CELLULOSE CHEMISTRY AND TECHNOLOGY

BIOMIMETIC OXIDATION OF LIGNIN IN PULP BY SIMPLE INORGANIC COMPLEXES

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In recent years, the growing concern about the fate of chlorinated organic compounds in the bleaching area determined a higher demand of new bleaching technologies. Several well-known systems, such as peroxydisulfate plus Cu(II), Co(II), Ag(I), Fe(II) and Mn(II), have been successfully applied in the oxidative degradation of phenols and lignin model compounds, providing a good example for oxidation of the lignin moiety of pulp. Model studies, however, do not reflect all the reactions that lignin might undergo as a macromolecule in the pulp matrix. To advance the understanding of delignification with simple inorganic complexes, eucalyptus (*E. urophylla* × *E. grandis*) pulp was subjected to catalytic delignification (Cat) with atmospheric dioxygen at 70 °C in a peroxydisulfate-acetate system, i.e. in CH_3COOH-H_2O medium containing $(NH_4)_2S_2O_8$ and $Cu(CH_3COO)_2$/$Co(CH_3COO)_2$, the latter being used as catalyst. The Cat-treatment was followed by alkaline extraction (Ep) under standard conditions. Lignins in the bleached pulps were isolated and analyzed by different techniques, including Elemental Analysis, FTIR, NMR and GPC. The transformation of lignin during the aerobic treatment of eucalyptus pulp with a simple inorganic mixture was investigated. A comparative analysis of residual lignins isolated from pulps in the catalytic bleaching sequences (CatEp) showed that lignin underwent changes that included a reduction in the content of PhOH groups, a demethylation, and an increase in carbonyl groups. The FTIR results indicated that the catalytic treatment of pulp led to the loss of aromaticity. The GPC studies showed lignin depolymerization.

Keywords: ammonium persulfate, copper acetate, cobalt acetate, eucalyptus kraft pulp, biomimetic oxidation, residual lignin

INTRODUCTION

Wood and other plant materials used to make pulp contain three main components: cellulose fibres (desired for papermaking), lignin (a three-dimensional polymer that binds the cellulose fibers together) and hemicelluloses (shorter branched carbohydrate polymers). The aim of pulping is to break down the lignin into small, water-soluble molecules, which can be washed away from the cellulose fibers without seriously depolymerizing the cellulose fibres.[1,2] The pulp produced up to this point in the process can be bleached to produce a white paper product. However, the chemicals used to bleach pulp have been a source of environmental concern, and the conventional bleaching of chemical pulps using elemental chlorine has the potential to cause significant environmental damage, primarily through the release of organic materials into waterways. Wastewaters from pulp and paper units are often rich in color, containing large amounts of chlorinated organic compounds, including chlorinated dioxins. Dioxins are highly toxic, and health effects on humans include reproductive, developmental, immune and hormonal problems. They are known to be carcinogenic.[3-5] To replace environmentally detrimental chlorine-based bleaching technologies in pulp and paper industry, new bleaching technologies are required. The use of different chemical agents and enzymes as alternative delignification agents has been examined and, in some cases, implemented. A number of simple inorganic complexes, such as peroxydisulfate plus Cu(II), Co(II), Ag(I), Fe(II) and Mn(II), have been successfully applied in the oxidative degradation of phenols and lignin model compounds. They also have the advantages of chemical and biological agents. In accordance with an approach for using simple inorganic complexes in the oxidation of lignin model compounds, the oxidations of veratrylglycerol-β-guaiacyl ether by $S_2O_8^{2-}$-Cu(II) system gave veratraldehyde (20-25%) and guaiacol (ca. 10%) (Figure 1). These are initiated by single-electron oxidation of aromatic substrates to aryl cation radicals as common intermediates to both enzymatic and biomimetic reactions.[6]

