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Radicalization of Lignocellulosic Fibers, Related Structural and Morphological Changes

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The radicalization of unbleached lignocellulosic fibers obtained from thermomechanical (TMP) and chemothermomechanical (CTMP) pulps was performed in heterogeneous phase by reaction with dioxygen in the presence of *N*,*N*′-ethylenebis(salicylideneiminato)cobalt(II), [Co(salen)], as catalyst. Phenoxy cobalt radicals immobilized in fibers were observed by electron paramagnetic resonance (EPR) spectroscopy; their amount depends on the fiber swelling induced by reaction medium. The absolute concentration of such radicals in fibers, about 10^{16} spin/g, reaches values 10 times higher than that of phenoxy radicals formed in similar oxidative reactions catalyzed by laccase. The generation of phenoxy cobalt radicals in fibers was related to structural changes of lignin units, detected by mono- and bidimensional nuclear magnetic resonance (^{13}C NMR and 2D-HSQC) investigations, and to morphological modifications in fibers observed by scanning electron microscopy (SEM).

Introduction

The increase in environmental awareness has raised the interest in the development of new materials from renewable resources.[1] Among the most important renewable resources, natural fibers have biodegradability and thermal recyclability. In particular lignocellulosic fibers have raised the interest of industrial sectors as replacement for currently used synthetic or semisynthetic fibers in the field of packaging applications.[2] Lignocellulosic fibers from the pulp and paper industry are composite materials containing lignin, cellulose, hemicellulose, and various extractive components; lignin and hemicellulose are situated as fillers between the highly ordered cellulose microfibrils.[3] Lignin is a structurally intricate aromatic polymer with oxygenated phenylpropane units; four main carbon−carbon and carbon−oxygen interunit bonds are present in its structure and the relative abundance of these linkages varies with the different types of wood[4] and wood treatments.[5] In the field of packaging applications, materials with high barrier and mechanical properties are generally required. Wood fibers can achieve these properties, after proper modification. Attempts to modify fiber properties by grafting synthetic polymers onto the cellulose backbone, started as early as the 1940s. Radical centers at the cellulose backbone behave as grafting initiators, and they were generated by high-energy irradiation, by oxygen reaction in the presence of transition metal complexes, by decomposition of peroxides, or by radical transfer reaction.[6]

Alternatively, radical active centers were produced on the lignin at the fiber surface. As an example, the reaction of wood fibers from thermomechanical pulp (TMP) with oxygen was performed by using laccase as catalyst, and it was demonstrated to proceed through radicalic activation of the surface lignin phenols. Under such treatment, glue-less fiberboards were obtained and the wet strength of paper was improved.[7,8]

Mechanism studies on the laccase-catalyzed oxidation[9] showed that one mole of oxygen reacts with four moles of phenols giving phenoxy radicals

$$4\mathrm{PhOH} + \mathrm{O_2} \xrightarrow{\text{laccase}} 4\mathrm{PhO^\bullet} + 2\mathrm{H_2O} \qquad (1)$$

It was demonstrated that phenoxy radicals are the intermediate species in modifying the lignin structure. The same radical species were found to be active in the oxidation of lignin model compounds by O_2, in the presence of transition metal complexes as catalysts.[10−12]

We recently reported[13] that representative lignin model compounds, such as phenylcoumarans and arylglicerol-β-aryl ethers, were oxidized in homogeneous phase with dioxygen, using *N*,*N*′-ethylenebis(salicylideneiminato) cobalt-(II), [Co(salen)], as catalyst. The reactivity and the characterization of the paramagnetic species by electron paramagnetic resonance (EPR) spectroscopy suggested that such oxidation occurs through the following three steps:

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$$[Co^{II}(salen)] + ROH + O_2 \rightleftharpoons [Co^{III}(salen)(ROH)(O_2^-)] \quad (2)$$

$$[Co^{III}(salen)(ROH)(O_2^-)] + ROH \rightleftharpoons [Co^{III}(salen)(ROH)(RO^\bullet)] + HO_2^- \quad (3)$$

$$[Co^{III}(salen)(ROH)(RO^\bullet)] + HO_2^- \rightleftharpoons [Co^{III}(salen)(RO^-)(RO^\bullet)] + H_2O_2 \quad (4)$$

where ROH is the phenol unit in the lignin model compound and $RO^\bullet$ is the corresponding intermediate radical. After the third step, $RO^\bullet$ probably dissociates from the cobalt center and then further reacts with O_2 giving oxidized molecular products.

On these bases it was expected that lignocellulosic fibers containing phenols might undergo radical formation under similar oxidative conditions.

The present study reports the identification and the quantification of radicals formed by reacting unbleached fibers obtained from thermomechanical (TMP) and chemothermomechanical (CTMP) pulps with dioxygen, using [Co(salen)] as catalyst. These materials were chosen because they are rich in lignin whose chemical structure is not heavily modified after pulp treatment and they are widely in use for industrial purposes. Lignin structure and morphology are different in fibers from CTMP with respect to those from TMP. In fact unbleached CTMP has a slightly higher surface content of lignin than unbleached TMP[14,15] and some sulfonate groups are grafted to the lignin during manufacture of CTMP.[16,17]

The radicals formed in fibers during oxidation were identified and quantified by EPR spectroscopy; the influence of solvent, molecular oxygen pressure, and time of reaction on the amount of formed radicals were also investigated.

The changes in chemical structure, achieved by lignin units under oxidative treatments, were assessed by heteronuclear single quantum coherence (HSQC) and ^{13}C NMR spectroscopy and compared with the radicalization.

Morphological modifications induced on fibers by the oxidative process were evaluated by Scanning Electron Microscopy (SEM) and related both to formation of radicals and to structural changes in the lignin units.

Materials and Methods

Pulps. The softwood thermomechanical (TMP) and chemothermomechanical (CTMP) pulps, both unbleached, were provided by Stora Enso Oyj. The amounts of lignin in pulps were evaluated by Stora Enso using the Klason method[18] and resulted in 27.5% for CTMP and 27.1% for TMP. The amount of extractives in both pulps evaluated by Stora Enso, are reported in Table 1.

Fibers and Fines. Fibers and fines (fiber fragments) were obtained by suspending pulps in dichloromethane for 30 min then in methanol for 60 min, under mechanical stirring, to eliminate extractives. Lignocellulosic fibers and fines were recovered by filtration and dried in air at 353 K. Hereafter "fibers and fines" will be named TMP and CTMP fibers, depending on the pulp they were obtained.

Reagents. *N*,*N*′-ethylenebis(salicylideneiminato) cobalt(II), [Co(salen)] (99%), was supplied by Aldrich. Methanol, chloroform, dioxane and deuterated DMSO-d_6 (Fluka) were used as received. Mill-Q water was used. Oxygen (99.99%) was supplied by Technogas.

