but the content of 2-Furfura and 5-HMF decreased to the lowest point, indicating that JFC-M inhibited the further degradation of glucan and xylan in the liquid fraction, resulting in the reduction of pseudo-lignin yield.

The liquid fraction of hydrothermal pretreatment consisted mainly of a mixture of hydrolyzed sugars and their degradation products (carboxylic acids and furans) with a final pH between 3.31 and 3.49. In the pretreatment solution, the content of dextran was 3.67 to 4.43%, the content of xylan was 17.6 to 20.3%, the content of furfural was 0.9% to 1.91%, and the content of 5-hydroxymethylfurfural was 0.21 to 0.49%. The contents of dextran and xylan increased with the increase of JFC-M concentration, while the contents of

furfural and 5-hydroxymethylfurfural increased and decreased with the increase of JFC-M concentration, and the content of furfural and 5-hydroxymethylfurfural reached the lowest value under the condition of 2.0% JFC-M concentration, and more than 95% of the dissolved hemicellulose was not further degraded under this condition.

To facilitate the comparison of the results, Fig. 1 shows the content of furfural and 5-hydroxymethyl furfural in the pretreatment solution and the yield of pseudo-lignin under different test conditions. When JFC-M was not added, the pseudo-lignin content was 14.2%, and the pseudo-lignin content decreased when the amount of JFC-M added was 12.9% to 9.8%, the content of pseudo lignin decreased with the increase of JFC-M concentration, and reached its lowest at a concentration of 2.0%.

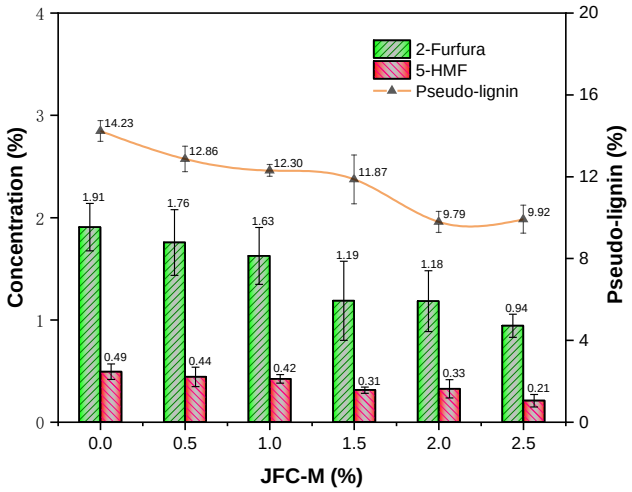

Fig. 1. Contents of furfural and 5- hydroxymethylfurfural in liquid fractions at different JFC-M concentrations, and yield of pseudo-lignin

SEM Analysis

Micrographs from SEM imaging of holocellulose after hydrothermal pretreatment with and without JFC-M are shown in Fig. 2.

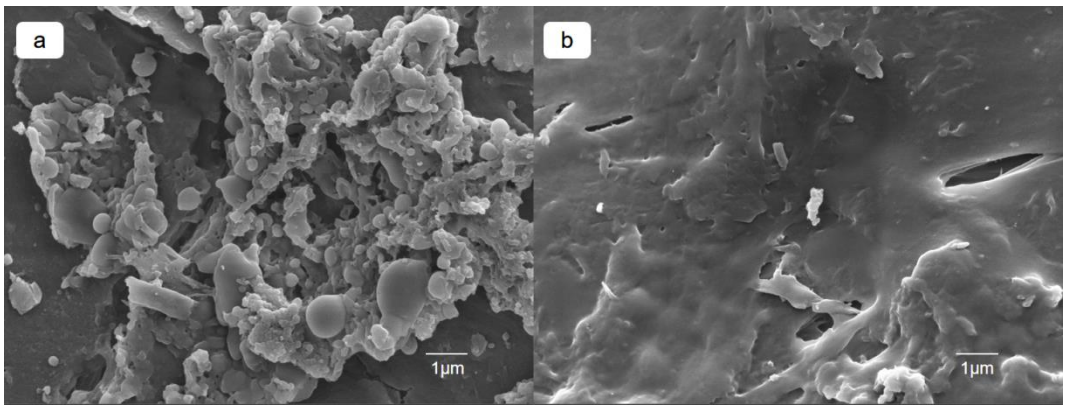

Fig. 2. The SEM image of holocellulose after hydrothermal pretreatment shows that JFC-M was not added on the left and JFC-M is added on the right.

After hydrothermal pretreatment without JFC-M, a large amount of pseudo-lignin was attached to the surface of the holocellulose in a hemispherical structure or a spherical structure. This is consistent with the phenomenon reported by Ko *et al.* (2015) and Shinde

et al. (2018), who also observed droplet-shaped pseudo-lignin on the pretreated biomass cell wall. Numerous studies have shown that drop-shaped pseudo-lignin is directly formed from the degradation of destroyed hemicellulose and cellulose (Cybulska *et al*. 2019; Lei *et al.* 2013; Liu 2015; Ma *et al.* 2015). However, after hydrothermal pretreatment with JFC-M, the whole cellulose was clean, and spherical pseudo-lignin with large particle size were hardly present. Only a small amount of spherical pseudo-lignin with small particle size adhered to the surface of the material. It can be inferred that JFC-M, as an additive, inhibited the production of pseudo-lignin to a certain extent in the hydrothermal process. Combined with the output of pseudo-lignin, the function of JFC-M was more reflected in the effective isolation between the formed pseudo-lignin and biomass.

