PRETREATMENT OF FURFURAL RESIDUES WITH ALKALINE PEROXIDE TO IMPROVE CELLULOSE HYDROLYSIS. CHARACTERIZATION OF ISOLATED LIGNIN

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Furfural residues were pretreated with alkaline peroxide (AP) to remove lignin and then hydrolyzed to produce glucose. The maximum glucose yield – of 92.38% – was achieved when the pretreated sample with a lignin removal of 56.7% was hydrolyzed for 120 h at an enzyme loading of 25 FPU/(g cellulose), which was 48.3% higher than that attained from the raw materials. However, the glucose yield decreased when the removal of lignin exceeded 56.7%. The effect of enzyme loading on enzymatic hydrolysis was also investigated. In addition, three AP lignin fractions were successively isolated and comparatively characterized by gel permeation chromatography, Fourier transform infrared spectroscopy, ^{13}C-nuclear magnetic resonance spectroscopy and thermal analysis. Depolymerization, including destruction of the aromatic skeleton in lignin, and repolymerization of AP lignin occurred during peroxide delignification; however, depolymerisation dominated the reaction, especially at high pretreatment severity. It was also found out that the AP treatment increased the thermal stability of the lignin fractions.

Keywords: alkaline peroxide pretreatment, furfural residues, enzymatic hydrolysis, lignin removal, lignin structure

INTRODUCTION

In search of a suitable alternative energy source to the fast depleting fossil fuel and on seriously considering the environmental issues of today, growing concerns over renewable bioethanol have led to intense research. However, about 60% of the global bioethanol production comes from sugarcane and only 40% from other crops. These conventional sources are unable to meet the worldwide ethanol demand, given the primary value of food and feed, so that the debate between 'food' and 'fuel' will go on.[1] Second-generation biofuels, globally available as agricultural and forest waste residues, may reduce global carbon emissions. Therefore, agricultural wastes, known as cost-effective, renewable and abundant, will be attractive feedstocks for bioethanol production.[2]

Commercial furfural production facilitates the use of corn cobs as a feedstock. Corn cobs are heated under acidic conditions to hydrolyze arabinoxylans (hemicelluloses) into sugars, after which the sugars are converted into furfural. The cellulose and lignin present in cobs are relatively stable under these conditions, so that the residues are enriched in cellulose and lignin. The furfural residues recovered from commercial furfural production contain approximately equal portions of lignin and cellulose. Compared to other lignocellulosic materials, the furfural residues are almost free of hemicelluloses, which is beneficial

for enzymatic hydrolysis.[3] Furthermore, they can be processed either chemically or biologically by breaking the chemical bonds to produce glucose for bioethanol. It has been estimated that about 2.3 million tons of furfural residues are annually available in China, but only small amounts are used as a raw material for boilers.[4] Furfural residues have the potential to reduce the cost of producing ethanol, because they are less expensive than other lignocellulosic materials. Studies on the utilization of this material have shown its potential as a source of biomass.[5]

One of the first considerations of such an ethanol production is the efficient generation of a fermentable hydrolyzate, rich in glucose, from the cellulose present in the biomass feedstock. However, the physical and chemical barriers caused by the close association of cellulose, hemicelluloses and lignin hinder the hydrolysis of cellulose to fermentable sugars.[6] Previous works[6,7] have suggested that the presence of lignin decreases the yield of cellulose hydrolysis, mainly because of the significant robustness of the lignocellulosic biomass to microbial and enzymatic deconstruction. To increase the liability of cellulose to the enzymatic attack, the lignocellulosic substrates are subjected to a proper pretreatment for the eventual fermentation to ethanol. Previous papers[9] have reported that the lignin content, biomass-crystallinity-dominated digestibility and lignin removal greatly enhanced the ultimate hydrolysis extent. The rate and extent of cellulose enzymatic hydrolysis give the measure of the effectiveness of the pretreatment. As to the variables affecting enzymatic hydrolysis, great attention should be paid to the lignin content, which is a significant factor influencing glucose yield. A challenging aim of the current research is the relationship between the extent of delignification and the enzyme saccharification of lignocellulosic substrates for hydrolysis. Hence, much research work is still required for making the whole conversion process effective, economical and efficient.

Recently, much attention has been paid to the application of the alkaline peroxide (AP) pretreatment, viewed as a simple, efficient and environmentally friendly method for enhancing the enzymatic hydrolysis of lignocellulosic crop residues.[10] The objective of the present study was to demonstrate the relationship between the extent of delignification and the enzyme saccharification of furfural residue after the AP pretreatment. The physico-chemical analyses of lignin isolated from AP were also discussed.

EXPERIMENTAL

Preparation of raw materials

The furfural residues used as raw materials were kindly supplied by the Chunlei Furfural Corporation (Hebei, China). The residues, with an initial pH of 2 to 3, were immersed in a 1% NaOH solution for half an hour, then washed with freshly distilled water until neutral. The samples were dried at 50 °C for 12 h and milled, to give a size under 40 meshes. All materials were stored in sealed bags at room temperature until further processing.

