ANALYSIS OF STRUCTURAL CHANGES OF MASSON PINE LIGNIN REACTED WITH SUPEROXIDE ANION RADICAL USING NMR SPECTROSCOPY

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Oxygen delignification can be considered to be the most important part of TCF and ECF bleaching sequences because it allows for cleaner production of pulp. During the process, oxygen gets one electron from lignin in the alkaline condition to form some active oxygen species (AOS), including a superoxide anion radical (O_2-•), which is crucial for lignin degradation without damage of carbohydrates. The reaction of O_2-• on cellulolytic enzymatic lignin (CEL) from Masson pine was studied. The change in active hydroxyl content after reaction with O_2-• was investigated using ^{31}P-NMR. After reaction, the aliphatic hydroxyl and uncondensed type phenol hydroxyl contents decreased, but the content of carboxylic group increased in Masson pine lignin. Through the analysis with HSQC-2D^{13}C-H technology, β-O-4 linkages could be cleaved by O_2-•, but β-β and β-5 linkages were observed to be more stable; benzaldehyde and cinnamic aldehyde structures could be oxidized to carboxylic acids by O_2-•. Guaiacyl units in lignin were more easily degraded than p-hydroxybenzene units.

Keywords: Masson pine; Lignin; Oxygen delignification; Superoxide anion radical; Structure change; NMR

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INTRODUCTION

Oxygen delignification is an important stage for a modern pulping plant, with which totally chlorine free (TCF) bleaching can be achieved easily (Tench and Happer 1987). Lots of research has been done to improve lignin removal (Olm and Teder 1979; Iribarne and Schroeder 1997) and the selectivity of delignification (Hartler et al. 1970; Ek et al. 1989; Yang et al. 2003; Soo et al. 2006). McDonough (1996) gave a review on the advantages and disadvantages of oxygen delignification for paper mill. The mechanism and kinetics of oxygen delignification is still a topic under research in the field of papermaking. Agarwal et al. (1999) studied the kinetics of medium-consistency oxygen delignification of southern hardwoods. They found that high levels of delignification and low final kappa number requires progressively more severe process conditions to remove refractory lignin moieties, which adversely affect pulp selectivity. Ji et al. (2009) described the role of radicals in the process of cellulose degradation during oxygen delignification. Gierer and associates did a lot of work related to the mechanism of active oxygen species; among of them the superoxide anion radical, O_2^-•, was recognized (Gierer et al. 1986, 1992, 1993, 1997). O_2^-• is favored for its reactivity with lignin without damage of carbohydrate in pulp (Gierer et al. 1997, 2001). Ek et al. (1989) considered the selectivity of oxygen delignification. Cao et al. (2007) found that increasing the proportion of the superoxide anion radical is beneficial to the selectivity of

lignin removal from pulp during oxygen delignification. The present paper provides insight into the reaction of the O_2^{-}• radical ion with lignin by analysis with ^{31}P-NMR and 2D-NMR. The information of changes in structure and functional groups is helpful to understand the degradation of lignin.

EXPERIMENTAL

Materials

Masson pine was provided by Guangzhou Paper mill. The chemicals 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane (TMDP), pyridine-d_5 (99.5%), and chromium(III) acetylacetonate (97%) were purchased from Sigma-Aldrich. Cellulase enzyme was donated by Pure Branch (Asia) Limited.

Chemical Preparation

Preparation of cellulolytic enzyme lignin (CEL) from Masson pine was carried out according to the method of Chang et al. (1975). The milled wood (10 g) was suspended in acetate buffer (100 ml, pH 4.8) and 1 mL of cellulolytic enzyme solution (50IU/mL) was added and incubated for 48 h at 50ºC. The reaction system was centrifuged, the supernatant was removed, and the residue was again suspended in acetate buffer (50 ml, pH 4.8) and treated with enzyme (1 ml) for an additional 24 h at 50ºC. The residue was again collected by centrifugation, washed with distilled water (200 ml), centrifuged, and freeze-dried. The freeze-dried residue was treated with 210 ml of pyridine/HAc/water (9:1:4, v/v) and extracted twice with $HCCl_3$ according to the method of Lundquist et al. (1983). The lignin solution in $HCCl_3$ was condensed under vacuum and dropped into ethyl ether. The precipitate lignin (CEL) was collected after drying.

