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Erratum

Lignin modification in the initial phase of softwood kraft pulp delignification with polyoxometalates (POMs)

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The corresponding author's address is correct and we apologize for any inconvenience this may have caused.

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Lignin modification in the initial phase of softwood kraft pulp delignification with polyoxometalates (POMs)

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Abstract

Commercial softwood kraft pulp with kappa number 30.5 ($KP_{30.5}$) was delignified with polyoxometalates (POM, $Na_{5(+2)}[SiV_{1(-0.1)}MoW_{10(+0.1)}O_{40}]$), and POM-treated kraft pulp of kappa number 23.6 was obtained ($KP_{POM,23.6}$). Residual lignin from pulps was isolated by mild acid hydrolysis and characterized by analytical and spectral methods to gain insight into lignin reactions taking place during the initial delignification phase. Lignin from POM-delignified pulp was isolated in lower yield. Comparative analysis of residual lignins (RL-$KP_{30.5}$, RL-$KP_{POM,23.6}$) showed that POM leads to products enriched in carbonyl/carboxyl groups and carbohydrates. POM lignins have a lower molecular mass and a lower content of phenolic hydroxyl and methoxyl groups. Based on these results and FTIR spectra, we suggest that aromatic ring cleavage and quinone formation occur during POM delignification. The degree of lignin-cellulose association increases after POM delignification. Lignin-cellulose association was found to be partially unstable under mild alkaline conditions, as residual lignin isolated after alkaline extraction of $KP_{POM,23.6}$ pulp (RL-$KP_{POM/NaOH}$) exhibited lower glucose content, higher Klason lignin content, and less extraneous material.

Keywords: acid hydrolysis; commercial softwood kraft pulp; lignin-cellulose association; polyoxometalate delignification; residual lignin.

Introduction

Polyoxometalates (POMs) are discrete, early-transition-metal-oxygen-anion clusters that may be used instead of chlorine-based bleaching chemicals as selective delignification agents. POMs are considered to be chemical analogs of ligninolytic enzymes excreted by white-rot fungi (e.g., lignin peroxidase and manganese peroxidase). They are single-electron oxidants in which transition metal ions are responsible for electron transfer and may be formulated to have approximately the same redox potential as enzymes. Different mixed-addenda heteropolyanions with a Keggin structure were studied as pulp bleaching agents in a two-step process. In the first, POMs act under anaerobic conditions and delignify the pulp by oxidation of residual lignins. The second step in the presence of O_2 at high temperature provides for oxidation of both the reduced POMs and the dissolved lignin fragments. Then the regenerated fully oxidized POMs are ready for the next bleaching cycle (Weinstock et al. 1993, 1998a,b). However, POMs may also function as catalysts in one-step aerobic delignification processes (POM/O_2) in which delignification and POM reoxidation take place together (Evtuguin et al. 2000a,b; Gaspar et al. 2004).

The mechanism of POM delignification must be elucidated for process optimization. In previous studies, lignin model compounds (LMCs) were investigated. In ether-bonded LMCs, the substituent at C_4 of the aromatic ring affects the reaction activation energy (Yokoyama et al. 2004). LMC dimers with free phenolic groups undergo side-chain cleavage in the presence of POMs (Weinstock et al. 1998b). Studies of lignin as a macromolecular part of the pulp matrix offer a complementary insight into reactions occurring in the course of POM-catalyzed bleaching.

Our previous studies included the isolation and characterization of residual lignin (RL) from softwood kraft pulps. Pulps with decreasing kappa number produced by POM treatment were investigated. The yield of lignin isolated by mild acid hydrolysis (AH) from POM-treated kraft pulps (RL-KP_{POM}) was two- to three-fold lower than that from kraft pulp (RL-KP). The purity of isolated lignins gradually decreased with the kappa number. The Klason lignin content in RL from KP_{POM} pulps with kappa number <15 was less than 80%. RL-KP_{POM} contained 2.2–4.8% carbohydrates, compared with 0.9% in RL-KP. The amount of extraneous non-lignin and non-carbohydrate material also increased more than two-fold in RL from KP_{POM} with kappa number <15. These are unusual results because lignin isolated from KP by mild AH is free of carbohydrates and other impurities (Tamminen and Hortling 1999).

The aim of the present study was to investigate chemical changes in an initial phase of POM delignification of KP. The kappa number decrease from 30.5 to 23.6 was the focus in understanding the significantly lower yield of isolated RL and the change in its chemical composition. Structural changes in lignin during the delignification process were assessed using wet-chemical and spectroscopic analyses.

Materials and methods

Commercial softwood kraft pulp of kappa number 30.5 ($KP_{30.5}$) was delignified by means of an equilibrated POM mixture composed of 0.5 M $Na_{5(+2)}[SiV_{1(-0.1)}MoW_{10(+0.1)}O_{40}]$, resulting in a pulp

with kappa number 23.6 ($KP_{POM,23.6}$). The conditions were as described by Bujanovic et al. (2005). RL was isolated by mild AH of extractive-free pulp as described by Gellerstedt et al. (1994) and Bujanovic et al. (2005) with a slight modification (RL-$KP_{30.5}$, RL-$KP_{POM,23.6}$). Residual lignin from $KP_{POM,23.6}$ was isolated after alkaline extraction of extractive-free pulp (RL-$KP_{POM/NaOH}$) (Tappi standard T 212 om-88). The alkaline extract was acidified, and the precipitate is designated as $KP_{POM,NaOH/pH2}$.