Alkaline peroxide pretreatment

The furfural residues were slurried in water (3.33%, w/v), containing 1.0-1.6% hydrogen peroxide and adjusted to pH 11.5 with 10 M sodium hydroxide. The pretreatment was conducted in a water bath at 80 °C for 1 h. No further adjustments of pH were made during the reaction. Samples with different lignin contents were obtained after being treated with various H_2O_2 levels. The insoluble solids were separated from the liquor fractions following the pretreatment with a Buchner funnel fitted with glass fibre filters. Before enzymatic hydrolysis, the solid material was washed with distilled water until neutral pH, then stored in a sealed plastic bag at 4 °C. The samples with different contents of lignin obtained by treatment with 1.0, 1.3, 1.6% H_2O_2 (w/v) were labelled as M_1, M_2 and M_3. For comparative purposes, M_0 represented the raw material of neutralized furfural residue without delignification.

Enzymatic hydrolysis

The solid residue obtained from the pretreatment was further submitted to enzymatic hydrolysis. Each enzyme hydrolysis treatment was performed at 47 °C, pH 4.8 for 120 h, with a substrate concentration of 2.5% (w/v), the cellulase loading ranging from 6 to 25 FPU/(g cellulose). The hydrolysates (about 2 mL) were withdrawn and the enzymatic hydrolyses were stopped

by heating the samples at 100 °C for 10 min. Further on, the samples were centrifuged at 10000 *g* for 10 min to remove the insoluble materials. Commercial cellulase was provided by Xiasheng Corporation (Ningxia, China) with a filter paper unit (FPU) activity of 90 FPU/g. Sugar analysis was carried out by HPAEC (High Performance Anion-Exchange Chromatography, Dionex ICS3000, USA) with a pulsed amperometric detector (PAD) and an ion exchange Carbopac PA-20 column. All experiments were performed at least in duplicate, and the standard errors or deviations observed were lower than 5%.

Analysis of biomass composition and crystallinity

The contents of all samples were measured by the National Renewable Energy Laboratory (NREL) lignin analysis method for biomass.[11] The glucose yield was determined and calculated by NREL methods.[12] The crystallinity of the hydrolyzed AP pretreated furfural residue was detected by wide-angle X-ray diffraction (XRD) on a XRD-6000 instrument (Shimadzu, Japan) operated at 40 kV and 200 mA. The specimens were scanned at a speed of 2°/min from 5° to 60° with a step size of 0.2°. Crystallinity, determined as the percentage of crystalline material in the biomass, was expressed as the crystallinity index (Cr_I):

$$Cr_I = [(I_{002} - I_{am})/ I_{002}] \times 100$$

where I_{002} is the intensity of the crystalline portion of biomass at about $2\theta = 22.5°$ and I_{am} is the intensity of the amorphous portion at $2\theta = 18.7°$.

Lignin extraction and characterization

After the AP pretreatment, the filtrate was adjusted to pH 7.0 with 6 M HCl and then concentrated. The concentrated solution was acidified to pH 2.0 by addition of 6 M HCl, and the dissolved lignin was centrifuged. The lignin samples were labelled as L_1, L_2 and L_3, corresponding to the samples obtained from the hydrogen peroxide pretreatment of M_1, M_2 and M_3, respectively. Milled wood lignin from furfural residues (MWL) was used for comparative purposes.

Characterization of AP lignins

The molecular-average weights of the lignin fractions were determined by gel permeation chromatography (GPC) on a PL-gel 10 mm Mixed-B 7.5 mm ID column. The injection volume was of 20 μL, and the samples were dissolved in tetrahydrofuran at a concentration of 0.2%. The column was operated at 30 °C and eluted with tetrahydrofuran at a flow rate of 1 mL/min. Monodisperse polystyrene was used as a standard for the molecular weight of lignin. The UV spectra were recorded on an ultraviolet/visible spectrophotometer (Techcomp, UV2300) from a 5 mg sample dissolved in 0.1 mol/L NaOH solution (10 mL). A 1 mL aliquot was diluted to 10 mL with deionized water, and the absorbance between 250 and 400 nm was measured. The FT-IR spectra were obtained on a FT-IR spectrophotometer (Tensor 27, Bruker, Germany) using a KBr disc containing 1% finely ground lignin samples. The solution-state ^{13}C-NMR spectrum was recorded on a Bruker MSL-300 spectrometer at 74.5 MHz, for 250 mg of sample dissolved in 1 mL DMSO-d_6 after 30000 scans. A 60 °C pulse flipping angle, a 3.9 μs pulse width, a 0.85 s acquisition time and 1.2 s relaxation delay time were used. Thermal analysis of the lignin preparations was performed by thermogravimetric (TGA) and differential thermal (DTA) analyses, on a simultaneous thermal analyzer (TGA-60, Shimadzu, Japan). The apparatus was continuously flushed with a nitrogen flow of 20 mL/min. The sample, weighing between 8 and 10 mg, was heated from atmospheric temperature to 800 °C at a rate of 10 °C/min.