Reaction with Lignin

Reaction of lignin with superoxide radical was carried out by the mixing 5mL lignin solution in DMSO and 10mL O_2^{-}• in DMSO at 20 ºC for 120 min (Hyland 1981, Han 1991). The products were freeze-dried for analysis by NMR spectroscopy.

NMR Analysis

NMR spectra were recorded on a Bruker 400-MHz spectrometer at 300 K using a 5-mm symmetrical NMR microtube and DMSO-d_6 as solvent. Chemical shifts were referenced to TMS (0.0 ppm). Chromium (III) acetylacetonate (0.01 M) was added to the lignin solution to decrease the acquisition time. The experimental conditions used were with a 90º pulse width. ^{31}P-NMR spectra were performed with lignin in pyridine-d_6 and $DCCl_3$ reacted with TMDP, in which cholesterol was the internal standard. Conditions for ^{31}P-NMR included NMR microtube 5mm, 298K, 90^{o}pulp width, acquisition time 2s, TD=16k, LB=3Hz, delay time 5s, and 228 scans. 2D ^{1}H-^{13}C correlation HSQC-NMR analysis was performed using a 5% lignin solution. Conditions used included a 90º pulse width, a 0.15-s acquisition time, and a 1.4-s relaxation delay.

RESULTS AND DISCUSSION

Analysis of Lignin with ^{31}P-NMR spectra

^{31}P-NMR is a very good technique to determine functional groups in lignin by phosphorylating the lignin with 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane, i.e. TMDP, before the determination. Functional groups in lignin, such as aliphatic hydroxyl groups, hydroxyl groups in p-hydroxyphenyl, guaiacyl, and syringyl moieties, as well as carboxyl groups can be measured qualitatively and quantitatively according to the method of Granata and Argyropoulos (1995). Lignin reacted with $O_2^{-}\cdot$, and the product was collected for ^{31}P-NMR analysis. The spectra are shown in Fig 1.

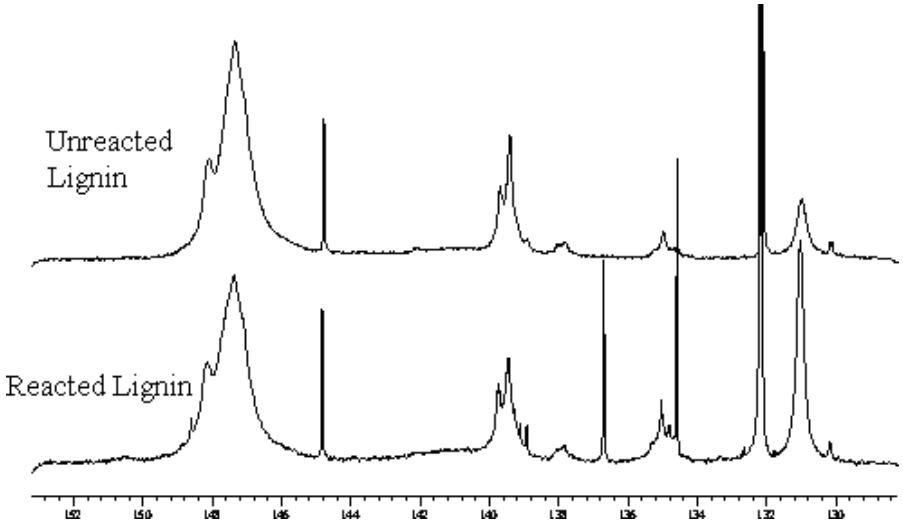

Fig. 1. ^{31}P-NMR spectra and comparison of Masson pine lignin with $O_2^{-}\cdot$ before and after reaction

A new peak appeared at δ136.7ppm in the ^{31}P-NMR spectrum for Masson pine lignin. The peak corresponds to the phenolic OH with C_α carbonyl group, which was obtained by oxidation of C_α-OH by the superoxide anion radical. The measurements corresponding to functional groups are listed in Table 1.