The following determinations were performed on the residual lignins: Klason lignin, acid-soluble lignin, carbohydrate determination (Davis 1998), free phenolic hydroxyl groups (PhOH) (Gärtner et al. 1999), and methoxyl group (OCH_3) determination (Chen 1992).

Gel permeation chromatography (GPC) profiles of acetylated RL-$KP_{30.5}$ and RL-$KP_{POM,23.6}$ were obtained on a Sephacryl-100 column (1.8×32.5 cm) using an eluent of dioxane/water (9:1), with 1.5-ml fractions collected. The eluate was monitored using a UV detector set to 280 nm. The calibration standards were acetylated spruce milled wood lignin (Lundquist 1992), LMC tetramer #3009 (Ralph et al. 2006) and veratraldehyde.

UV spectroscopy

For determination of the PhOH content, a Spectronic Genesys 5 spectrophotometer was used.

FTIR spectroscopy

A Mattson Galaxy series FTIR 5000 spectrometer (200 scans at 4 cm^{-1}) was used to record spectra according to the KBr transmission technique. The difference FTIR spectrum was obtained after normalization of the band at ~1510 cm^{-1} and subtraction (RL-$KP_{30.5}$–RL-$KP_{POM,23.6}$). The content of carboxylic acid and unconjugated carbonyl groups (C=O groups) in relation to aromatic structures in lignin was determined by means of the horizontal baseline method as suggested by Hortling et al. (1997). Results are expressed as moles of C=O groups per gram of "aromatic lignin" (C=O g^{-1} AL). Sodium borohydride reductions were carried out according to Zhang and Gellerstedt (1999).

NMR spectroscopy

Samples were run with standard Bruker pulse sequences on a Bruker DPX-250 (62.9 MHz ^{13}C) spectrometer fitted with a quadranuclear 5-mm Z-gradient coil probe. Samples (~50 mg) were dissolved in 400 µl of acetone-d_6/D_2O (4:1), while acetylated samples were dissolved in DMSO-d_6. The central solvent peak served as the internal reference (acetone at δ_H 2.04, δ_C 29.8 ppm and DMSO at δ_H 2.50, δ_C 39.5 ppm). Qualitative ^{13}C spectral experiments were acquired with a standard power-gated sequence with a 0.5-s delay and 50,000 scans. Quantitative ^{13}C spectral experiments for acetylated lignin samples were acquired with an inverse-gated sequence, a 12-s relaxation delay, 90° pulse, and 25,000 scans. For spectral editing (DEPT), the standard Bruker DEPT135 sequence was run and spectra were phased to indicate positive CH_3 and CH and negative CH_2 signals. Inverse-detected ^1H-^{13}C correlation spectra (HSQC) were acquired from 9.0 to 0 ppm in F2 (^1H) with 900 data points (acquisition time 200 ms), and from 150 to 0 ppm in F1 (^{13}C) with 512 increments, 128 scans, and a 1-s interscan delay for a total acquisition time of 11 h 12 min. The d_{24} delay was set to 1.72 ms (J=145 Hz). A standardized parameter set was used for both acquisition and processing to facilitate direct comparison of RL-$KP_{30.5}$ and RL-$KP_{POM,23.6}$ spectra.

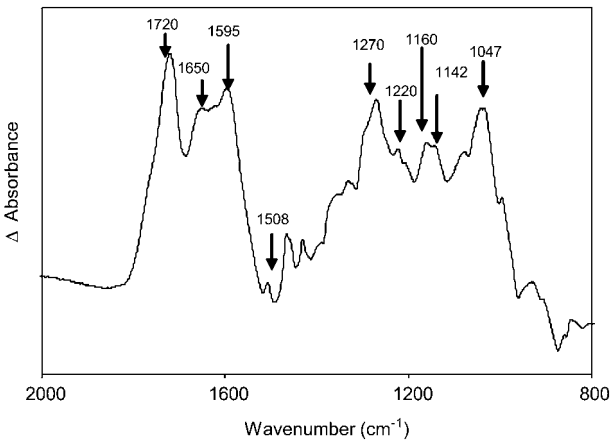

Figure 1 Difference FTIR spectrum of residual lignin from kraft pulp RL-$KP_{30.5}$ and from POM-treated kraft pulp RL-$KP_{POM,23.6}$: RL-$KP_{POM,23.6}$–RL-$KP_{30.5}$

Results and discussion

Carbonyl/carboxyl groups

The difference FTIR spectrum (Figure 1) shows that POM treatment caused a considerable increase in bands for carbonyl group stretching (Faix 1991). The 1720 cm^{-1} band and the 1650 cm^{-1} shoulder are signs of the formation of conjugated and unconjugated C=O groups, respectively. These groups were also identified after POM treatment of LMCs yielding benzaldehyde (**1**), a *p*-benzoquinone (**2**), a conjugated ketone (**3**), and an unconjugated aldehyde (**4**) (Weinstock et al. 1998b) (Figure 2). An increase in the band at 1650 cm^{-1} is consistent with quinone production as proposed by Weinstock et al. (1993) based on NIR, FT-Raman and UV/Vis spectral studies of softwood KP during POM delignification. Similar FTIR results were obtained after different oxidative treatments and attributed to quinone group formation in lignin (Furman and Lonsky 1988; Poppius-Levlin et al. 1999). A lower intensity of the aromatic skeletal vibration indicates a loss of aromaticity (Kirk and Chang 1975), which may be caused by quinone formation or by aromatic ring