RESULTS AND DISCUSSION

Compositional analysis

The composition of furfural residue resulting from various AP procedures was expressed as the percentage of dry material (Table 1). As expected, the data indicated that the non-pretreated furfural residue (M_0) was mainly made up of cellulose and lignin, which occupied 45.0 and 48.4% by weight, respectively.

Table 1
Chemical composition of raw materials and pretreated samples

Component (%)	M_0	M_1	M_2	M_3
Cellulose	45	61.4	76.4	85.3
Hemicelluloses	3.6	0	0	0
Lignin	48.4	32.6	21.0	11.9
Lignin removal	0	32.6	56.7	75.3

M_0 – furfural residue, M_1, M_2 and M_3 – furfural residue treated with 1.0, 1.3 and 1.6% H_2O_2 (w/v), respectively; Values for cellulose, hemicellulose and lignin are expressed as dry matter content

It was shown that hemicellulose accounted for only a small portion of the furfural residue (3.6%), which was significantly lower than that of corn cob,[13] meaning that hemicelluloses were mostly removed through acid pretreatment during furfural production. Hemicelluloses in biomass have been cited as barriers to cellulase activity[3]. Hence, furfural residues will be a potential alternative source of biomass. Table 1 shows that the pretreatment of furfural residues with 1.0, 1.3 and 1.6% hydrogen peroxide at pH 11.5 for 1 h at 80 °C resulted in the removal of 32.6, 56.7 and 75.3% of the original lignin, respectively. One may also observe that the cellulose content strongly varied with each condition employed (from 45 to 85.3%). In contrast, the levels of lignin decreased from 48.4 to 11.9%, along with an increase of H_2O_2 addition.

Effect of lignin content and cellulase loading on enzymatic hydrolysis

The effect of the pretreatment with hydrogen peroxide on enzymatic cellulose conversion of furfural residues is shown in Figure 1. Enzymatic hydrolysis of cellulose is ultimately influenced by the pretreatment severity.[14] Moreover, the degree of lignin removal reflects the severity of the AP pretreatment. A comparison between the glucose yield after enzymatic hydrolysis of the AP pretreated samples and the raw material of furfural residues clearly showed that the AP pretreatment increased the glucose yield for all pretreated samples, which could be also found in different lignocellulosic materials during delignification.[15]

A maximum glucose yield of 92.38% was achieved when the pretreated sample with a lignin removal of 56.7% was hydrolyzed for 120 h at an enzyme loading of 25 FPU/(g cellulose), which was 48.3% higher than that of the raw material. This strongly indicated that AP delignification significantly enhanced the enzymatic hydrolysis of furfural residues, a possible interpretation being that delignification led to a dramatic increase of fibre swelling and of the cellulose surface area available to the enzyme. Indeed, the effective concentration of cellulase was also increased by reducing the non-specific absorption of enzymes.

According to Silverstein *et al*.,[16] the AP pretreatment of furfural residues can increase cell wall porosity through delignification, breaking down some ester bonds cross-linking lignin and causing swelling of microfibrils. The experimental data obtained in the present study show that the yield of glucose increased with the decrease in lignin content. The increase in glucose yield may be the result of the increased accessible surface area of particles, which in turn increases the accessible area for the enzymatic attack. The AP pretreatment also appeared to enhance the entry to the porous inner surface, which could give rise to increased enzyme–substrate interactions. In contrast, further reduction of the lignin content (from 21.0 down to 11.9%) decreased the enzymatic glucose yield (from 92.38 down to 86.90%) after 120 h of enzymatic hydrolysis. One possible reason for this phenomenon was the formation of collapsed pores when the lignin was extensively removed, the reduction of the available surface area for enzyme adsorption thus resulting. The results of the present investigation are probably related to the method used for removing the lignin, as the hydroxyl groups and reducing end groups of cellulose could be oxidized during the AP pretreatment. Chemical oxidation of the reducing ends of cellulose could negatively impact the interaction between the reducing end and targeting cellobiohydrolase, while cellobiose accumulation exerted an enhanced influence upon enzymatic digestion. In addition, in the absence of hemicelluloses, lignin acts as a spacer among the cellulose microfibrils, while lignin removal allows the aggregation of the neighbouring cellulose fibrils into a less hydrolysable form.[17]

These results reveal that lignin removal favoured cellulose conversion.