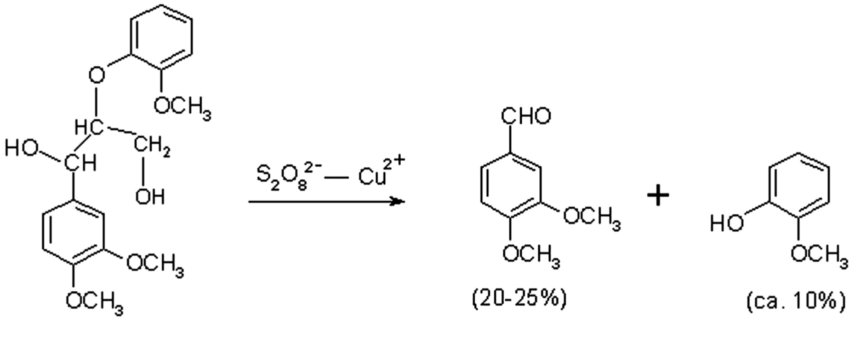

Figure 1: Proposed mechanism for veratrylglycerol-β-guaiacyl ether transformation by $S_2O_8^{2-}$-Cu^{2+}

Table 1
Pulp properties obtained in cooking and bleaching

Pulp property	K	O	Cu	Co	CuEp	CoEp
Brightness (%ISO)	35.2	45.3	48.20	48.97	62.31	61.63
Viscosity (mL/g)	1127	967	826	779	799	778
Kappa number	17.58	11.26	10.94	10.55	9.82	9.97

Peroxydisulfate is one of the strongest oxidizing agents, and its oxidation of aromatic compounds is believed to be due to its major decomposition product $SO_4^{\bullet}$.[6,7] Model studies, however, do not reflect all the reactions that lignin might undergo as a macromolecule in the pulp matrix. In the current study, we focused on the effect of simple inorganic complexes ($(NH_4)_2S_2O_8/Cu(CH_3COO)_2$ and $(NH_4)_2S_2O_8/Co(CH_3COO)_2$) on lignin in the catalytic bleaching of pulp in the two-stage process (CatEp). The structural transformation of lignin in simple inorganic complex-bleached pulps was evaluated by comparative analysis of the properties of residual lignins isolated from laboratory-made oxygen-bleached and simple inorganic complex-bleached eucalyptus kraft pulps. The results of these studies, combined with the results obtained using lignin model compounds, are expected to improve our understanding of delignification and facilitate the implementation of the simple inorganic complex bleaching.

Lignins in the bleached pulps were isolated and analyzed by different techniques used in lignin characterization, including Elemental Analysis, FTIR and NMR-spectral analyses and GPC.

EXPERIMENTAL

Materials

The raw material used for pulping was hybrid eucalyptus chips (*E. urophylla* × *E. grandis*) supplied by Yunjing Forestry Development Co. Ltd. of Yunnan Province, China. The chips were cooked in a laboratory digester using the kraft pulping method to obtain kraft pulp (KP). Oxygen delignification of the kraft pulp was performed using the conditions described previously.[8] The pulp properties are shown in Table 1. Laboratory-made oxygen-bleached pulp (OP) was used in these bleaching experiments. Chemicals were purchased from Sinopharm Chemical Reagent Co. Ltd.

Catalytic delignification and alkaline extraction

The OP was subjected to catalytic delignification (Cat: Cu and Co) and alkaline extraction (Ep). The following conditions were applied for the Cat stage: pulp consistency 5%; $Cu(CH_3COO)_2$ (Cu) or $Co(CH_3COO)_2$ (Co) 0.01%; $(NH_4)_2S_2O_8$/Cu or Co (M/M) 2; time 120 min; temperature 70 °C. The following conditions were applied for the Ep stage: pulp consistency 10%; NaOH 2.5%; DTPA 0.05%; $MgSO_4$ 0.1%; NaOH 1.5%; H_2O_2 0.5%; time 60 min;

temperature 70 °C. The pulp properties are presented in Table 1.