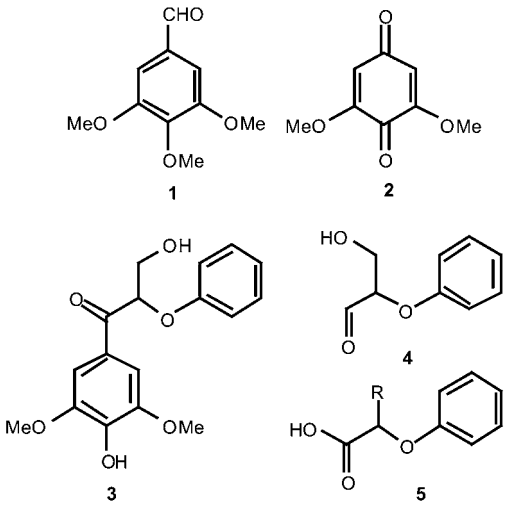

Figure 2 Products of POM treatment of lignin and model compounds; R=H,H; CH2OH (Tuor et al. 1992; Weinstock et al. 1998b; Evtuguin et al. 2000b; Gaspar et al. 2004).

cleavage, in the course of which muconic acid or cyclic carbonate-type compounds are formed. Such reactions were observed in the course of different oxidative treatments of pulp, lignin, and LMCs (Umezawa et al. 1986; Evtuguin and Robert 1997).

The content of carboxyl and unconjugated carbonyl groups was calculated as mmol C=O g^{-1} AL according to Hortling et al. (1997). It is obvious that POM treatment leads to ~23% delignification and to an essential increase in C=O groups (RL-$KP_{POM,23.6}$ 6.34 mmol C=O g^{-1} AL vs. RL-$KP_{30.5}$, 1.59; average of three results, SD 0.087 and 0.045, respectively). After $NaBH_4$ reduction, the content of C=O groups in RL-$KP_{POM,23.6}$ was 3.62 mmol C=O g^{-1} AL (SD 0.098). Accordingly, more than 50% of the total C=O groups are $NaBH_4$-non-reducible and probably belong to acids and esters.

By qualitative ^{13}C NMR (Figure 3), an increase in signal height between 191 and 193 ppm in the spectrum of RL-$KP_{POM,23.6}$ clearly demonstrates the oxidative effect of POMs on lignin. The HSQC (short-range C-H correlation) revealed the presence of aldehydes, most likely benzaldehydes (Ar-CHO) based on the appearance of correlations at 9.80/191.3 and 9.95/191.5 ppm and LMC data (Ralph et al. 2006) (Figure 4). This correlated with DEPT spectral data indicating the presence of protonated C atoms (CH) in this region. An increase in Ar-CHO structures is commonly interpreted as resulting from C_α-C_β bond cleavage. Evtuguin et al. (2000a) reported on such reactions during POM/O_2 degradation of eucalyptus and spruce wood lignins. Alkyl-phenyl cleavage may also lead to Ar-CHO groups (Weinstock et al. 1998b). Product (**1**) in Figure 2 was identified, for example, in a solution after POM treatment of diphenylmethane compounds.

^{13}C NMR ketone signals typically appear between 193 and 201 ppm (Zhang and Gellerstedt 1999; Ralph et al. 2006), and there is no apparent increase in this region for RL-$KP_{POM,23.6}$ (Figure 3). Accordingly, the ketone content in RLs is not influenced by POM treatment. Our previous study on the β-O-4 side-chain in RLs showed

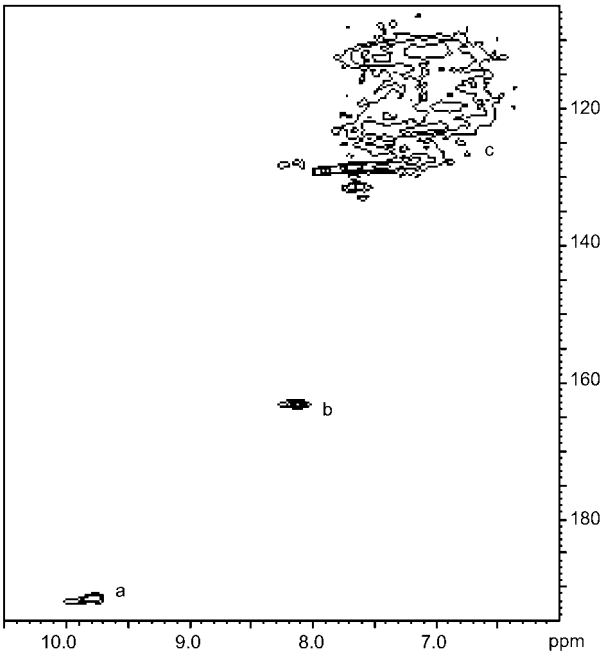

Figure 4 HSQC spectrum of residual lignin from POM-treated kraft pulp RL-$KP_{POM,23.6}$ (acetylated; DMSO-d_6): (a) benzaldehyde; (b) possible formate; and (c) aromatic region.

correlations characteristic of C_α-OH, whereas a C_α=O structure such as **3** (Figure 2) was not detected (Bujanovic et al. 2005). Based on these data, we suggest that the POM attack on lignin in pulp does not include oxidation of the C_α-OH/β-O-4 substructure. In LMCs the situation is different; Weinstock et al. (1998b) observed oxidation for a free phenolic LMC (C_α-OH/β-O-4). The absence of compound **3** (Figure 2) in RL-$KP_{POM,23.6}$ may also indicate that this type of structure, initially formed and incorporated in lignin, is readily available for further degradation. This was indeed observed in the case of etherified dimer analogs during oxidative degradation by enzymes (Kirk et al. 1986).