However, it is not necessary to promote a complete removal of lignin to achieve high glucose yields of furfural residue. Previous research clearly showed that selective lignin removal from the wood cell walls had a direct influence on the levels of lignin removal, on also increasing the digestibility of the remaining material by cellulases.[18-20] As seen from the GPC, depolymerisation dominated the reaction, causing some lignin fractions with low molecular mass. However, these lignin fractions – such as phenol – exerted a negligible influence on adsorption. With further increase of pretreatment severity, the low molecular mass compounds were thought to have a significant impact on enzyme adsorption. Nevertheless, the results obtained in this work show that the reduction of the lignin content really has the potential to enhance the enzymatic glucose release. To further increase the hydrolysis yield, binding of enzymes to the substrate needs to be taken into consideration. Porous lignocellulosic substrates are highly complex and require further study.

In the ethanol production from lignocellulosic materials, all factors hindering the access of the hydrolytic enzymes to polysaccharides would increase the production cost.[21,22] It has been suggested[23] that the pretreatment, fractionation and hydrolysis of lignocellulosic substrates represent 60% of the overall cost of producing ethanol, while the cost of cellulase reached 50%. Consequently, the effect of cellulase loading on enzymatic hydrolysis was also studied. The AP sample M_2 was treated with enzyme loading ranging from 6 to 25 FPU/(g cellulose) (Fig. 2). The experimental results revealed that the glucose yield was improved by increasing enzyme loading. The highest glucose yield, of 92.38%, was obtained at an enzyme loading of 25 FPU/g and a reaction time of 120 h. The results indicated that a small amount of the available sites for enzyme absorption on cellulose is still free, and also that the glucose yield could be enhanced by gradually increasing cellulase loadings.

Crystallinity

The crystallinity of furfural residues and AP samples has been extensively investigated by X-ray diffraction (XRD). Figure 3 illustrates the effect of the lignin content on the crystallinity of samples before and after 120 h of enzymatic hydrolysis. The cellulose-rich fractions of M_1, M_2 and M_3 obtained from AP samples were found to have crystalline indexes of 38.85, 42.32 and 44.81%, respectively, higher than that of the non-pretreated material (36.37% for M_0). It was observed that the crystallinity of samples increased as lignin removal gradually increased. On the other hand, a general decrease in crystallinity could be observed for all hydrolyzed samples (120 h), which was due to the hydrolysis of cellulose in the substrates (Fig. 3).

The diffraction patterns (Figures not shown) are typical for the crystalline structures of cellulose I, with the main diffraction signals at 2θ values of 16.3°, 22.5° and 34.6°, normally assigned to the diffraction planes $10\bar{1}$, 002 and 040, respectively. For the delignified samples, the crystalline size was higher than that of the raw material. The diffraction intensity of the delignified samples was higher at all lattice planes than that of the raw materials, which coincided with the data on the increased crystallinity degree. The $10\bar{1}$ and 002 lattice planes were regarded as the amorphous zone and crystalline zone diffraction peak, respectively. These observations indicated that hydrolysis occurred simultaneously in the crystalline and amorphous zones.

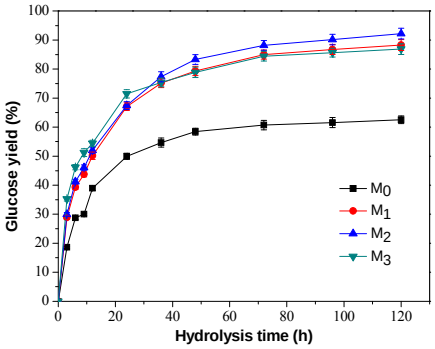

Figure 1: Enzymatic hydrolysis of furfural residues (M_0) and delignified samples (M_1, M_2 and M_3 – furfural residues treated with 1.0, 1.3 and 1.6% H_2O_2 (w/v), respectively

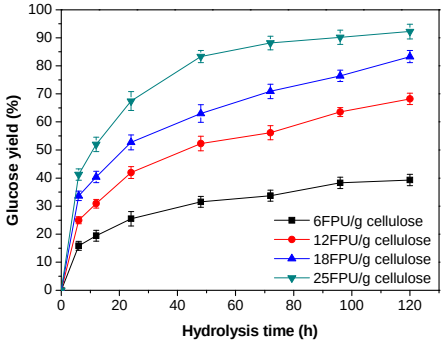

Figure 2: Effect of cellulase loadings on the enzymatic hydrolysis of furfural residues treated with 1.3% H_2O_2 (w/v) (M_2)

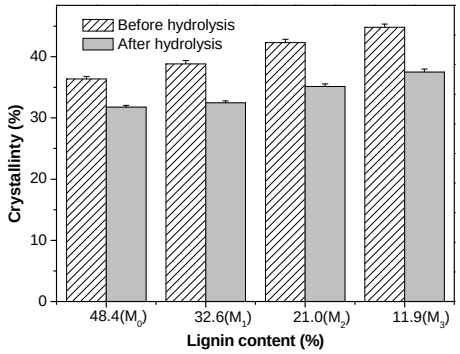

Figure 3: Effect of lignin content on crystallinity of samples before and after enzymatic hydrolysis for 120 h. M_0 – furfural residues, M_1, M_2 and M_3 – furfural residues treated with 1.0%, 1.3% and 1.6% H_2O_2 (w/v), respectively