Apparatus and Measurements. The EPR spectra were recorded at 123 K on a Bruker EMX spectrometer working at the X-band frequency, equipped with a variable temperature BVT 2000 unit (Bruker).

The *g* values were determined by standardization with α,α′-diphenyl-β-picryl hydrazyl (DPPH).

The concentration of the paramagnetic species, expressed as number of spins per gram of sample (spin/g), was calculated with ±10% accuracy by double integration of the resonance lines. The area under the absorption signal was referred to a calibration curve ($R^2 = 0.996$), plotting the areas vs the number of spins per cm of the filled EPR tube. The calibration curve was determined using standard solutions of DPPH in toluene/nujol at a ratio of 2:1 v/v. Care was taken in order to obtain that the sensitive part of the EPR cavity (1 cm length) was always filled; the weight of the sample filling 1 cm length of the EPR tube was always accurately determined.

NMR analyses were performed on lignin extracted from TMP and CTMP fibers by a modification of the acydolisis method developed by Gellerstedt et al.:[19] dried fibers (5 g) were suspended in 175 mL of dioxane/water 82:18 v/v (0.1 M HCl) and refluxed under nitrogen for 3 h. The fibers were filtered and washed 3 times with 15 mL of dioxane/water 82:18 v/v and then with distilled water to reach a neutral pH. The filtrate was then evaporated under reduced pressure at 313 K until dioxane had been removed. The aqueous solution was kept overnight in a refrigerator to induce coagulation of lignin; the precipitate was collected by filtration through a fine porous glass filter and washed with distilled water. After drying in air at 353 K for 2 h, lignin was refluxed with hexane in a Soxhlet extractor for 8 h in order to remove low molecular weight compounds. The yield of lignin, evaluated as (extracted lignin)/(lignin in pulp) w/w%, was around 35−40%.

The extracted lignin was acetylated with acetic anhydride: pyridine 1:1 v/v and each sample, approximately 60 mg, dissolved in 0.75 mL of DMSO-d_6.

The inverse detected $^1H-^{13}C$ correlation spectra (HSQC) were measured on a Varian Inova 300 MHz instrument at 308 K. The spectral width was set at 5 kHz in F2 and 25 kHz in F1. Altogether 128 transients in 256 time increments were collected. The polarization transfer delay was set at the assumed coupling of 140 Hz, and a relaxation delay of 2 s was used. The spectra were processed using Γ/2 shifted squared sinebell functions in both dimensions before Fourier transform.

To evaluate the number of phenolic and alcoholic groups and the relative amount of intermonomeric bonds in lignin extracted from CTMP and TMP fibers,[20−23] 1D ^{13}C spectra were recorded using a Varian Mercury 400 MHz instrument at 308 K. The chemical shifts were referred to the solvent

Table 1. Amount of Extractives in TMP and CTMP Pulps

pulps	percentage of total extractives[a] (w/w)	fatty acids (mg/g)	resin acids (mg/g)	lignans and sterols (mg/g)	sterylesters (mg/g)	triglycerides (mg/g)
TMP	1.39	0.94	0.88	0.42	0.64	0.65
CTMP	0.14	0.34	0.09	n.d.[b]	n.d.[b]	n.d.[b]

[a] In acetone. [b] n.d. = not detected.

signal at 39.5 ppm. Relaxation delay of 10 s was used between the scans. Line broadening of 2−5 Hz was applied to FIDs before Fourier transform. For each spectrum, typically about 8000 scans were accumulated. The number of primary, secondary, and phenolic OH groups per aromatic ring was calculated from that of the corresponding acetylic carboxyl groups, as reported in the literature.[20−23] Specifically, first the average number of methoxyl groups per aromatic ring in CTMP and TMP lignins was evaluated by elemental and gas-chromatographic analyses.[24] Then the intensity of signals due to acetylic carboxyl groups was divided by the intensity of signal due to methoxyl group and multiplied with the average number of methoxyl groups per aromatic ring.

Scanning electron microscopy (SEM) investigation was performed by JEOL (Tokyo, Japan) JSM 5600 LV (Low Vacuum) microscope. Samples were coated with a Pd/Au thin film to avoid charging effects and the images were taken in high vacuum conditions.

Optical microscopy images were taken using a polarized light Leica DMP instrument, equipped with a 100 W lamp. The swelling of fibers in the reaction medium was studied by comparing the distance between the outer walls of a fibril suspended in a given solvent with that after its complete evaporation. To avoid mistakes due to the natural variability of fibers, the two values were measured leaving the sample at exactly the same position on the sample holder of the optical microscope. The "time-zero value" was the outer wall distance for the fibril suspended in the solvent; the constant value reached by the distance after complete evaporation of the solvent was taken as "dry fiber value". For each fiber, these measurements were repeated in three different regions.

Graphite furnace-atomic absorption spectroscopy (GF-AAS) analyses were performed on unreacted lignocellulosic fibers, dried at 343 K for 48 h. Samples (150 mg) were suspended in a 70% HNO_3 solution (10 mL) and digested under reflux at 353 K for 24 h. The cold solution was diluted to 50 mL with Mill-Q water and analyzed for Zn, Mn, Fe, and Cu content[25] by a Perkin-Elmer SIMAA 6000 instrument. Oxidized lignocellulosic fibers were similarly treated and analyzed for Co content.

Radicalization of Fibers. Fibers (150 mg) were suspended in 30 mL of different solvents containing [Co(salen)] (15 mg) and were allowed to react with molecular oxygen at 298 K, at the pressure and for the time summarized in Table 2.

After reaction the solid was recovered by filtration and washed three times with 30 mL of ethyl acetate and three times with 20 mL of acetone. Solvents and washing liquids were collected in order to check the presence of paramagnetic species by EPR spectroscopy. In all cases paramagnetic species were absent in the filtered liquid. The washed fibers were allowed to dry in air, then inserted into the EPR tube, and frozen at the liquid nitrogen temperature in order to inhibit further reaction before the spectromagnetic investigation.

Table 2. Solvents, Oxygen Pressure (MPa), and Time of Reaction (min) for the [Co(salen)]-Catalyzed Radicalization of Fibers

solvent	oxygen pressure (MPa)	time (min)
methanol	0.1, 0.5, 1	5, 15, 30, 45, 65, 85
chloroform	0.1	30

Blank samples were prepared in the same way, but in the absence of [Co(salen)].