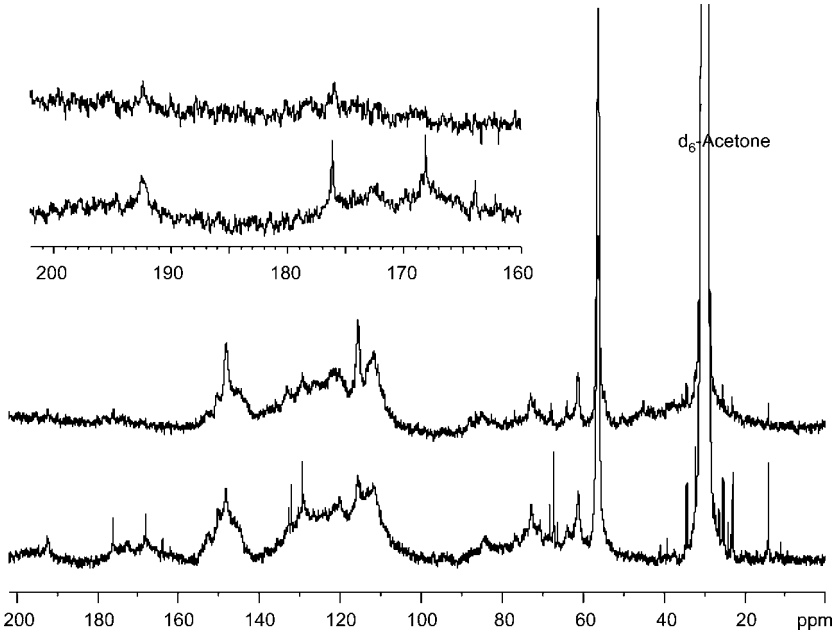

Figure 3 Qualitative ^{13}C NMR of residual lignins: top, RL-$KP_{30.5}$ bottom, RL-$KP_{POM,23.6}$ (acetone-d_6/D_2O 4:1).

The C atom of carbonyl groups in quinone-type lignin models has been reported at 180–187 ppm (Zhang and Gellerstedt 1999; Ralph et al. 2006). ^{13}C NMR data showed no change in this area when the spectra of RL-KP_{POM} and RL-$KP_{POM,23.6}$ were compared (Figure 3). Therefore, the suggested presence of quinones in RL-$KP_{POM,23.6}$ based on FTIR studies was not confirmed by ^{13}C NMR results.

The ^{13}C NMR signals at 160–180 ppm were more intense for RL-$KP_{POM,23.6}$ than for RL-KP_{POM} (Figure 3). We interpret this as POM delignification leading to the incorporation of conjugated and unconjugated carboxyl and/or ester groups (COOR) in lignin. Because the corresponding HSQC spectrum (Figure 4) did not show correlations for C_α/H_α in cinnamic acid structures (143–144/7.0–7.7 ppm; Ralph et al. 2006), the conjugated COOR groups at ~164–170 ppm (the most intense signal at 168.2 ppm) are most likely benzylic esters (Ar-COOR). The Ar-COOR structure is usually diagnostic of C_α-C_β cleavage, which presumably takes place during treatment of lignin and LMCs with different oxidizing agents (Kirk et al. 1986; Tuor et al. 1992; Evtuguin et al. 2000a,b; Srebotnik and Hammel 2000; Gaspar et al. 2004).

The increase in signal intensity at 170–180 ppm in the RL-$KP_{POM,23.6}$ spectrum indicates the presence of unconjugated COOR groups (Figure 3, signal at 176.2 ppm increased the most), as they typically appear in this region. Unconjugated acids identified as products of lignin and LMCs upon treatment with chemical and biological oxidizing agents were discussed as products of alkyl-phenyl cleavage of β-O-4 dimers; for example, compound **5** in Figure 2 (Tuor et al. 1992; Evtuguin et al. 2000b; Gaspar et al. 2004). In oxygen bleaching, however, an increase in the content of unconjugated acids was attributed to the oxidative fragmentation of different conjugated acids, such as benzylic conjugated carboxylic acids or muconic-type acids (Zhang et al. 2006).

Data from DEPT experiments can be interpreted as indicating that signals in the area of COOR groups at 160–180 ppm are all due to quaternary carbon atoms, except for one CH at 163.9 ppm. The HSQC data (Figure 4) show a correlation at 163.2/8.11 ppm, which may be a formate, because formic acid NMR signals appear in this region (**H**COOH, 8.1 ppm; H**C**OOH, 163.2 ppm in DMSO-d_6). Formate generation may be supported by earlier reports on the formation of the γ-formyl compound as a result of aromatic ring cleavage in the treatment of etherified C_α-OH/β-O-4 dimers with ligninolytic enzymes (C_γ-O-C**H**O, 8.03 ppm in $CDCl_3$; Kawai et al. 1985, 1999; Umezawa et al. 1986). Formate as a result of aromatic ring cleavage caused by POMs would be consistent with a decrease in aromaticity observed by FTIR. Although formate was detected as a product in LMC experiments, formate incorporated in lignin has not been reported. It should be pointed out, however, that a Rotholz Fraser fir MWL showed a correlation at 161.5/8.25 ppm (DMSO-d_6) attributed to unknown olefinic CH groups (Balakshin et al. 2005).