FTIR Analysis

The FTIR spectra of holocellulose extracted from poplar, holocellulose after hydrothermal pretreatment and extracted pseudo lignin are shown in Fig. 3. The FTIR spectrum of the extracted pseudo lignin sample was obviously different from the absorption band of holocellulose. FTIR characterization showed that pseudo-lignin was composed of hydroxyl, carbonyl, and aromatic structures. The hydroxyl group was strong and wide at 3366 cm^{-1}, which indicates that there were hydrogen bonds in the extracted pseudo-lignin. The absorbances at 1608 cm^{-1} and 1509 cm^{-1} can be attributed to the conjugation of C=O with aromatic ring, while the region of 1300 to 1000 cm^{-1} corresponds to the C-O stretching in alcohol, ether, or carboxylic acid. These observations show that dehydration and aromatization of carbohydrates occurred during the formation of pseudo-lignin. In addition, bending out of the C-H plane of 859 cm^{-1} shows that the benzene ring of pseudo-lignin was 1,3,5- trisubstituted. Table 2 summarizes the above peak distribution, similar to the research of Samuel *et al.* (2010) and Ko *et al.* (2015). After hydrothermal pretreatment, the broad band associated with fatty hydroxyl groups decreased, the strength of primary hydroxyl groups decreased, and the strength of methoxy groups also decreased due to the degradation of aromatic rings and the breaking of ether bonds.

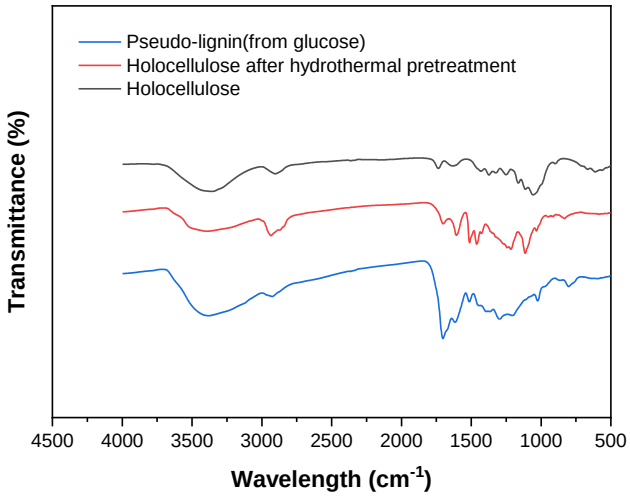

Fig. 3. FTIR spectra of holocellulose, hydrothermal pretreated holocellulose, and pseudo-lignin

Table 2. FTIR Peak Distribution

Wavelength (cm^{-1})	Assignment
3366	Stretching of O-H in alcohols, phenols or carboxylic acids
2920	Aliphatic C-H stretching
1702	C=O stretching in carboxylic acid, conjugated aldehyde or ketone
1608, 1509	Aromatic C=C stretching
1361	Aliphatic C-H vibration
1294, 1200, 1019	C-O stretching in alcohol, ether or carboxylic acid
859, 798	C-H out-of-plane bending of aromatic hydrocarbons

XRF Analysis

Table 3 shows the XRF of the hydrothermal pre-treatment material before and after the addition of JFC-M surfactant. As expected, the content of inorganic components in the hydrothermal pretreatment material was relatively low. In fact, this can be attributed to the sodium chlorite treatment of the poplar. The treatment requires repeated washing in a solution at 75 °C, resulting in the elimination of most inorganic components. The content of various inorganic substances did not show significant changes before and after the addition of JFC-M surfactant, the lower content of inorganic substances in the material may be one of the reasons.

Table 3. XRF of Hydrothermal Pretreated Materials without the Addition of JFC-M Surfactant

Element	Before JFC-M added	After JFC-M added
O	96.3260%	96.1595%
Al	0.1383%	0.1125%
Si	0.3802%	0.3438%
S	0.0152%	0.0147%
Cl	2.3123%	2.7757%
K	0.0254%	0.0214%
Ca	0.6955%	0.5180%
Fe	0.0318%	0.0235%
Zn	0.0753%	0.0309%

Effect of Surfactants on Pseudo-lignin

Pseudo-lignin is a complex substance with a molecular weight exceeding 5000, like lignin, it is essentially relatively hydrophobic, during enzymatic hydrolysis, it adsorbs enzymes, reduces their fluidity, and thus reduces their activity. This makes surfactants a good pre-treatment additive. There are relevant studies that report that surfactants can extract hydrophobic degradation products from lignin and hemicellulose, thereby enhancing the removal of lignin during the pretreatment process (Escalante *et al.* 2005). It was found in the present work that JFC-M surfactants also have a similar positive effect on pseudo-lignin. In hydrothermal pretreatment, the yield of pseudo-lignin decreased from 14.23% to 9.79%, which may be due to the interaction between surfactants and pseudo-lignin forming an emulsion, reducing the re-deposition of pseudo-lignin on the surface of biomass.

CONCLUSIONS

1. Adding JFC-M nonionic surfactant in the course of hydrothermal pretreatment, the hemicellulose content in biomass dropped from 0.99% to 0.47%, the cellulose content in biomass increased from 80.4% to 88.4%, and the pseudo-lignin yield dropped from 14.2% to 9.8%.
2. The addition of JFC-M surfactant increased the sugar recovery percentage. The glucan content in the hydrothermal pretreatment solution increased from 3.67% to 4.36%, and the xylan content increased from 17.6% to 20.1%.
3. The presence of JFC-M surfactant inhibited the formation of enzymatic by-products. In the hydrothermal pretreatment system adding JFC-M surfactant, the content of furfural and 5-hydroxymethylfurfural in the pretreatment liquid decreased to 1.18% and 0.33%, respectively.

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Article submitted: January 19, 2024; Peer review completed: February 11, 2024; Revised version received and accepted: February 27, 2024; Published: March 13, 2024.
DOI: 10.15376/biores.19.2.2714-2723