Lignin structure

Molecular weight distribution

The values of the weight-average ($\overline{M}_w$) and number-average ($\overline{M}_n$) molecular weights, calculated from the gel permeation chromatography (GPC) curves, and the polydispersity ($\overline{M}_w/\overline{M}_n$) of the four lignin preparations are given in Table 2. The molecular weight distribution (MWD) curves of the lignin samples, plotted in Figure 4, show that the maximum molar mass value, which stood for the largest ratio of lignin with such molecular mass, was of about 1000 g/mol in the separated samples, as compared to 2500 g/mol for furfural residues milled wood lignin (MWL). The molecular-average weights of L_2 and L_3 were much lower than the MWL. All these results indicated a substantial degradation of the lignin polymers by cleavage of some amounts of β-O-4′ linkages between the lignin precursors during the AP treatment under the applied conditions. AP lignins presented a broad molecular weight distribution and, in particular, the molecular-average weight of L_1 (11213 g/mol) was much higher than that of MWL, indicating that repolymerization had taken place during the alkali peroxide pretreatments. As the radical reactions are the dominant effects of peroxide lignin removal, the compounds depolymerized from the lignin fractions under AP conditions re-reacted with parts of high molecular weight through the *β*-1 or *α*-1 repolymerization

mechanism, causing some lignin fractions with high molecular mass, even higher than 10000 g/mol. However, depolymerization dominated the reaction, especially the further increase of pretreatment severity, a result consistent with the values of thermal stability. The polydispersity of L_1 was much higher than that of MWL (Table 2). The data indicate that lignin extraction under low severity of the AP conditions led to a dissolved lignin fraction with a broader molecular weight distribution, comparatively with the high severity of the AP conditions here applied. Furthermore, the exceeding data on L_1 polydispersity may be also attributed to the lignin-degraded substances formed during delignification. However, the polydispersities of the other samples were lower than that of MWL, which suggests that the homogeneity of these lignin samples improved after pretreatment under highly severe AP conditions, which was good for a comprehensive utilization of the lignin resources.

Table 2

Weight-average ($\overline{M}_w$) and number-average ($\overline{M}_n$) molecular weight and polydispersity ($\overline{M}_w/\overline{M}_n$) of acid-insoluble lignin preparations

	Lignin fractions			
	MWL	L_1	L_2	L_3
$\overline{M}_w$	2831	11213	1405	1348
$\overline{M}_n$	2132	854	735	712
$\overline{M}_w/\overline{M}_n$	1.33	13.12	1.91	1.89

MWL – milled wood lignin from furfural residues; L_1, L_2 and L_3 – lignin fractions released by alkali peroxide from furfural residues treated with 1.0%, 1.3% and 1.6% H_2O_2 (w/v), respectively

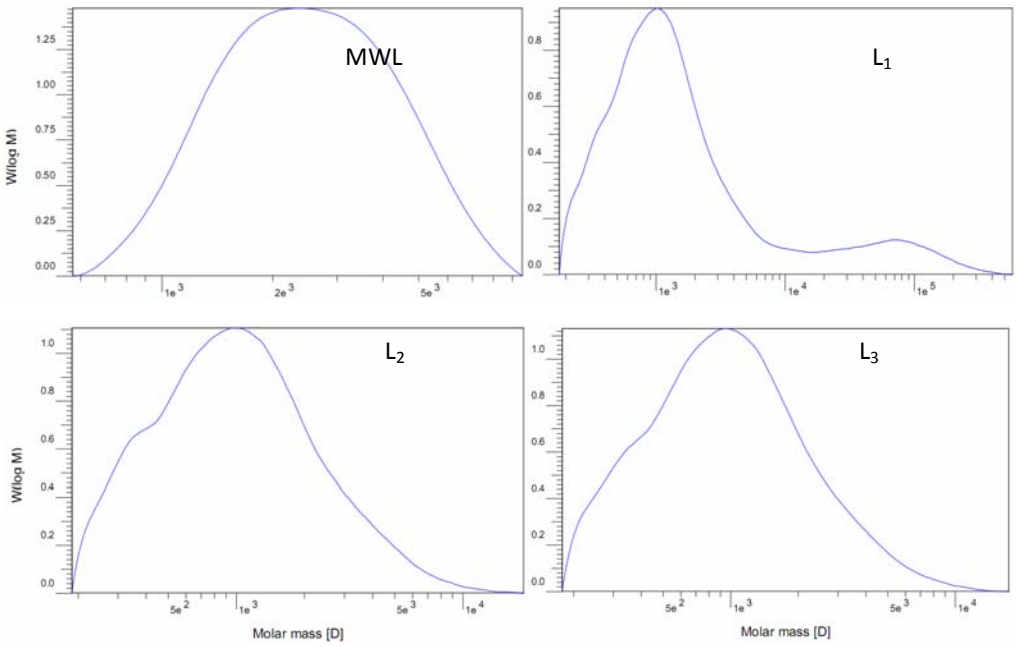

Figure 4: Molecular weight distribution curves of milled wood lignin from furfural residues (MWL) and lignin fractions released by alkali peroxide from furfural residues treated with 1.0, 1.3 and 1.6% H_2O_2 (w/v) (L_1, L_2, and L_3)