The carbonyl group, based on our results as mostly being of benzaldehyde nature, and carboxyl groups formed during POM treatment may lead to a decrease in lignin isolation yield (Bujanovic et al. 2005). The RL isolation yield was not, however, significantly lower in the case of other oxidative bleaching procedures, for example, oxygen, laccase-mediator or chlorine dioxide (Runge and Ragauskas 1999; Chakar and Ragauskas 2001; Fu and Lucia 2003), even when the degree of delignification was higher than in this study.

Carbohydrates

An increased level of carbohydrates in RL-KP_{POM} found previously was confirmed in this study (Table 1). Carbohydrate content in the residual lignin increased almost six-fold from 0.85% in RL-$KP_{30.5}$ to 4.6% in RL-$KP_{POM,23.6}$. This result and the lower yield of RL-KP_{POM} (Bujanovic et al. 2005) imply the formation of an acid-resistant association between lignin and carbohydrates during POM delignification of kraft pulp. This closer association may contribute to the low yield of RL-KP_{POM} (Bujanovic et al. 2005), although Fu and Lucia (2003) obtained carbohydrate-enriched RL from oxygen-bleached KP in even higher yield than from KP. The FTIR results are consistent with the observed carbohydrate content increment. The characteristic polysaccharide bands at approximately 1047 and 1160 cm^{-1} – assigned to C-O stretching and C-O-C vibration in polysaccharides, respectively (Rodrigues et al. 1998) – increased in RL-$KP_{POM,23.6}$ (Figure 1). The relative content of monosaccharides was diminished. An exception is glucose, the content of which increased from ca. 60% to ~90%. Obviously, POMs are efficient in weakening the association between lignin and carbohydrates that do not contain glucose, whereas the association between glucose-containing carbohydrates and lignin becomes stronger during POM delignification.

Table 1 Composition of residual lignin (RL) in kraft pulp (KP) delignified with polyoxometalate (POM).

Residual lignin	Lignin in RL (%)			Carbohydrate content in RL (%) (% based on total carbohydrates)						Total content in RL (%)
	Klason	AS	Total	Gal	Glu	Ara	Man	Xyl	Total	
RL-$KP_{30.5}$	92.8	0.8	93.6	0.06 (7.06)	0.53 (62.35)	0.04 (4.70)	0.06 (7.06)	0.16 (18.82)	0.85 (100)	94.45
RL-$KP_{POM,23.6}$	86.2	1.4	87.6	0.05 (1.09)	4.20 (91.30)	0.05 (1.09)	0.10 (2.17)	0.20 (4.35)	4.60 (100)	92.2
$KP_{POM/NaOH}$	91.6	1.8	93.4	0.08 (4.19)	1.22 (63.87)	0.06 (3.14)	0.14 (7.33)	0.41 (21.47)	1.91 (100)	95.31

The KP indices refer to kappa numbers. AS- acid-soluble.

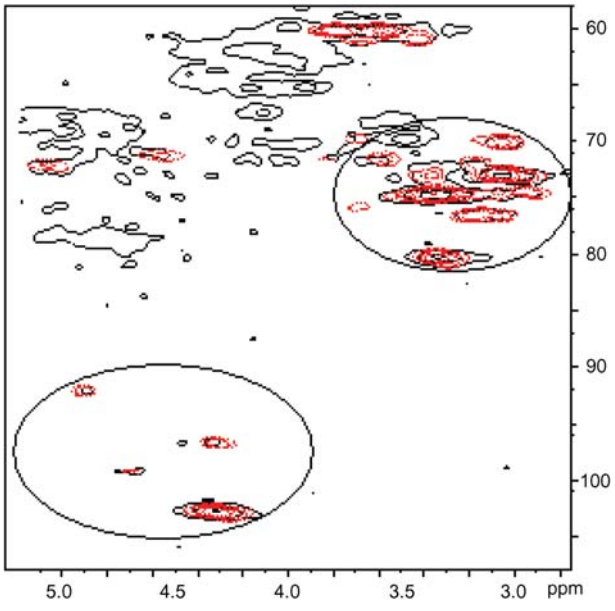

Figure 5 Partial HSQC spectra of residual lignin from POM-treated kraft pulp RL-$KP_{POM,23.6}$ and of cellulose (acetylated; DMSO-d_6). Circled correlations due to cellulose are also present in residual lignin.

The HSQC spectrum of acetylated RL-$KP_{POM,23.6}$ presented in Figure 5 is superimposed on that of acetylated cellulose. Accordingly, carbohydrate correlations in the lignin spectrum are similar to those obtained for cellulose. This indicates that glucose units in RL are β-D-(1 → 4) bonded, probably originating from cellulose. Although cellulose may be chemically bonded to lignin even in KP (Isogai et al. 1987), these bonds were largely cleaved during mild AH of $KP_{30.5}$, as the characteristic correlations of β-D-(1 → 4) bonded glucose units were not observed in the HSQC of RL-$KP_{30.5}$. POM treatment leads to a closer lignin-cellulose association, which is manifest in less efficient mild AH, i.e., RL-KP_{POM} is enriched in glucose.

Free phenolic hydroxyl groups (PhOH)

POM delignification resulted in a decrease in PhOH of ~45% (RL-$KP_{30.5}$, 1.95 mmol PhOH g^{-1} lignin; RL-$KP_{POM,23.6}$ 1.05 mmol PhOH g^{-1} lignin; average of two results; SD 0.063 and 0.017, respectively). Some similarity between the delignifications obtained by POM and by laccase/HBT (1-hydroxybenzotriazole mediator) may be detected. Poppius-Levlin et al. (1999) investigated the delignification of pine KP by the laccase/HBT system. The kappa number decreased from 24.7 to 18.7 and the PhOH was diminished by 42%.