Residual lignin isolation

The residual lignins were isolated from KP, OP, and from Cat- and Ep-treated pulps by a two-step mild enzymatic/acidolysis isolation procedure suggested by Argyropoulos *et al.*[9] In brief, a mild enzymatic treatment is first applied to the pulp using industrial cellulases with an activity of about 3000 units/g (most cellulase and significant hemicellulases), followed by a mild acid hydrolysis of the resulting lignin (0.05 mol/L HCl solution in dioxane-water 85:15 v/v). The yield of lignin represented at least 70% with a high purity (>96%) of the theoretical content in pulp. The carbohydrate impurities in the extracted lignin represented 2-4%. Residual proteins were of the order of 4% (average protein N content around 0.6%). Lignin samples were designated by the name of the treatment, KL, OL, CuL, CoL, CuEpL, CoEpL.

Lignin acetylation

Acetylation of the purified residual lignin was carried out in pyridine with acetic anhydride, according to the published method.[10]

Measurements

The lignin samples were analysed for C, H, and N, using a Vario EL Elemental Analyzer (Elementar Co., Germany). Oxygen was determined by difference.

The FTIR spectra of unacetylated lignin samples were recorded using the KBr transmission technique on a Bruker Equinox 55 FTIR spectrometer (Germany), 200 scans at 4 cm^{-1}. Background spectra were collected before every sampling. The spectra were analyzed using MestReC software to compare the absorbance of each functional group. The absorption bands were assigned as suggested by Faix[11] and Nada *et al.*[12]

^{1}H-NMR spectra of acetylated lignin samples were acquired on a 400MHz Avance Bruker spectrometer (Switzerland) using standard Bruker sequences.[13] The samples (~80 mg) were dissolved in 400 μL of DMSO-d_6. ^{1}H-NMR spectroscopy of acetylated lignin provides improved spectral resolution of key lignin functionality. A less tedious method for methoxyl determination of lignin that involves the use of ^{1}H-NMR uses the theoretical ratios between aromatic and methoxyl protons of guaiacyl and syringyl.[14] These ratios can actually be measured from the ^{1}H-NMR spectra of acetylated lignins. Aromatic and methoxyl protons occur between 6.4-7.1 ppm and 3.5-4.1 ppm, respectively, see Figure 2. For this study, the acetylated lignins were then analysed by ^{1}H-NMR to obtain these ratios (x) from which the % OCH_3 was calculated from the equation $\%OCH_3 = 28.28436-19.750047x$ submitted by Aberu and Freire.[14] The phenolic (2.3 ppm) and aliphatic (2.0 ppm) hydroxyl groups can be calculated following the ratios between methoxyl and hydroxyl groups.[15] The lignin functional groups characterized by ^{1}H-NMR had standard errors of 1.2%.

For gel permeation chromatography (GPC) (Agilent 1100, USA), the dried acetylated lignin was dissolved in tetrahydrofuran (1 mg/mL) and filtrated through a 0.45 μm membrane filter for analysis.[16,17] In the analysis, an Agilent 1100 HPLC pump, a differential refractometer and stainless steel columns (length 30 cm; i.d. 7.5 mm) were used. The calibration curve was obtained using monodisperse polystyrene compounds of known molecular weight as reference substances.

RESULTS AND DISCUSSION

Molecular weight distribution

Changes in the polymer structure of the lignin were followed by molecular weight distribution analysis with GPC using acetylated samples.[18,19] The GPC data presented in Table 2 illustrate that simple inorganic complex delignification leads primarily to lignin depolymerization. Cleavage of some bonds (such as in the side chains of phenyl propane units) can partly explain the catalysis-induced lignin degradation.[20] The behaviour of residual lignin during Ep-treatment is quite unexpected, no depolymerization seems to take place in Ep treatment in contrast to what happened in Cu- and Co- treatment. This could be attributed to a preferential solubilization of low molecular weight fragments during the Ep-treatment. The absence of depolymerization limits the removal of lignin, which would mean that CatEp must be combined with other bleaching stages. However, additional experiments are needed to further clarify this postulation.