Results and Discussion

Unreacted Lignocellulosic Fibers. To give a rationale for the changes observed after oxidation in the presence of [Co(salen)], CTMP, and TMP unreacted fibers were characterized in terms of: (i) amount of phenoxy radicals, photochemically induced and/or caused by thermal, mechanical, chemical treatments during pulp production;[26] (ii) amount of transition metal centers, native in pulps, which could catalyze, as well as [Co(salen)], the formation of phenoxy radicals in fibers according to the following reactions:[27]

$$M(II) + O_2 \rightarrow M(III)-O_2^- \quad (5)$$

$$M(III)-O_2^- + PhOH \rightarrow PhO^\bullet + M(III)OOH \quad (6)$$

(i) EPR spectra allowed to assess the presence of radical centers in unreacted fibers. In both fibers, sharp isotropic symmetric signals were observed (Figure 1a,b) which, on the basis of g values ($g = 2.004$ for CTMP; $g = 2.005$ for TMP) and line width ($\Delta Hpp = 11$ G for both) are attributable to phenoxy radicals.[28] For both fibers, the spin concentration is 1×10^{16} spin/g (Table 3).

The resonance lines of ^{55}Mn ($g = 2.004$ $A_{Mn(I=5/2)} = 90.8$ G) were also detectable in TMP fiber (Figure 1b); on the basis of the hyperfine nuclear interaction value, the lines are attributable to Mn centers interacting with lignin coordinating groups.[29]

(ii) Transition metal centers able to generate radicals following reactions 5 and 6 (Zn, Mn, Fe, Cu) were determined in fibers by GF-AAS. Iron was present in similar amounts in both fibers (31.80 and 26.50 μg/g dry fiber for CTMP and TMP, respectively), whereas manganese was detected in very small amounts only in TMP (5.62 μg/g dry fiber). Zn and Cu were absent. The results are reported in Table 3

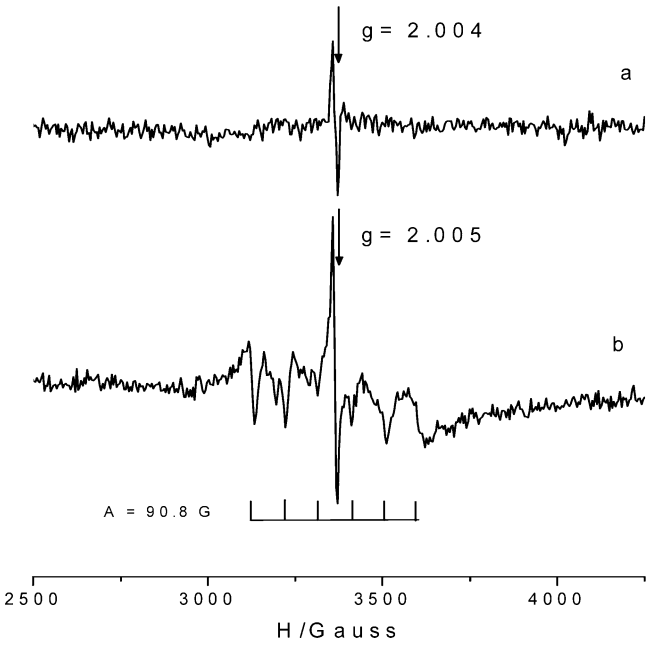


Figure 1. X-band EPR spectra recorded at 123 K on (a) CTMP and (b) TMP unreacted fibers.

Table 3. Amounts of Phenoxy Radicals and Metal Content in CTMP and TMP Unreacted Fibers

	phenoxy radicals	metal content (μg metal/g fiber)	
fibers	(spin/g fiber)	Mn	Fe
CTMP	1×10^{16}	n.d.[a]	31.80
TMP	1×10^{16}	5.62	26.50

[a] n.d. = not detected.

Oxidized Lignocellulosic Fibers. *Radical Formation.* The radical formation in fibers, under reaction with molecular oxygen at 298 K in the presence of [Co(salen)], was investigated aiming to maximize the radical amount, while preserving the fiber integrity. The optimal conditions were determined by varying (i) the reaction medium, (ii) the oxygen pressure, and (iii) the time of reaction according to Table 2.

The fiber/[Co(salen)] ratio 10:1 w/w was always used, corresponding to molar ratio phenols/[Co(salen)] $\sim$ 0.8.

The fiber concentration in the solvent was always 5.0 mg/mL.

(i) CTMP and TMP fibers were suspended in [Co(salen)] solutions in methanol and chloroform; then the suspension was let react with oxygen (0.1 MPa) for 30 min. At the end, fibers were recovered by filtration, washed, and allowed to dry (see experimental), and the EPR spectra were recorded at 123 K.

For both fibers, only a low intensity signal of the phenoxy radical was observed in chloroform, whereas an apparently eight resonance line signal was detected in methanol (Figure 2a,b).

Such a signal is very similar in shape to that observed in frozen solution during the oxidative degradation of monomeric and dimeric lignin model compounds[13,30] and is attributable to the phenoxy cobalt radical [Co^{III}(salen)(ROH)-(RO$^{\bullet}$)] (ROH is a phenol lignin model compound). As a comparison, the spectrum observed for ROH = *E*-methyl ferulate after oxidation in methanol for 20 min in the presence of [Co(salen)] at 1 MPa O_2 pressure was reported (Figure 2c). Radicals formed by lignin model compounds in solution were deeply investigated in previous studies[13,30] both by X-band and high frequency (HF) EPR spectroscopy. All showed axial magnetic symmetry of g and A (^{59}Co) tensor components, with $g_{\perp} > g_{||}$ and $A_{||} > A_{\perp}$, the hyperfine coupling constant values varying with the ROH molecule.[30] Previous studies[13,30] also showed that the signal of the phenoxy cobalt radical dramatically changes from isotropic to anisotropic shape upon cooling from 298 to 123 K; this effect was attributed to the change from the fast motion regime at 298 K to the rigid regime in frozen solution. Instead, no changes were observed in the signal shape of radicals formed during the oxidation of fibers when temperature was lowered from 298 to 123 K. This result suggests that radicals in fibers are motionless also at 298 K, probably because they are immobilized within the fiber host, being the Co center coordinated to two phenol groups of the fiber.