Table 1. Results of ^{31}P-NMR Quantitative Analysis of Masson Pine Lignin

	Unreacted lignin	Reacted lignin
OH Aliphatic	8.19	6.77
OH Condensed phenolic	0.68	1.13
OH Guaiacyl and 5-demethoxyl	1.72	1.68
OH Phenolic	0.22	0.37
OH Total phenolic	2.62	3.19
COOH	0.21	1.68

The radical $O_2^{-}\cdot$ can react with lignin from Masson pine and cause a functional group change. It was found that the content of aliphatic hydroxyl group decreased from 8.19 mmol/g to 6.77 mmol/g in lignin. The aliphatic hydroxyl groups mainly were those associated with the side chain of lignin. The reason for these decreases can be explained by the hydroxyl group on the side chain being attacked by the radical, which also can increase the content of carbonyl and carboxyl groups, as shown in Table 1. The content of guaiacyl hydroxyl groups and 5-demethoxyl guaiacyl groups decreased, which meant that this kind of lignin can be broken into small fragments or degraded. However, the *para*-hydroxylbenzene group was still stable under the reaction conditions, which is in agreement with results obtained for hard wood pulp by Soo et al. (2006).

NMR Analysis of Lignin

Lignins were analyzed by ^{13}C-NMR and 2D-HSQC spectroscopies to identify the principal intermonomeric units and to evaluate the structural changes in lignin by reaction with $O_2^{-}\bullet$. Figures 2 and 3 show the NMR spectra of Masson pine CEL and CEL treated with $O2^{-}\bullet$. There are strong signals to show the C_2, C_5, C_6 on the benzene ring with C-H bonds, C_α, C_β, C_γ in β-O-4 linkages, and also OCH_3 and COOH groups for the original lignin (CEL), which exhibited the typical features of softwood lignin.

After reaction of lignin with $O_2^{-}\cdot$, new peaks at δ174.5 and δ167 appeared in the ^{13}C-NMR spectrum. They correspond to the aliphatic carboxyl group and aromatic carboxyl group, which were probably formed from the oxidation of the side chain of lignin by the superoxide anion radical. The side chain of phenylpropane was oxidized to form a carbonyl group at C_α, and the bond between C_α and C_β could be broken, so that the the amount of carboxyl groups increased. There was much overlap of peaks in the 1D-NMR spectrum. The 2D-NMR technique can provide more detailed information about the structures of lignin. There were three areas in 2D-HSQC sprectrum corresponding to aromatic C-H, oxidized side chain, and oxygen-free aliphatic chains.