For these lignins, on the other hand, the dispersities are similar, with values around 1.7. This clearly indicates that each sample essentially contains lignin of similar chain length.

Elemental analysis of lignins

The elemental analysis results of the lignins (Table 3) are average values from duplicate analyses of the samples. Using the original lignin KL and OL as reference, a number of trends are observed.

As expected, the data from Table 3 show that carbon and hydrogen contents decrease from KL to OL. The oxygen content is high in the OL because lignin is oxidized in alkali and so will be easily increased in oxygen content during the oxygen delignification process.[21,22] The carbon and hydrogen contents increase after Cat- and Ep-treatment. The nitrogen content of the lignin obtained from Ep-pulp is lower than that from Cat-stage, because proteins are soluble in alkali and so will be easily removed during the alkali extraction.

Elemental analyses of the different lignins allow the empirical formula to be calculated as $C_xH_yO_z$ with neglecting the nitrogen contents (Table 3). In lignin chemistry, the empirical formula of the macromolecule is commonly given as a hypothetical hydroxyphenyl structural unit. This is known as the C_9 formula, with six carbon atoms in the benzene ring plus three carbon atoms making up the propyl side-chain. The results are shown in Table 3. It is worth noting that the C_9 formula of Ep-lignin is similar to that of Cat- lignin.

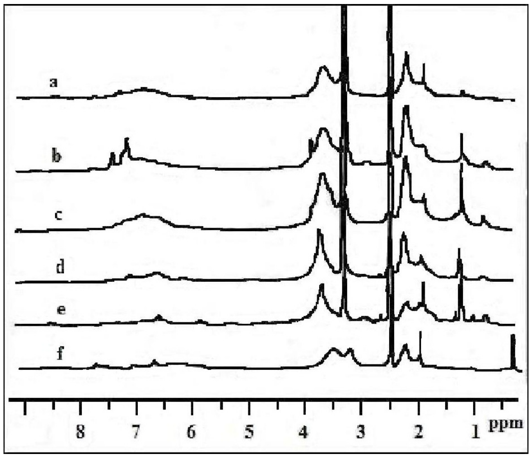

Figure 2: H-1 NMR spectra of acetylated lignins (a, CuEpL; b, CoL; c, CuL; d, OL; e, KL; f, CoEpL)

Table 2
Molecular weight of lignins

Lignin	KL	OL	CuL	CoL	CuEpL	CoEpL
Mw (g/mol)	18580	3399	2696	2659	2744	3146
PD (Mw/Mn)	1.636	1.669	1.871	1.736	1.626	1.794

Mw: weight-average molecular weight; Mn: number-average molecular weight; PD: polydispersity (Mw/Mn)

Table 3
Elemental analysis of lignins

Lignin	C (%)	H (%)	N (%)	O (%)	Empirical formula	C_9 formula
KL	44.71	6.80	1.28	47.21	$C_{3.73}H_{6.80}O_{2.95}$	$C_9H_{16.41}O_{7.12}$
OL	32.74	4.91	1.40	60.95	$C_{2.73}H_{4.91}O_{3.81}$	$C_9H_{16.19}O_{12.56}$
CuL	48.44	6.76	1.70	43.10	$C_{4.04}H_{6.76}O_{2.69}$	$C_9H_{15.06}O_{5.99}$
CoL	68.10	6.72	1.45	23.73	$C_{5.68}H_{6.72}O_{1.48}$	$C_9H_{10.65}O_{2.35}$
CuEpL	61.12	6.43	1.35	31.10	$C_{5.09}H_{6.43}O_{1.94}$	$C_9H_{11.37}O_{3.43}$
CoEpL	52.19	6.66	1.31	39.84	$C_{4.35}H_{6.66}O_{2.49}$	$C_9H_{13.78}O_{5.15}$