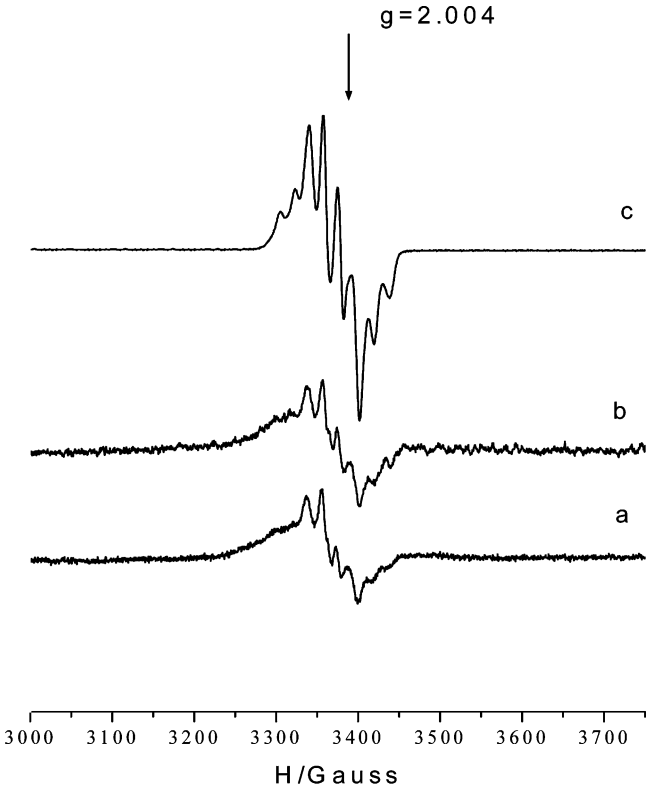


Figure 2. X-band EPR spectra recorded at 123 K on (a) CTMP fibers (b) TMP fibers, after oxidation in methanol in the presence of [Co-(salen)] at 0.1 MPa O_2 pressure for 30 min; (c) E-methyl ferulate after oxidation in methanol for 20 min in the presence of [Co(salen)] at 1 MPa O_2 pressure.[13,23]

In the case of lignocellulosic fibers, the observed signal is probably the envelope of several phenoxy cobalt radicals, [Co^{III}(salen)(ROH)(RO$^{\bullet}$)]-like, originated by the coordination of [Co(salen)] to different phenols of lignin. This causes a higher width of resonance lines with respect to the species containing a unique ROH ligand. In the study of fibers, the use of HF-EPR spectroscopy is not encouraged, in the light of the small amount of paramagnetic species, and we did not proceed into further investigation of the signal.

For TMP, after 30 min from the onset of reaction in methanol, the signal intensity of the phenoxy cobalt radical reached a value about three times higher than for CTMP.

Table 4. Percentage of Shrinkage for CTMP and TMP Fibers Suspended in Methanol and in Chloroform

	percentage of shrinkage[a]	
solvent	CTMP	TMP
methanol	8 ± 3	19 ± 4
chloroform	3 ± 1	4 ± 1

[a] Calculated according to the equation: (wet fiber size − dry fiber size) × 100/dry fiber size.

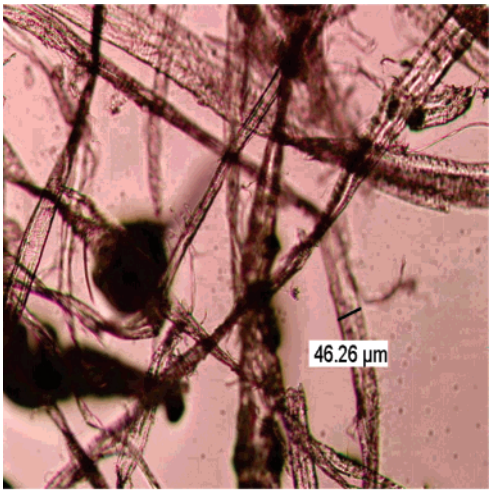
Surprisingly phenoxy cobalt radical did not form in chloroform, although this had resulted to be the election solvent for the stabilization of the same radical in homogeneous phase. In fact the not coordinating $CHCl_3$ allows phenols to interact with [Co(salen)] on both sides of the planar catalyst molecule. Basing on this argument, the formation of phenoxy cobalt radicals on fibers, observed in methanol, cannot be promoted by the [Co(salen)]−methanol interaction. It may be suggested that a higher swelling of fibers in methanol increases the surface area and favors the fiber interaction with the catalyst.

To verify this, a morphological investigation of fibers during the reaction was performed and the swelling of the two fibers both in methanol and in chloroform was investigated by optical microscopy. The variation in fiber size was measured from the distance between the outer walls as (wet fiber size − dry fiber size) × 100/dry fiber size (percentage of shrinkage in Table 4) and taken as a measure of the capability of the solvent to swell fiber (Figure 3a,b for CTMP). The results show that both fibers are better swelled by methanol than by chloroform and suggest that the variation in the amount of phenoxy cobalt radicals is strongly influenced by the swelling of fibers. On these bases, methanol showed to be the election solvent for fiber radicalization.

Although the above results suggest that the high fiber swelling by methanol has a primary role in the formation of phenoxy cobalt radical, we demonstrated in previous papers[13,30,31] that methanol could coordinate to the planar complex [Co(salen)] in fifth position and promote the formation of the superoxo derivative of [Co(salen)]; this, in principle, may react with the lignin phenols, giving phenoxy cobalt radical.

To verify if methanol, as well as swelling the fiber, also coordinates cobalt and promotes the formation of a methoxy phenoxy cobalt radical, both fibers were suspended in chloroform, which does not coordinate cobalt, and then methanol was added in MeOH/[Co(salen)] 2:1 molar ratio. The same fiber/[Co(salen)] and fiber/chloroform ratios as in the radical formation studies were used. The amount of methanol was the highest in which the fiber did not swell. After 30 min of oxidation with 0.1 MPa of oxygen, the phenoxy radical was the only observed paramagnetic species. This result excludes the role of methanol in promoting the formation of the superoxo derivative of [Co(salen)], under the described reaction conditions.

(ii) The effects of the oxygen pressure both on the amount of phenoxy cobalt radical and on the fiber recovery were investigated by carrying out the radicalization in methanol for 30 min at different oxygen pressures (0.1, 0.5, and 1 MPa). The amounts of phenoxy cobalt radicals vs oxygen pressure are reported in Figure 4.



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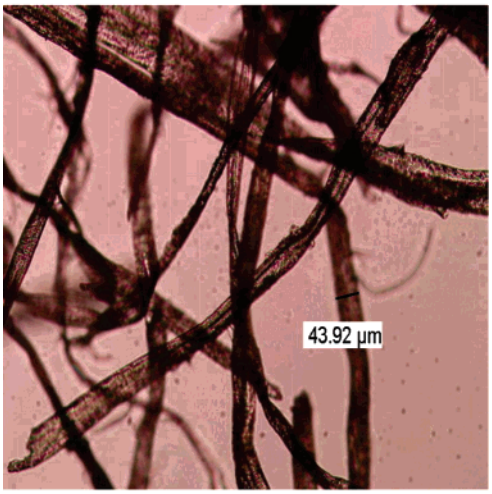

b

Figure 3. Optical microscopy images of CTMP fiber (a) suspended in methanol and (b) after solvent evaporation.

