Radical formation on laccase treatment of wood defibrated at high temperatures

Part 1. Studies with hardwood fibers

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Keywords: Defibration, Hardwood, Laccase, Lignin, Phenoxyl radicals

SUMMARY: Hardwood chips were defibrated at high temperatures and extracted with water. Unextracted fibers, fiber water-extracts and water-extracted fibers were treated with laccases, causing formation of phenoxyl radicals. The laccase reactivities of the unextracted and water-extracted fibers were assessed by quantification of radicals in laccase-treated fibers by ESR spectrometry and by measuring the oxygen uptake during the treatments. The oxygen uptake of fiber water extracts during laccase treatment was determined as well. The pH optima and catalytic abilities varied between laccases. The laccase reactivities of unextracted and water-extracted fibers in terms of radical formation increased with an increase in defibration temperature and content of water-extractable aromatic substances, the water-extracted fibers being far less reactive than the unextracted fibers. A roughly equal number of radicals were formed in hardwood fibers of different wood species defibrated at the same temperature. Radicals in laccase-treated samples were decayed by second-order reactions, slowly in vacuo or at ambient conditions, and fast on heat treatment. The oxygen consumption measurements showed that the water-extracts are far more reactive toward laccase than the unextracted fibers. The water-extracts, accounting for 6–23% of the fibers, were responsible for 50–70% of the oxygen uptake of the unextracted fibers.

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Laccases are phenol-oxidases produced by various white-rot fungi and plants, and play an important role in lignin biodegradation. In the presence of molecular oxygen, laccases catalyze the oxidation of phenolic compounds such as lignin to phenoxy radicals with concomitant reduction of oxygen to water (Yaroplov et al. 1994). In recent years, research has been carried out into the possibility of using laccases for technical applications in various fields of wood chemistry. The interaction of laccase and phenolic compounds such as lignin or chlorinated phenols may result in degradation, depolymerization or polymerization of the aromatic substances. The outcome of the laccase treatment can be controlled by various means, which include using laccases together with other enzymes or laccase-mediators. The lignin-degrading ability of laccases with suitable mediators has prompted research into their use in pulp bleaching and delignification (Paice et al. 1995; Luisa et al. 1996; Bourbonsais et al. 1998; Nelson et al. 1998). Treatment of chlorolignin and chlorophenol-containing effluents from the pulp and paper industry with laccases can be applied to achieve polymerization of the phenolic substances, which facilitates their removal from the effluents by precipitation or sedimentation. A substantial decoloration and reduction in the toxicity of effluents (Milestone et al. 1988; Forsell et al. 1989; Luisa et al. 1996) can thus be obtained. Also the wood-composite industry may benefit from their ability to polymerize phenolic substances by developing gluing methods for wood particles based on coupling of radicals on the surfaces of laccase-treated fibers (Yamaguchi et al. 1992; Hüttermann, Khazizaripour 1996; Felby et al. 1997a; Khazizaripour et al. 1997; Khazizaripour et al. 1998).

The present paper is concerned with the generation – by laccase treatment – and decay of radicals in hardwoods, which were defibrated at temperatures beyond the range used in conventional TMP pulping. The effects of defibration temperature, wood species and conditions of laccase treatment on radical formation were studied. A major part of the work focused on the identification of laccase-reactive fiber components. The decay of radicals at different conditions is also discussed. Studies on the behavior of softwood fibers on laccase treatment are reported in another paper (Widsten et al. 2002a).

Materials and methods

Laccase-treated samples

The samples used in this study included non-defibrated (chips ground in a Wiley mill to pass a 20 mesh screen) and defibrated birch (mainly Betula verrucosa), aspen (Populus tremula) and eucalypt (Eucalyptus sp.), water-extracts and water-extracted fibers from the defibrated samples, and milled wood lignins (MWL) prepared from the fiber raw materials. The fiber samples used in this study were subjected to the treatments described in Table 1. The defibrations were carried out at unusually high preheating temperatures (171–196°C) using a Sunds Defibrator pilot refiner in Sundsvall, Sweden. Except for the MWL, the samples have been characterized in a previous paper (Widsten et al. 2002b) in which also the defibration conditions are described. The MWLs was obtained by an application of the method of Björkman (1956). Dry milling in argon for 48 h was used instead of milling in toluene as in the original method.