Table 4
Quantities of functional groups

Lignin	Methoxyl (%)	Phenolic OH (%)	Aliphatic OH (%)	Total OH (%)
KL	21.49	2.63	3.98	6.61
OL	19.27	4.25	4.82	9.07
CuL	11.81	1.79	1.52	3.31
CoL	11.31	1.99	2.31	4.30
CuEpL	15.99	3.13	4.08	7.21
CoEpL	14.97	3.27	1.90	5.17

Functional groups

The functional groups of lignins were obtained by ^{1}H-NMR spectroscopy, as shown in Table 4. For the first time, the functional group character of the residual lignin after catalytic treatment by simple inorganic complex is demonstrated. Total hydroxyl content comprises phenolic and aliphatic hydroxyl groups. As can be seen from Table 4, the CatEp-treated lignins contain less methoxyl and phenolic hydroxyl groups than the corresponding untreated samples. The decrease in methoxyl group of lignin in Cu- and Co-treatment means that for pulp, lignin is demethoxylated during the catalytic process. The methoxyl group content of lignin increases with Ep treatment. Similar results have been observed in Co(salen) biomimetic processes of lignin oxidation.[23] The aliphatic OH content of CuEp-lignin is higher compared with that of Cu-lignin. The aliphatic OH can be produced by disproportionation reactions of side chain and ring cleavage products derived from oxidative cleavage of the aromatic rings followed by hydration.[24]

Table 4 indicates that the Ep treatments lead to the formation of new phenolic OH groups. The increase in the number of phenolic OH groups can be attributed to the cleavage of α- and β-ether linkages. In fact, the cleavage of these linkages leads to the appearance of phenolic OH groups in the aryl substituent removed from the β position. The same trend was observed in studying the lignin and lignin model compound behavior during the catalyzed oxidation by Cu^{2+}, Co^{2+} and Fe^{3+}.[6,20] On the positive side, this explains why catalysis activates the lignin prior to bleaching and makes the final bleaching easier, as shown in several enzymatic studies.[25,26] It should be noted that, as shown in Figure 3, Cu-pulp with a high phen-OH/kappa ratio has much better bleachability after Ep extraction than Co-pulp, when the brightness/kappa ratio is compared between the Cu- and Co-bleaching sequences.

FTIR analysis

The FTIR spectra of all studied lignin preparations are shown in Figure 4. The differences are found in the region 1600-1750 cm^{-1} and at about 1400 cm^{-1}, particularly in the absorption bands of carbonyl groups. The absorption band at about 1600 cm^{-1}, which is assigned to aromatic skeletal vibration and carbonyl stretch,[27] is broader and abundant for catalytic bleached lignin versus starting lignin preparation (OL). This is probably due to overlapped intensive bands assigned to carbonyl originating from carboxylate ion (asymmetrical stretching band).[28,29] The presence of carboxylic groups in catalytic bleached lignin is also supported by notable broadening of the band at about 1400 cm^{-1}, which is due to an increase in the intensity of the symmetrical stretching band.

Interestingly, the more or less pronounced bands in conjugated (1660 cm^{-1}) and unconjugated (1720 cm^{-1}) carbonyl region are seen in the spectra of catalytic bleached lignin.[27]

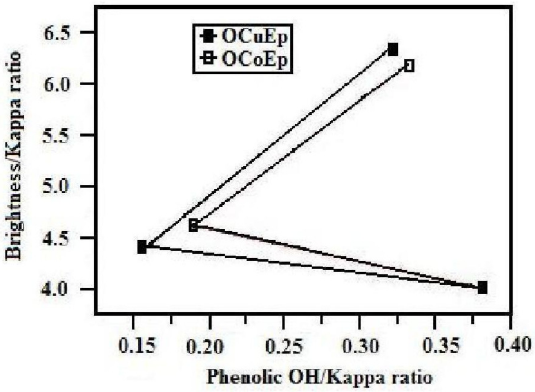

Figure 3: Brightness/kappa ratio vs. phenolic OH/kappa ratio for the pulps in OCuEp and OCoEp – bleaching sequences