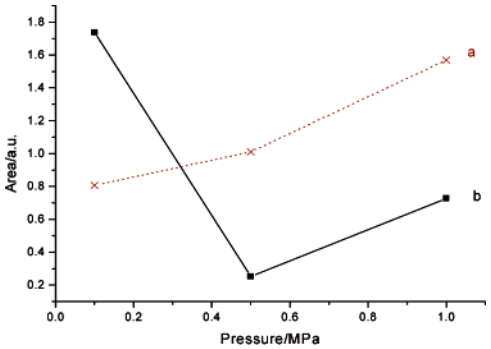

Figure 4. Amounts of phenoxy cobalt radicals, reported as area of the EPR resonances, in (a) CTMP and (b) TMP fibers after oxidation for 30 min in methanol, in the presence of [Co(salen)], at different oxygen pressures.

For TMP the amount of phenoxy cobalt radicals reaches the maximum value at 0.1 MPa of oxygen pressure; at the same pressure, the highest recovery of fiber (87 ± 3% w/w) was also obtained. The recovery of this fiber at 1 MPa of oxygen pressure was lower (55 ± 4% w/w). For CTMP, the maximum amount of phenoxy cobalt radicals was obtained

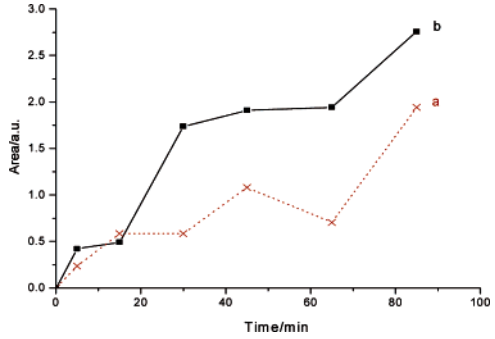

Figure 5. Amounts of phenoxy cobalt radicals, reported as area of the EPR resonances, in (a) CTMP and (b) TMP fibers after oxidation in the presence of [Co(salen)] at 0.1 MPa of oxygen pressure in methanol at different reaction times.

at 1 MPa, but in this case, the fiber recovery after treatments was much lower (60 ± 4% w/w) than working at 0.1 MPa of pressure (90 ± 3% w/w). These results suggest that no significant delignification occurs for both fibers at 0.1 MPa of oxygen pressure. The amount of lignin present in oxidized fibers, evaluated by the Klason method[18] (28.3% for CTMP, 30.6% for TMP), confirmed that the delignification effect, under the above reaction conditions, is not relevant.

(iii) The role of the reaction time was investigated by suspending fibers in methanol and by evaluating the resonance intensities of the phenoxy cobalt radicals formed under 0.1 MPa of oxygen pressure at different times of reaction (5, 15, 30, 45, 65, and 85 min; Figure 5).

During the reaction, both fibers show an increase in the amount of phenoxy cobalt radicals, TMP being more reactive than CTMP at any time of reaction. This trend is similar to that observed for the phenoxy cobalt radicals of lignin models compounds with the lowest reactivity.[13] It may be suggested that the low tendency of the cobalt radicals in fibers to undergo further oxidation, right-hand shifting reactions 2−4, is probably due to the difficult dissociation of the fiber phenoxy groups from cobalt.

Accordingly, phenoxy cobalt radicals formed in fibers in the best radicalization conditions (in methanol, at 0.1 MPa of oxygen pressure, after 85 min of reaction) showed long lifetimes in the solid state at room temperature. For TMP, the kinetic is roughly characterized by an initial, rather rapid decay, followed by a slower one, whereas for CTMP, an initial slight increase is followed by a slow decay (Figure 6). The inclusion of radicals in fibers is thus suggested to be responsible for the long lifetime of the paramagnetic species. Similar behavior was reported for phenoxy radicals generated in fibers, by using laccase as catalyst.[32]

The absolute amount of the paramagnetic species in fibers, expressed as spin/g of fiber, was evaluated for (i) each unreacted fiber, (ii) the corresponding blank sample (that is, the fiber reacted for 85 min in the absence of catalyst), and (iii) each fiber after 85 min of reaction in the presence of [Co(salen)] under the optimized conditions (methanol as solvent, 0.1 MPa of oxygen pressure). Phenoxy radicals were present in unreacted fibers and in blank samples in similar amounts (about 1×10^{16} spin/g); thus, iron and manganese in unreacted fibers do not promote the formation of further radicals (Figure 7a,b). In the presence of [Co(salen)], in both

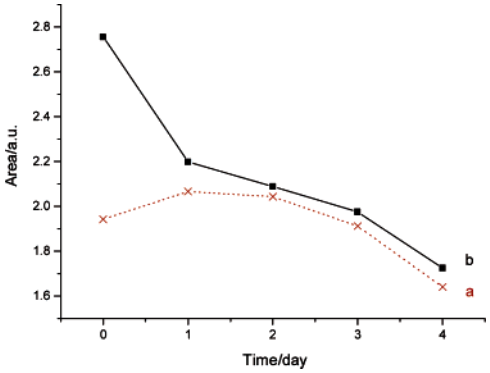

Figure 6. Decay in the amounts of phenoxy cobalt radicals, reported as area of the EPR resonances, in (a) CTMP and (b) TMP fiber samples after oxidation in methanol in the presence of [Co(salen)] for 85 min under 0.1 MPa of oxygen pressure.

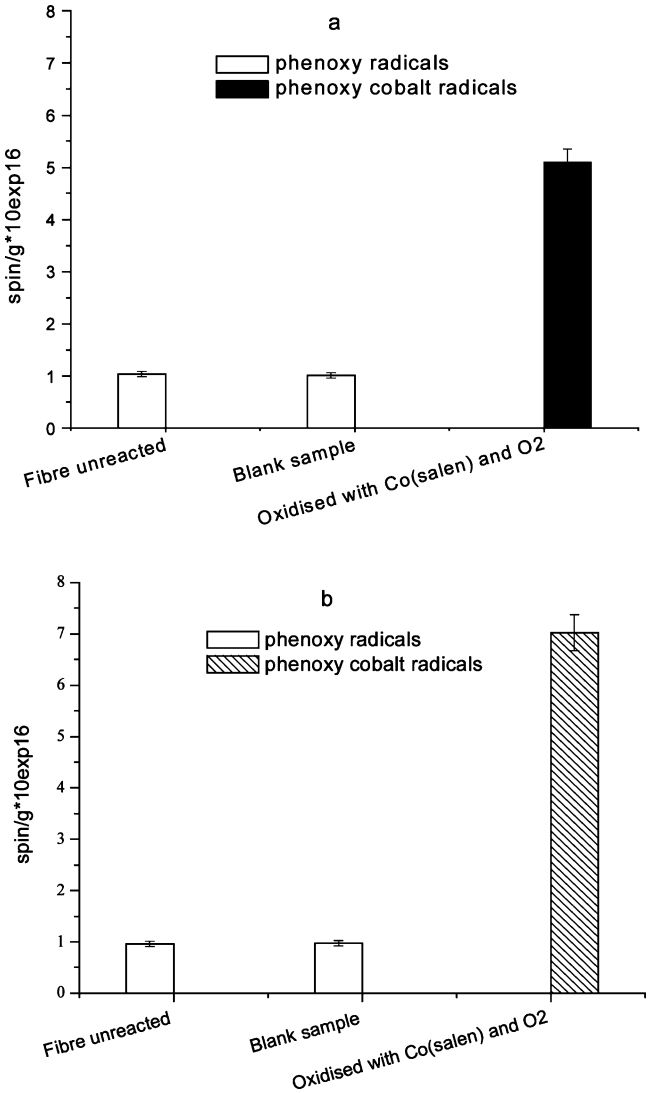

Figure 7. Concentration of radicals for (a) CTMP and (b) TMP fibers, in the unreacted samples, in the corresponding blank samples and the two fibers after 85 min of reaction in methanol in the presence of [Co(salen)] at 0.1 MPa of oxygen pressure.