UV absorption

The absorption coefficients at various wavelengths in the ultraviolet region of L_2 and MWL are illustrated in Figure 5. Obviously, the MWL fraction showed that the basic UV spectrum of typical lignin with a maximum at 280 nm originated from non-conjugated phenolic groups (aromatic ring), as well as the absorptions at 350 nm, providing information on the conjugated phenolic structures.[24] The absorption coefficient at 250-320 nm slightly increased, indicating that more ether linkages between lignin and polysaccharides could be cleaved, and also that aromatic ring degradation occurred during the pretreatment. Hydroxycinnamic acids, such as *p*-coumaric and ferrulic acids, seemed to be strongly linked to lignin molecules, in which *p*-coumaric was found to be bonded to lignin by ester linkage, while the ferrulic acid was linked by its phenolic group *via* an ether bond to lignin, and mainly by its carboxyl group *via* an ester bond to lignin.[25] The much lower absorption of lignin fraction L_2 at 310-320 nm suggested that the L_2 lignin fraction was free of conjugated phenolic structures. It was stated that the AP pretreatment cleaved most of the ester or ether linkages between lignin and hydroxycinnamic acids.

FT-IR spectra

Figure 6 shows the FT-IR spectra of the furfural residue lignin fractions extracted with 1.0, 1.3 and 1.6% H_2O_2 at pH 11.5 for 1 h at 80 °C, as well as that of the MWL. The spectra of the AP lignin fractions and MWL were rather similar, except for the intensities of the absorption bands, supporting the previous finding that the overall structure of lignin had been maintained after the AP treatment.[25] As shown in Figure 6, the lower absorption intensity in AP treated fractions spectra at 3418 cm^{-1} originates from the O-H stretching vibration in aromatic and aliphatic OH groups, comparatively with MWL, which may be attributed to the high oxidation and degradation power of furfural residue during the AP pretreatment. The 3000-2820 cm^{-1} region of the C-H stretching showed only small differences in the methyl and methylene groups. The intensities of these bands, mainly attributed to the methoxyl groups in lignin spectra, were substantially lower for pretreated lignin. The band at 1710 cm^{-1} has been assigned to unconjugated ketone and unconjugated carbonyl stretching, while the band at 1640 cm^{-1} has been attributed to carbonyl stretching conjugated with aromatic rings. A significant difference between the AP lignin and MWL was represented by the absorptions at 1604, 1513 and 1424 cm^{-1}, which stood for aromatic skeleton vibrations. These peaks were not as intense as those in the MWL, which suggested that the aromatic skeleton of AP lignins was destroyed. Simultaneously, the phenomenon appeared at 1460 cm^{-1}, which indicated C-H deformations and aromatic ring vibrations, as well as at 835 cm^{-1} from aromatic C-H out-of-bending. This result implies significant oxidation of the lignin structure during the AP pretreatment.

A characteristic weak band at 1326 cm^{-1}, which was absent in the spectra of L_1, L_2 and L_3, is assigned to syringyl ring breathing with C-O stretching. Clearly, the spectra of pretreated lignins appeared at weak absorption, at 1123 cm^{-1}, which was caused by the aromatic CH in-plane deformation for the syringyl (S) type. In the meantime, comparatively with MWL, the absorption intensity of the AP lignin samples at 1264 cm^{-1} decreased, which represents guaiacyl (G) ring breathing with C-O stretching.

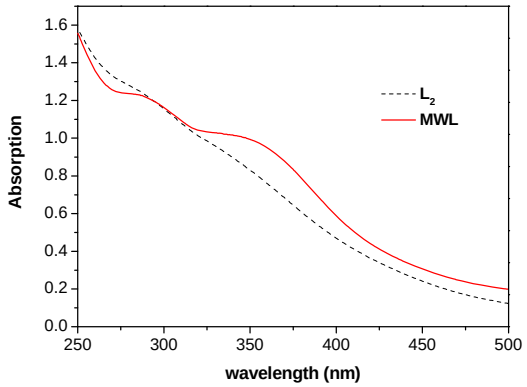

Figure 5: UV spectra of milled wood lignin from furfural residues (MWL) and lignin fraction released by alkali peroxide from furfural residues treated with 1.3% H_2O_2 (w/v) (L_2)