A decrease in PhOH detected by ionization difference UV-spectroscopy (Gärtner et al. 1999) is confirmed by the ^{13}C NMR data for acetylated RL, which show a decrease in signal intensity at 168.51 ppm assigned to phenolic acetates (Figure 6). These results confirm that the PhOH groups are susceptible to POM delignification reactions (Weinstock et al. 1998b; Yokoyama et al. 2004).

Methoxyl groups (OCH_3)

Demethoxylation during POM treatment of KP may be expected. Such reactions were observed in POM experiments with vanillin and creosol (Kang et al. 1997) and in POM/O_2 experiments with LMCs and lignin (Evtuguin et al. 2000c; Gaspar et al. 2004). Based on our results, RL-$KP_{POM,23.6}$ displayed lower OCH_3 group content than RL-$KP_{30.5}$ by approximately 16% (RL-$KP_{30.5}$, 12.1% OCH_3; RL-$KP_{POM,23.6}$, 10.1% OCH_3; average of two results, SD 0.2475 and 0.1345, respectively). The demethoxylation reaction is generally seen as a pathway to quinone formation and/or aromatic ring cleavage, both of which cause aromaticity loss, which was detected in our FTIR studies.

Gel permeation chromatography

The GPC data presented in Figure 7 illustrate that POM delignification leads primarily to depolymerization of RL, with only minor repolymerization. Contrary to our results (PhOH of RL-$KP_{POM,23.6}$ < PhOH of RL-$KP_{30.5}$), lower-molecular-weight lignin is generally characterized by higher PhOH content. The only way to rationalize this finding is that POM delignification proceeds via aromatic ring cleavage (Kirk and Chang 1975). Higher UV absorbance values noted in the area of very-low-molecular-weight compounds may be caused by benzyl carbonyl and carboxyl groups arising from POM treatment. Such

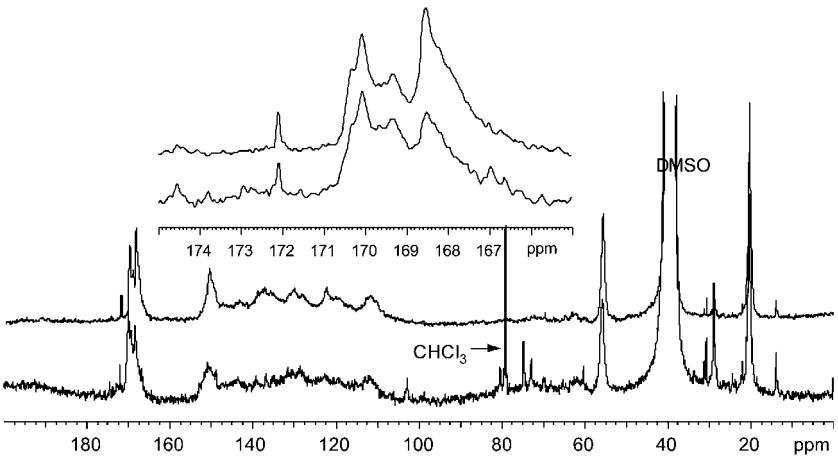

Figure 6 ^{13}C NMR spectra of residual lignins: top, RL-$KP_{30.5}$; bottom, RL-$KP_{POM,23.6}$ (acetylated; DMSO-d_6).

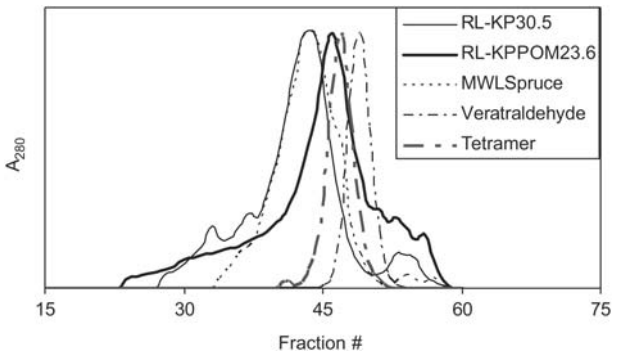

Figure 7 Gel permeation chromatography of lignins and model compounds.

substances have high UV absorptivity (Srebotnik and Hammel 2000).

Alkaline extraction of $KP_{POM,23.6}$ prior to mild AH

Extraction of $KP_{POM,23.6}$ with 1% NaOH was performed in an attempt to increase the yield and purity of RL. The low-molecular-weight carbohydrates that may inhibit lignin from solubilization during mild AH should thus be eliminated. Approximately 4% of the total pulp, including 17% of the RL, was dissolved in this pretreatment. The alkaline extract was acidified and the precipitate was isolated ($KP_{POM,NaOH/pH2}$). The FTIR spectrum of the precipitate indicated the presence of both lignin and carbohydrates. Approximately 82% lignin was calculated by the formula developed by Rodrigues et al. (1998). Xylans were detected by HSQC (not shown) in addition to lignin.

Alkaline pretreatment did not improve the removal of lignin by mild AH as the lignin yield remained approximately the same. However, RL-$KP_{POM/NaOH}$ was less contaminated. It contained more Klason lignin, less residual carbohydrates (3.5-fold less glucose), and less non-lignin/non-carbohydrate extraneous material (Table 1). Accordingly, the characteristic correlations of the internal $\beta(1\rightarrow4)$-bonded glucopyranose units in the HSQC NMR spectrum of this lignin were not observed (HSQC not shown). Our interpretation is that the lignin-cellulose association formed during POM treatment of pulp is alkali-sensitive. This behavior is in opposition to alkali-stable cellulose-lignin bonds formed in kraft pulping (Isogai et al. 1987).