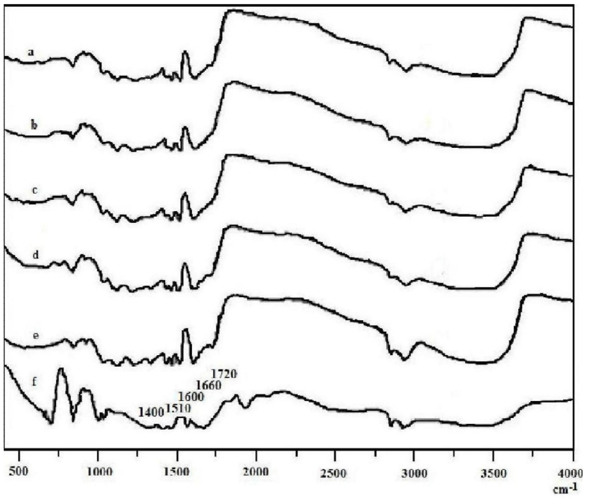

Figure 4: FTIR spectra of acetylated lignins (a, CoEpL; b, CoL; c, CuEpL; d, CuL; e, KL; f, OL)

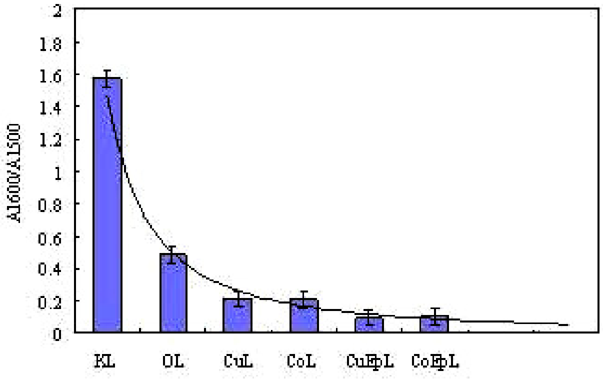

Figure 5: A_{1600}/A_{1510} of lignins in FTIR-spectra

The A_{1600}/A_{1510} ratio is particularly affected in CatEp-delignification (Figure 5), which represents the plot of these ratios calculated for the KL, OL, CuL, CoL, CuEpL, CoEpL. The decrease of the A_{1600}/A_{1510} ratios (1510 cm^{-1} aromatic skeletal vibration band is usually a reference band in lignin studies) is observed with the progression of both Cat- and Ep-treatment, and is consistent with aromatic ring cleavage during white-rot fungi decomposition of lignin.[30] Notably, the Cat-delignification of pulp in our experiments resulted in a greater decrease of these ratios, which along with a decrease of the phenolic OH content (Table 4) might indicate comparatively more intense ring cleavage.

CONCLUSION

Spectroscopic characterizations are used to detect the structure transformation from lignins in both $(NH_4)_2S_2O_8$-$Cu(CH_3COO)_2$ and $(NH_4)_2S_2O_8$-$Co(CH_3COO)_2$ oxidation, which presents information on the effect of the catalytic chemicals on residual lignin in kraft pulp. This information is particularly useful in explaining some previous results and for defining optimum bleaching sequences. It appears that some simple inorganic complexes exhibit catalytic features that make them useful in green bleaching of pulp. The catalytic system has the capability to depolymerize the residual lignin, while forming new carboxy groups. Ep-extraction, at the opposite extreme, does not depolymerize the residual lignin efficiently. Consequently, CatEp must be associated with other stages, which have this capability. From the results obtained in this study, the reactions for our biomimetic model system and for ligninases are similar. $(NH_4)_2S_2O_8$ plus $Cu(CH_3COO)_2$ and $Co(CH_3COO)_2$ should be equally good candidates for chlorine-based agents in pulp bleaching.

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