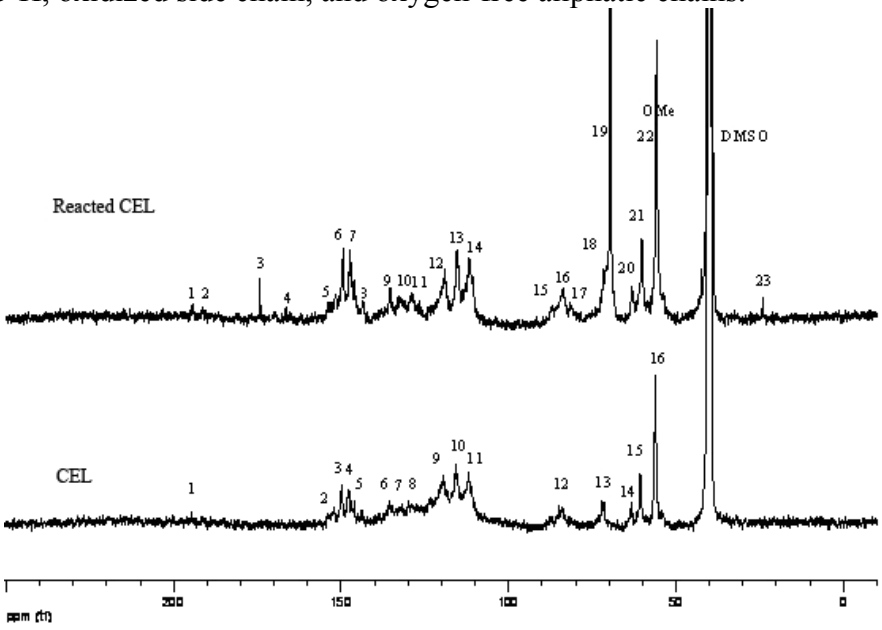

Fig. 2. C^{13}-NMR spectra of CEL and reacted CEL

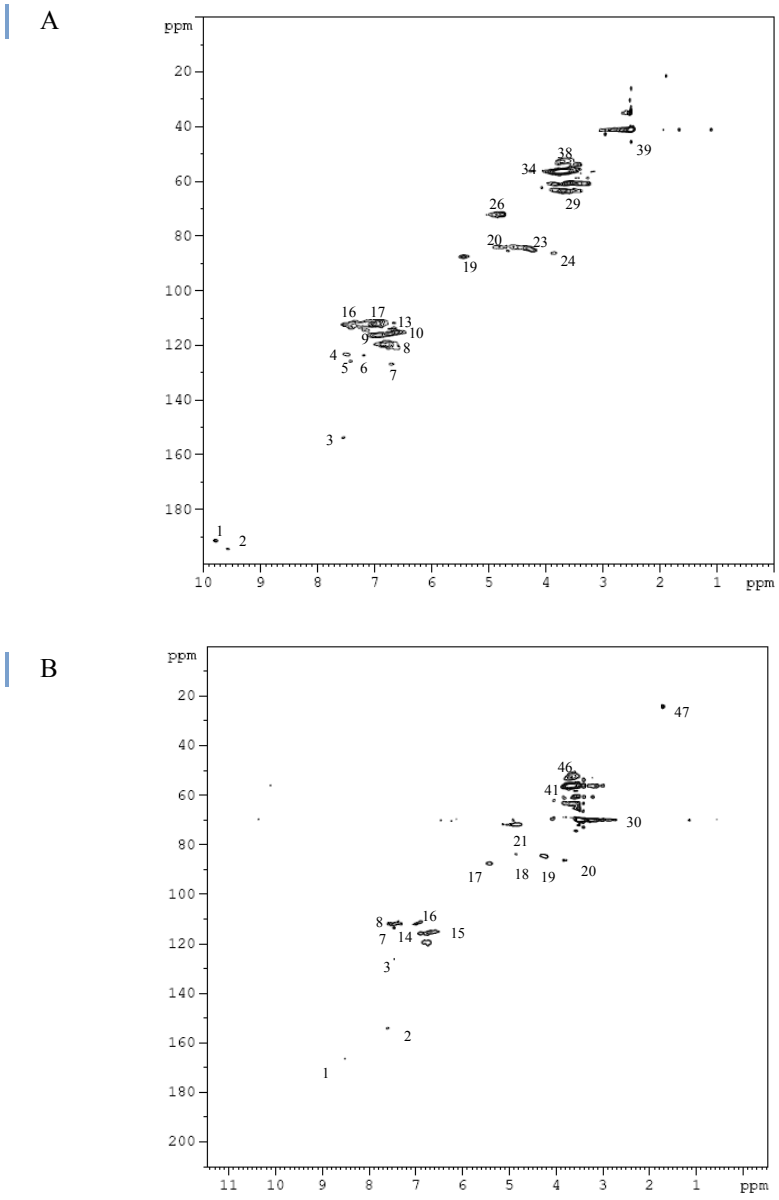

Fig. 3. C^{13}-H-NMR spectra of CEL (part A) and reacted CEL (part B)

The signals from NMR were determined to correspond to carbon in lignin, according to Balakshin et al. (2001) and Ibarra et al. (2007). Peaks appeared in the spectrum of CEL at δ191.6ppm/δ9.78ppm and 194.6ppm/9.54ppm for carbon-13 and hydrogen, corresponding to the aldehyde in benzaldehyde and cinnamaldehyd, respectively. They disappeared when the lignin was treated with O_2^{-}•. However, the Cα(δC/δH = 154.0ppm /7.57ppm) and Cβ(δC/δH=126.5ppm /6.71ppm) in cinnam-aldehyde persisted after treatment of O_2^{-}•, which means that the carbonyl group in lignin can be oxidized into a carboxyl group, but the double bond between Cα and Cβ of the aromatic ring did not break during this oxidative process.