Alkaline pretreatment of pulp led to a decrease in the C=O group content of the RL by approximately 5.5% (RL-$KP_{POM,23.6}$, 6.34 mmolC=O g^{-1} AL, RL-$KP_{POM/NaOH}$, 6.00 mmol C=O g^{-1} AL, SD 0.0865 and 0.0829, respectively), whereas $KP_{POM,NaOH/pH2}$ was enriched in these groups (7.75 mmol C=O g^{-1} AL, SD 0.0934). This result is not unexpected, because treatment of lignin with oxidizing agents produces material susceptible to alkaline extraction, presumably due to the formation of quinones and ketones (Weinstock et al. 1993; Srebotnik and Hammel 2000; Chakar and Ragauskas 2001). However, alkaline extraction of laccase-mediator-treated pulps reduces the level of quinone groups in RLs by approximately 21% (Chakar and Ragauskas 2001). This finding is four-fold greater than the data observed in the present study. It is likely that POM delignification gives rise to fewer quinones than the laccase/mediator system.

Conclusions

POMs are effective in oxidizing RL from kraft pulp. A four-fold increase in carbonyl/carboxyl groups was observed in RL isolated from POM-treated kraft pulp in an early phase of delignification (after a decrease in kappa number of ~23%). This lignin has lower molecular mass, lower aromaticity, lower methoxyl content and low content of free phenolic hydroxyl groups. Lignin-cellulose association and a more oxidized lignin structure may be the reasons for the low yield of residual lignin of POM-delignified pulps.

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References

Balakshin, M.Yu., Capanema, E.A., Goldfarb, B., Frampton, J., Kadla, J.F. (2005) NMR studies on Fraser fir *Abies fraseri* (Pursh) Poir. lignins. Holzforschung 59:488–496.

Bujanovic, B., Reiner, R.S., Hirth, K.C., Ralph, S.A., Atalla, R.H. (2005) Studies of lignin transformation in polyoxometalate (POM) bleaching of kraft pulp. In: Proceedings of the 13th ISWFP Conference, Auckland, New Zealand, 2005, Vol. 3. pp. 49–56.

Chakar, F.S., Ragauskas, A.J. (2001) Formation of quinonoid structures in laccase-mediator reactions. In: Oxidative Delignification Chemistry – Fundamental and Catalysis, ACS Symposium Series No. 785. Ed. Argyropoulos, D.S. ACS, Washington, DC. pp. 444–455.

Chen, C.-L. (1992) Determination of methoxyl groups. In: Methods in Lignin Chemistry. Eds. Lin, S.Y., Dence, C.W. Springer-Verlag, Berlin. pp. 463–472.

Davis, M.W. (1998) A rapid modified method for compositional carbohydrate analysis of lignocellulosics by high pH anion-exchange chromatography with pulsed amperometric detection (HPAEC/PAD). J. Wood Chem. Technol. 18:235–252.

Evtuguin, D.V., Robert, D. (1997) The detection of muconic acid type structures in oxidized lignins by ^{13}C NMR spectroscopy. Wood Sci. Technol. 31:423–431.

Evtuguin, D.V., Pascoal Neto, C., Rocha, J. (2000a) Lignin degradation in oxygen delignification catalyzed by $[PMo_7V_5O_{40}]^{8-}$ polyanion. Part I. Study on wood lignin. Holzforschung 54:381–389.

Evtuguin, D.V., Daniel, A.I.D., Sivestre, A.J.D., Amado, F.M.L., Pascoal Neto, C. (2000b) Lignin aerobic oxidation promoted by molybdovanadophosphate polyanion. $[PMo_7V_5O_{40}]^{8-}$. Study on the oxidative cleavage of β-O-4 aryl ether structures using model compounds. J. Mol. Catal. A 154:217–224.

Evtuguin, D.V., Pascoal Neto, C., Carapuça, H., Soares, J. (2000c) Lignin degradation in oxygen delignification catalyzed by $[PMo_7V_5O_{40}]^{8-}$ polyanion. Part II. Study on lignin monomeric model compounds. Holzforschung 54:511–518.

Faix, O. (1991) Classification of lignins from different botanical origins by FT-IR spectroscopy. Holzforschung 45 (Suppl.): 21–27.

Fu, S., Lucia, L.A. (2003) Investigation of the chemical basis for inefficient lignin removal in softwood kraft pulp during oxygen delignification. Ind. Eng. Chem. Res. 42:4269–4276.

Furman, G.S., Lonsky, W.F. (1988): Charge-transfer complexes in kraft lignin Part 1: Occurrence. J. Wood Chem. Technol. 8:165–189.

Gärtner, A., Gellerstedt, G., Tamminen, T. (1999) Determination of phenolic hydroxyl groups in residual lignin using a modified UV-method. Nordic Pulp Pap. Res. J. 14:163–170.

Gaspar, A., Evtuguin, D.V., Pascoal Neto, C. (2004) Lignin reactions in oxygen delignification catalyzed by Mn(II)-substituted molybdovanadophosphate polyanion. Holzforschung 58: 640–649.

Gellerstedt, G., Pranda, J., Lindfors, E.L. (1994) Structural and molecular properties of residual birch kraft lignin. J. Wood Chem. Technol. 14:467–482.