fibers, phenoxy radicals disappeared and phenoxy cobalt radicals were 7×10^{16} spin/g in TMP and 5×10^{16} spin/g in CTMP. For both fibers the amount of Co in phenoxy cobalt radicals was 1×10^{-7} mol Co/g of fiber. This value, compared with the Co content of oxidized fibers determinated

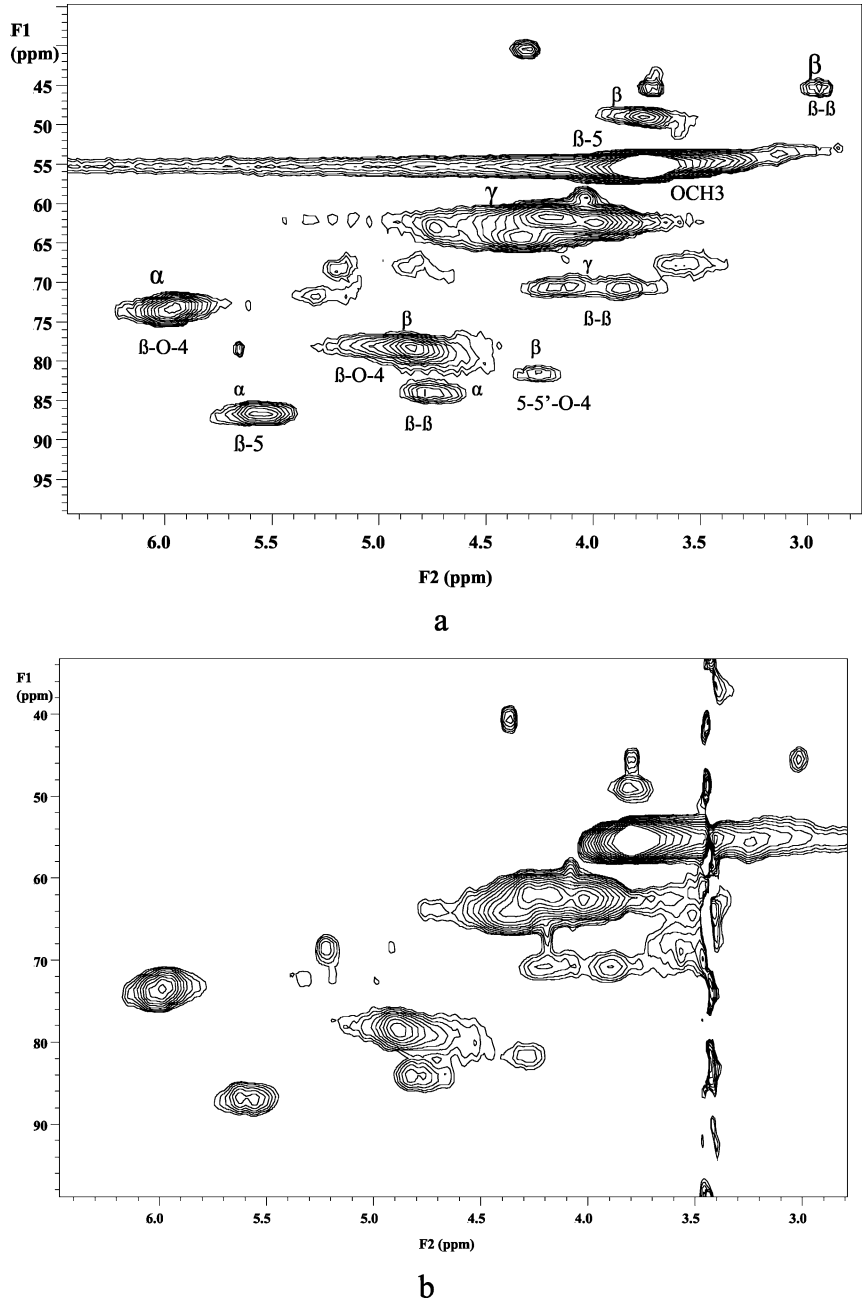

Figure 8. 2D HSQC spectrum of acetylated lignin from (a) TMP unreacted fibers (b) TMP after 85 min reaction in methanol at 0.1 MPa of oxygen pressure in the presence of [Co(salen)].

by GF-AAS tecnique (2×10^{-5} mol Co/g of fiber) suggests that only the 0.5% of cobalt centers are involved in the formation of phenoxy cobalt radicals.

The number of phenoxy cobalt radicals was assumed to be a true quantification of the [Co(salen)] catalytic activity; in fact, the quenching of the EPR signal by spin−spin interaction between radicals, possible in the case of simple phenoxy radicals,[32] is highly improbable in the case of phenoxy cobalt radicals, due to the double cobalt coordination to fiber phenolic groups.[13]

The concentration of radicals in fibers, when using [Co(salen)] catalyst, reaches values 10 times higher than those reported in the literature by Felby[32] and by Ferm[33] for the oxidation of respectively TMP and milled wood lignin by molecular oxygen, in the presence of laccase. It cannot be excluded this was partly due to the smaller molecular dimension of [Co(salen)] with respect to laccase, which allows the catalyst to interact also with subsurface lignin phenol groups.