The strong signals at δC/δH 119.8ppm/6.73ppm, δC/δH 116.1ppm/6.92ppm, and δC/δH 112.0ppm/7.0ppm corresponded to C6-H, C5-H, and C2-H, respectively. They became weak after reaction with O_2-•. The signals at δC/δH=113.0ppm/7.42ppm corresponding to the C_6-H of guaiacyl units in 5-5' linkage structures appeared in both spectra of CEL and reacted CEL. The signals at δC/δH=125.7ppm/7.41ppm corresponded to the C_6-H of oxidized guaiacyl units in 5-5' linkages. The results indicated that 5-5' linkage structures of guaiacyl in Masson pine lignin exist and are stable under oxygen delignification.

In the spectrum of CEL (Fig 3, A), the signals at δC/δH=71.9ppm/4.75ppm, δC/δH=85.0ppm/4.28ppm, δC/δH=60.9ppm/3.83ppm, and 60.9ppm/3.58 ppm correspond to Cα-H, Cβ-H, and Cγ-H of side chains of guaiacyl units lignin in β-O-4 linkages. When lignin was oxidized by superoxide anion radical, these signals became very weak (Fig 3, B). The location of the signal for Cβ-H changed to δC/δH=82.0ppm/4.70ppm, for Cγ-H changed to δC/δH=65.0ppm/3.52ppm, and the signal for Cα-H disappeared because it was oxidized and changed into an α-carbonyl group, which can cause a chemical shift to low field. The structure of dibenzodioxocin units in Masson pine lignin also was evident, and it did not degrade during the treatment with O_2-•.

Phenylcoumaran units in β-5 linkage and resinol units in β-β linkage are stable structures in the Masson pine lignin. In the HSQC spectrum of CEL, the signals at δC/δH =87.6ppm/5.42ppm, 52.5ppm/3.66ppm, and 63.6ppm/3.67ppm came from Cα-H, Cβ-H and Cγ-H of phenylcoumaran units. There are peaks at δC/δH=83.5ppm/4.85ppm, 53.7ppm/3.43ppm, and 70.0ppm/3.50ppm relating to Cα-H, Cβ-H, and Cγ-H of resinol units in the lignin. These signals still existed after reaction of CEL with oxygen radical species.

The side chain of lignin preparation from wood by the cellulolytic enzyme method remained after the enzymatic treatment, as is shown in Fig. 3 A. Most of the peaks disappeared after the lignin was oxidized with oxygen radical species. This disappearance is attributed to the breakage of Cα-Cβ chains in lignin. However, a peak at δC/δH =24.4ppm/1.7ppm was stable, which corresponds to the phenyl-CH_2-phenyl structure.

CONCLUSION

The superoxide anion radical, O_2^-•, is one of the important active oxygen species in the process of oxygen delignification. The reaction of a lignin preparation with O_2^-• can show the possibility of lignin degradation by this kind of radical species. The ^{31}P-NMR technique can show that the changes of hydroxyl groups, including the oxidation of aliphatic hydroxyl groups, phenolic hydroxyl groups, and hydroxyl groups into carboxyl moieties. The results showed that the content of aliphatic hydroxyl groups in CEL decreased after treatment with O_2^-•, but carboxyl groups increased. The hydroxyl groups in guaiacyl structures decreased, but there were increases in condensed lignin structures and para-hydroxybenzene units, which meant that the guaiacyl units in lignin are susceptible to cleavage under oxygen-active radical species, but not the para-hydroxy-benzene units.

^{13}C-NMR and 2D-HSQC-NMR were used to analyze the changes of structures in lignin. The Cα hydroxyl group in β-O-4 structures and the aldehyde in lignin can be oxidized into carbonyl and carboxyl groups, respectively, under the O_2^{-}• treatment. Phenylcoumaran units in β-5 linkage and resinol units in β-β linkage are stable during the treatment of O_2^{-}•, which means that these parts of lignin cannot be removed under oxygen delignification.

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