Hortling, B., Tamminen, T., Kenttä, E. (1997) Determination of carboxyl and non-conjugated carbonyl groups in dissolved and residual lignins by IR spectroscopy. Holzforschung 51: 405–410.

Isogai, A., Ishizu, A., Nakano, J. (1987) Residual lignin in unbleached kraft pulp. Part 2. Analysis of unbleached kraft pulp by new permethylation method. J. Wood Chem. Technol. 7: 463–484.

Kang, G., Ni, Y., Van Heiningen, A. (1997) Polyoxometalate delignification: study of lignin model compounds. Appita J. 50: 313–318.

Kawai, S., Umezawa, T., Higuchi, T. (1985) Metabolism of a non-phenolic β-O-4 lignin substructure model compound by *Coriolus versicolor.* Agric. Biol. Chem. 49:2325–2330.

Kawai, S., Nakagawa, M., Ohashi. (1999) Aromatic ring cleavage of a non-phenolic β-O-4 lignin model dimmer by laccase of *Trametes versicolor* in the presence of 1-hydroxybenzotriazole. FEBS Lett. 446:355–358.

Kirk, T.K., Chang, H.-m. (1975) Decomposition of lignin by white-rot fungi II. Characterization of heavily degraded lignins from decayed spruce. Holzforschung 29:56–64.

Kirk, T.K., Tien, M., Kersten, P.J., Mozuch, M.D., Kalyanaraman, B. (1986) Ligninase of *Phanerochaete chrysosporium.* Biochem. J. 236:279–287.

Lundquist, K. (1992) Proton (^{1}H) NMR spectroscopy. In: Methods in Lignin Chemistry. Eds. Lin, S.Y., Dence, C.W. Springer-Verlag, Berlin. pp. 242–249.

Poppius-Levlin, K., Wang, W., Tamminen, T., Hortling, B., Viikari, L., Niku-Paavola, M.-L. (1999) Effects of laccase/HBT treatment on pulp and lignin structures. J. Pulp Pap. Sci. 25: 90–94.

Ralph, S.A., Ralph, J., Landucci, L.L. (2006) NMR database of lignin and cell wall model compounds. Available at http://www.arc.usda.gov/software.html.

Rodrigues, J., Faix, O., Pereira, H. (1998) Determination of lignin content of *Eucalyptus globules* wood using FTIR spectroscopy. Holzforschung 52:46–50.

Runge, T.M., Ragauskas, A.J. (1999) NMR analysis of oxidative alkaline extraction stage lignins. Holzforschung 53:623–631.

Srebotnik, E., Hammel, K.E. (2000) Degradation of nonphenolic lignin by the laccase/1-hydroxybenzotriazole system. J. Biotechnol. 81:179–188.

Tamminen, T.L., Hortling, B.R. (1999) Isolation and characterization of residual lignin. In: Advances in Lignocellulosics Characterization. Ed. Argyropoulos, D.S. TAPPI Press, Atlanta, GA. pp. 1–42.

Tappi standard (1991) T 212. Om-88, One percent sodium hydroxide solubility of wood and pulp.

Tuor, U., Wariishi, H., Schoemaker, H.E., Gold, M.H. (1992) Oxidation of phenolic arylglycerol β-aryl ether lignin model compounds by manganese peroxidase from *Phanerochaete chrysosporium*: Oxidative cleavage of α-carbonyl model compound. Biochemistry 31:4986–4995.

Umezawa, T., Shimada, M., Higuchi, T., Kusai, K. (1986) Aromatic ring cleavage of β-O-4 lignin substructure model dimers by lignin peroxidase of *Phanerochaete chrysosporium*. FEBS Lett. 205:287–292.

Weinstock, I.A., Minor, J.L., Reiner, R.S., Agarwal, U.P., Atalla, R.H. (1993) FT Raman and UV visible spectroscopic studies of highly selective polyoxometalate bleaching system. In: Proceedings of the Pulping Conference, Atlanta, GA, Book 2. TAPPI Press, Atlanta, GA. pp. 519–532.

Weinstock, I.A., Atalla, R.H., Reiner, R.S., Houtman, C.J., Hill, C.L. (1998a) Selective transition-metal catalysis of oxygen delignification using water-soluble salts of POM anions. Part. I. Chemical principles and process concepts. Holzforschung 52:304–310.

Weinstock, I.A., Hammel, K.E., Moen, M.A., Landucci, L.L., Ralph, S., Sullivan, C.E., Reiner, R.S. (1998b) Selective transition-metal catalysis of oxygen delignification using water-soluble salts of POM anions. Part. II. Reactions of α-$[SiVW_{11}O_{40}]^{5-}$ with phenolic lignin-model compounds. Holzforschung 52:311–318.

Yokoyama, T., Chang, H.-m., Reiner, R.S., Atalla, R.H., Weinstock, I.A., Kadla, J.F. (2004) Polyoxometalate oxidation of non-phenolic lignin subunits in water: Effect of substrate structure on reaction kinetics. Holzforschung 58:116–121.

Zhang, L., Gellerstedt, G. (1999) Detection and determination of carbonyls and quinones by modern techniques. In: Proceedings of the 10th ISWP Conference, Yokohama, Japan, 1999, Vol. II. pp. 164–170.

Zhang, D., Pu, Y., Chai, X.-S., Naithani, V., Jameel, H., Ragauskas, A.J. (2006) Elucidating carboxylic acid profiles for extended oxygen delignification of high-kappa softwood kraft pulps. Holzforschung 60:123-129.

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