Change in Lignin Structure. To evaluate changes in lignin chemical structure induced by catalytic oxidation, lignins extracted by acid hydrolysis of fibers (see Materials and Methods Section) were characterized by 2D-HSQC NMR spectroscopy, to identify the principal intermonomeric units, and by ^{13}C NMR spectroscopy, to quantify the principal intermonomeric units and the amount of alcoholic and phenolic groups. The spectra were run in DMSO-d_6 on the acetylated samples, to avoid lignin fractionation before NMR analysis,[34] to increase the lignin solubility in DMSO-d_6, and to enhance the chemical shift dispersion of the side chain units.[35] As the lignins under investigation were obtained from both unreacted and oxidized fibers, the structure modifica-

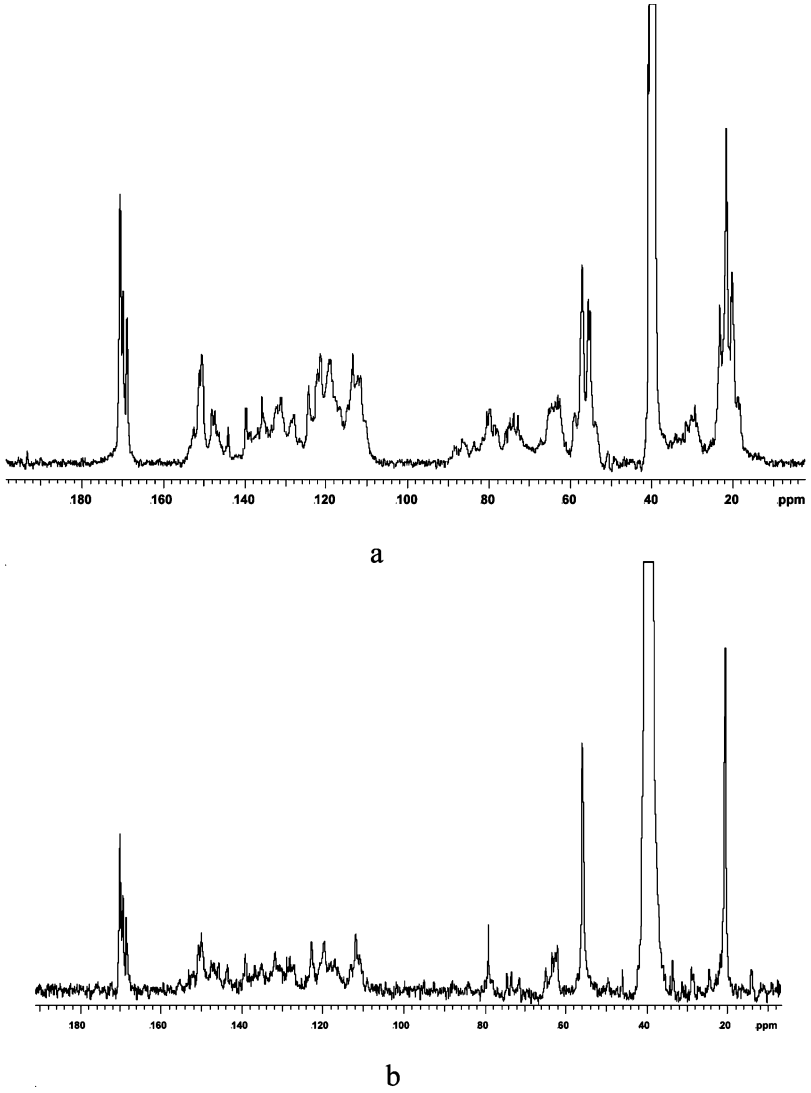

Figure 9. ^{13}C NMR spectrum of acetylated lignin from (a) TMP unreacted fibers (b) TMP after 85 min reaction in methanol at 0.1 MPa of oxygen pressure in the presence of [Co(salen)].

Scheme 1

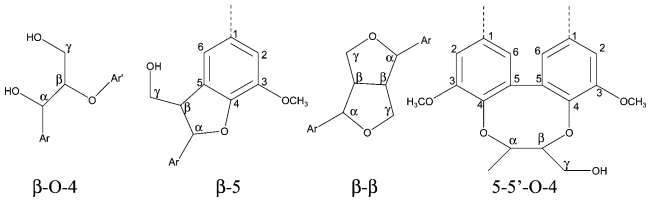

Table 5. Number of Methoxyl Groups Per Aromatic Ring

	unreacted CTMP	[Co(salen)]-treated CTMP	unreacted TMP	[Co(salen)]-treated TMP
OCH_3/C_6H_6	1.05	1.09	0.98	0.95

tions due to the oxidative treatment were distinguished from those due to the isolation method. To be concise, only the spectra of acetylated lignin extracted from TMP fiber, before and after oxidation, were reported in Figures 8 and 9.

The assignment of predominant signals in 2D-HSQC NMR spectra was based on the chemical shift data of lignin model compounds and of milled wood lignin (MWL), as reported in the literature by Drumond,[36] Ralph,[37] and Kilpelainen.[38] The considered intermonomeric units were as follows (Scheme 1): arylglycerol-β-aryl ether (β-O-4 unit), phenylcoumaran (β-5 unit), pinoresinol ($\beta-\beta$ unit), and dibenzodioxocine (5−5′-O-4 unit).

It was found that both unreacted and reacted TMP and CTMP lignins were rich in arylglycerol β-O-4 unit (Figure 8). They also contained significant amounts of β-5 unit, whereas the $\beta-\beta$ and 5−5′-O-4 units were not observed in lignin extracted from CTMP fibers. In the case of lignin extracted from unreacted TMP fibers, residual carbohydrates are present.

Quantitative ^{13}C NMR analysis was performed on CTMP and TMP fibers (Figure 9) by referring the intensity of each characteristic ^{13}C signal (80 ppm for α in β-O-4, 86 ppm for α in β-5, 84 ppm for α in $\beta-\beta$ and 82 ppm for β in 5−5′-O-4) to that of the methoxyl signal (56 ppm) and multiplied with the average number of methoxyl groups per aromatic ring (calculated as reported in "Materials and Methods" and given in Table 5).

The relative amounts of lignin structural units determined by ^{13}C NMR analysis are summarized in Table 6 and compared with the data of spruce MWL used as reference.[22,39]

Table 6. Relative Amounts of the Predominant Structural Units in MWL, CTMP, and TMP Acetylated Lignins Unreacted and after Reaction with Molecular Oxygen in the Presence of [Co(salen)] under the Optimized Reaction Conditions[a]

structural units	spruce MWL	unreacted CTMP	[Co(salen)]-treated CTMP	unreacted TMP	[Co(salen)]-treated TMP
β-O-4	++++	+++	+++	++++	++
β-5	++	+	+	++	+
β-β	+	n.d.[b]	n.d.[b]	+	+
5-5′-O-4	+	n.d.[b]	n.d.[b]	+	traces

[a] The number of + marks indicates the relative amounts of the structure in the lignin sample. [b] n.d. = not detected.