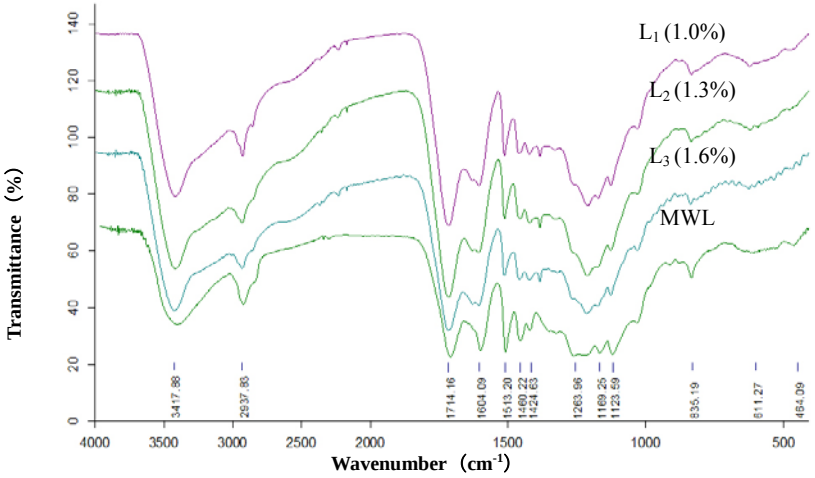

Figure 6: FT-IR spectra of milled wood lignin from furfural residues (MWL) and lignin fractions released by alkali peroxide from furfural residues treated with 1.0, 1.3 and 1.6% H_2O_2 (w/v) (L_1, L_2 and L_3)

^{13}C-NMR spectra

The three lignin preparations, L_1, L_2 and L_3, were investigated by ^{13}C-NMR spectrometry (Fig. 7). Most of the observed signals have been previously assigned in wood lignin spectra.[25-27] Two slight signals could be observed between 171 and 173 ppm. This behaviour was previously reported by Sun *et al*.[25] for evidencing the aliphatic carboxyl. These signals could therefore have resulted from the oxidation of the lignin side chains by AP treatment. In the aromatic region (153 to 103 ppm), the G units produced signals at 148.2 ppm (C-3, etherified), 147.3 ppm (C-4, etherified), 135.1 (C-1, etherified), 115.4 ppm (C-5) and 111.3 ppm (C-2), while the S units were detected by signals at 153.3 ppm (C-3/C-5, etherified), 138.1 ppm (C-4, etherified), 130.1 ppm (C-1, etherified) and 104.3 ppm (C-2/C-6). The *p*-hydroxyphenyl (H) units appeared as two signals at 128.9 and 129.4 ppm (C-2/C-6). All these facts demonstrated that the AP lignins of furfural residue contain three basic structural units *i.e*. H, G and S. However, the peak intensities of the S units are rather weak, indicating a trace amount of S in the lignin fractions.

The absence of signals between 57 and 104 ppm indicated a low concentration of residual sugars in these lignin preparations. A small signal

present at 71.7 ppm contributed to the common carbon-carbon linkages (*β*-*β*′). In the spectra, the signals of the *β*-5′ structure were found only as traces. Furthermore, three slight *β*-O-4′ signals could be observed at 86.2, 72.3 and 60.2 ppm. The strong signal at 56 ppm was attributed to the $–OCH_3$ groups in the S and G units. In addition, the spectra did show two signals between 14.1 and 33.8 ppm, assigned to the *γ*-methyl, as well as the α-and β-methylene groups in the n-propyl side chains of the lignin preparation. The treatment with AP (1.0-1.6%), performed under the given conditions, was shown to attack the ether structure to a significant extent. Furthermore, as the concentration of hydrogen peroxide increased from 1.0 to 1.6% (w/v), the signals for the S units had relatively weaker intensities and, conversely, the signals for G units exhibited stronger intensities.

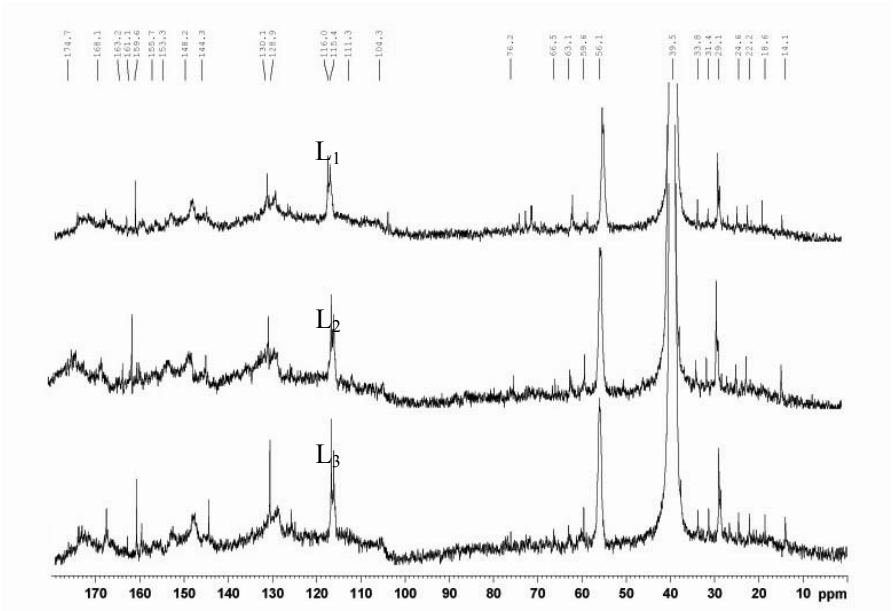

Figure 7: ^{13}C-NMR spectra of lignin fractions released by alkali peroxide from furfural residues treated with 1.0, 1.3 and 1.6% H_2O_2 (w/v) (L_1, L_2 and L_3)