Table 7. Number of Primary, Secondary, and Phenolic OH per Aromatic Ring in CTMP and TMP Acetylated Lignins Unreacted and after Reaction with Molecular Oxygen in the Presence of [Co(salen)]

OH type/C_6H_6	δ/ppm	unreacted CTMP	[Co(salen)]-treated CTMP	unreacted TMP	[Co(salen)]-treated TMP
primary	169.9−171	0.47	0.56	0.52	0.36
secondary	169.5	0.29	0.29	0.28	0.24
phenolic	168.6	0.22	0.22	0.20	0.15

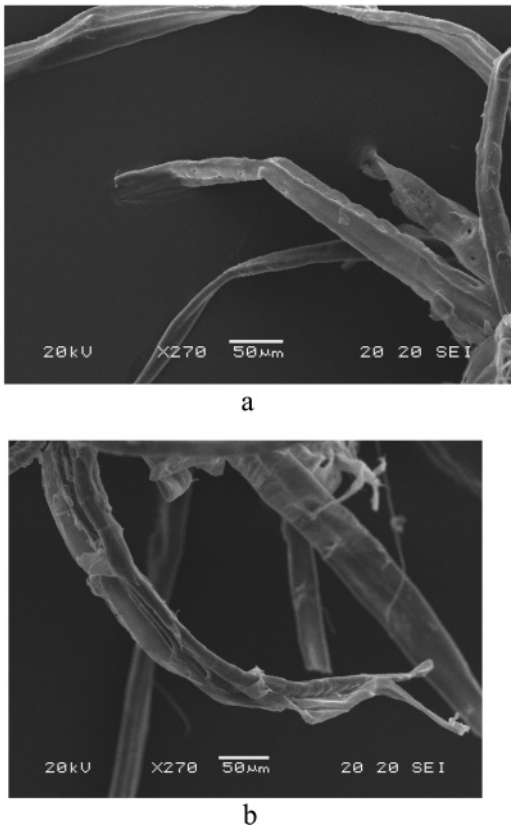

Figure 10. Scanning electron microscopy (SEM) photographs (scale bar 50 μm), of CTMP (a) unreacted and (b) after 85 min of reaction in methanol at 0.1 MPa of oxygen pressure in the presence of [Co-(salen)].

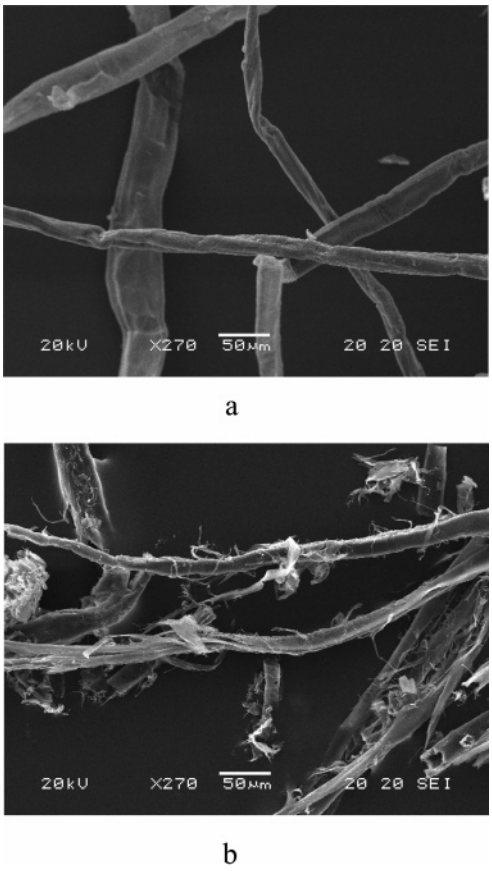

Figure 11. Scanning electron microscopy (SEM) photographs (scale bar 50 μm) of TMP (a) unreacted and (b) after 85 min of reaction in methanol at 0.1 MPa of oxygen pressure, in the presence of [Co-(salen)].

The relative amounts were roughly estimated due to the overlap of the signal.

The results indicate that the oxidative treatment of CTMP fibers by [Co(salen)] does not modify the relative abundance of intermonomeric bonds. Instead, lignin from TMP fibers was heavily modified by the oxidative treatment: in oxidized material, the amounts of β-O-4 and β-5 are significantly reduced, and the 5−5′-O-4 unit is detected only in traces.

The unexpected carbon signals appearing in the spectrum of untreated TMP lignin (Figure 9a) at 19, 22 and at 54, 55 ppm are most probably due to sample impurities. This was suggested by dividing signal area of acetoxy carbons attached to γ-hydroxyls (170 ppm) by that of the signal at 56 ppm (typical value for methoxyl carbons in lignin): the ratio value of 0.70 is close to that observed in spruce MWL (0.78).[22] Also the intensity pattern of aromatic carbon signals is the same as that reported for spruce MWL.[22]

The results in Table 6 also indicate that the structural assembly of lignin extracted from CTMP fibers is different from MWL since $\beta-\beta$ and 5-5′-O-4 structures are present in negligible amounts both before and after treatment. Instead, the structural assembly of lignin from unreacted TMP shows the same intermonomeric bonds as MWL lignin, in about the same relative abundance.

In addition to the elucidation of the structural changes in lignin intermonomeric composition, a quantitative evaluation of phenolic and alcoholic groups in CTMP and TMP fibers,

before and after the oxidative treatment, was also performed (Table 7). The number of primary, secondary, and phenolic groups in the polymers were determined as the number of carboxyl groups in acetylated samples calculated per aromatic ring, as reported in "Materials and Methods".[21−23]

According to the data in Table 7, after reaction, the amount of phenolic and secondary alcoholic groups do not significantly vary in CTMP fibers, whereas the number of phenolic groups decreases by 25% in TMP fibers.

Fibers were also analyzed before and after the radicalization by scanning electron microscopy (SEM). Each fiber was examined in three different regions. The results show that under oxidative treatment CTMP does not undergo morphology changes at the surface (Figure 10), while TMP suffers very heavy surface damage as a consequence of the treatment (Figure 11). The images, selected as an example of average behavior, demonstrate that the morphology changes agree with the relative amount of phenolic groups in the two fibers obtained by ^{13}C NMR analysis, and with the absolute amount of phenoxy cobalt radicals in the two fibers evaluated by EPR investigation.

Conclusions

The oxidation of unbleached CTMP and TMP fibers by molecular oxygen, catalyzed by [Co(salen)], induces the formation of phenoxy cobalt radicals similar in structure to those observed in the oxidation of lignin model compounds. The radicalization mechanism is thought to be the same as in the homogeneous phase;[13,30] however, radicals in fibers proved more stable and motionless than in solution. Probably, they are immobilized by coordination to fiber phenols, and moreover their reactions, typically coupling or interaction with oxygen, are less favored.

The best radicalization conditions were assessed by EPR spectroscopy and depend on the reaction medium, the oxygen pressure, and the time of reaction. In particular, the swelling of fiber in the reaction medium favors the formation of radicals.

TMP fiber forms a higher amount of radicals and in parallel undergoes deeper structural and morphological changes than CTMP, as assessed by 2D-HSQC, ^{13}C NMR, and SEM analysis. These results confirm that in fibers the oxidative process proceeds through radical active intermediates, as it was in solution for the lignin model compounds.

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