Thermal stability

Figure 8 shows the thermal gravimetric (TGA) and differential thermal (DTA) analyses for L_1, L_2 and L_3, respectively. The analysis of the TGA curves shows that the thermal degradation of lignins proceeds over a wide temperature range (100-800 °C), which might be a consequence of the activity of the chemical bonds and functional groups in lignin.[28] At temperatures lower than 120 °C, weight loss occurred as a result of evaporation of the free water. Major pyrolysis occurred between 200 and 600 °C. In the temperature range from 290 to about 430 °C, L_1 showed a higher thermal degradation than the others, indicating that the thermal stability of the lignin fractions was increased by AP severity. In addition, the final yields of the solid residue for L_1, L_2 and L_3 were of 24, 40.5 and 43%, respectively. The DTA curves had remarkable peaks at temperatures higher than 350 °C for L_1, L_2 and L_3 (Fig. 8). Only one peak in each lignin DTA curve indicated that decomposition occurred in one step. These reaction steps have been commonly assigned[29] to lignin characteristic decomposition, which has a flat endothermic peak at 425 °C. In DTA, L_1 showed a single and broad endothermic peak at 450 °C, which was higher than that of the peaks presented in L_2 and L_3. The result indicates that

the repolymerization of lignin fractions occurred during the AP pretreatment, so that the lignin with a higher severity of the AP pretreatment appeared to have a better thermal stability. The degradation characteristics of lignins from L_1, L_2 and L_3 at elevated temperatures were different. The differences in the inherent structures and chemical pretreatment strength possibly account for the diversities of the thermal degradation behaviours. The S, as well as the G units, were built into the lignin macromolecule mainly by ether bonds, the ether bonds between the S units being easier to split than those between the G units.[30] This may contribute to a higher thermal stability of L_3 *versus* L_1, as L_3 has fewer S units than L_1. Moreover, the G units of L_3 have easily undergone condensation and coupling reactions, which led to a high charcoal residue. Based on these analyses, it is not surprising that the charcoal residue yield of L_3 was much higher than that of L_1, which could also explain the higher thermal degradation rate of L_1.

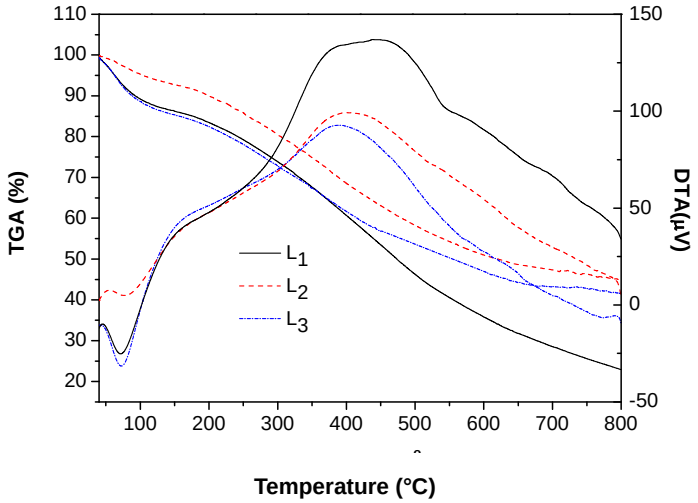

Figure 8: DTA and TGA curves of lignin fractions released by alkali peroxide from furfural residues treated with 1.0, 1.3 and 1.6% H_2O_2 (w/v) (L_1, L_2 and L_3)

CONCLUSIONS

Alkaline peroxide pretreatment of a novel lignocellulosic material, such as furfural residues, showed promising results in terms of glucose yield. The levels of lignin appeared to decrease from 48.4 to 11.9%, with an increase of H_2O_2 addition, and the 1.3% (w/v) result of the AP pretreatment showed the ability of lignin separation, reaching a 92.38% hydrolysis yield after 120 h. Possibly, the highest yield of glucose was reached at a lignin removal of 56.7%. However, the yield of glucose decreased when lignin was further removed. In addition, the three lignin preparations obtained from the AP pretreatment had similar chemical compositions, but different physico-chemical properties. Furthermore, depolymerization and repolymerization of the AP lignin occurred with an increase in the consistency of peroxide delignification. However, depolymerization dominated the reaction, especially the further increase of pretreatment severity. Lignin fractions obtained from higher AP pretreatment severity appeared to have a higher thermal stability.

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