Enzymes

The fungi from which the laccases used in this study were obtained are listed below together with the pH optima of the laccases (according to supplier). The pH of the buffer solutions in which they were used in this study are shown in parentheses: Trametes hirsuta, pH 4.5 (4.5), VTT Biotechnology, Espoo, Finland; Trametes villosa, pH 4–5 (4.5), Novo Nordisk, Denmark; Myceliophthora thermophila, pH 7 (7.0), Novo Nordisk, Denmark; Coriolus versicolor, pH 5–6 (4.5), Biocatalyst, Cardiff, UK; experimental laccase 1,
pH 4–5 (4.5); experimental laccase 2, pH 6 (6.0). The activities of the laccases were measured against 2,2’-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS).

**Laccase treatments for ESR measurements**

Unless otherwise stated, the laccase-treatments and ESR measurements of fibers (based on o.d. weight) were mixed with buffer solution (≈2 ml, final consistency 5% after addition of enzyme solution) in a 50 ml decanter and stirred magnetically while air was slowly bubbled into the mixture through a Pasteur pipette. 25 mM succinate buffer (pH 4.5) was used for T. hirsuta laccase and 100 mM phosphate buffer (pH 7.0) for M. thermophila laccase. For studies involving also other laccases and performed at different pH values, buffer solutions with a pH in the range 3–6 were prepared from the succinate buffer and those with a pH in the range 6.5–8.5 from the phosphate buffer by adjusting the pH with NaOH or HCl. Enzyme solution was added after about 30 seconds when a homogeneous suspension had been obtained. The enzyme dose was 1 nkat/mg of fiber (the enzyme activity was measured against 2,2’-azinobis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS)). The mixture was stirred under air bubbling for 1 h after which it was placed in a freezer. The frozen samples were then lyophilized overnight. The lyophilized samples were maintained in vacuum, pending their use for ESR measurements, which were carried out on the day after laccase treatment. For ESR measurement, 100 mg of lyophilized fiber was uniformly packed in a quartz tube in a standard volume.

For the measurements in solution, 50 mg of fiber water-extract was dispersed in 5 ml of 100 mM phosphate buffer (pH 7.0) and the resulting clear solution introduced in a flat quartz cell. The reaction was started by adding 50 nkat of M. thermophila laccase.

**ESR measurements**

The electron spin resonance (ESR) spectra were recorded at room temperature on a Varian E-line cw X-band ESR spectrometer using a microwave frequency of ~9.5 GHz. A modulation frequency of 100 kHz and a modulation amplitude of 0.5 gauss were applied in the measurements. The microwave power was 1 mW. Relative concentrations of radicals were determined by double integration of the baseline-corrected first derivative spectra. At least three spectra were run on each solid state sample and the integration results averaged. The solution spectra were run during the laccase treatment at 2–5 min intervals.

**O₂ consumption measurements**

Unextracted or water-extracted fiber (75 mg, o.d.) or fiber water-extract (19 mg, o.d.) was dispersed in 30 ml of buffer solution (see laccase treatments above) in a 25 ml erlenmayer flask. An O₂ electrode (Orion Model 97-08-99) was immersed in the mixture and the flask, half to the rim, sealed with paraffin film. The mixture was allowed to equilibrate for 20 min while being stirred magnetically, after which the laccase was added and the rate of O₂ uptake monitored for 16 min under constant stirring. After the initial burst in O₂ consumption due to the oxidation of the directly laccase-accessible reactive groups in solution or on the fiber surface, the rate of O₂ uptake gradually decreased to a slow linear consumption. In the case of the water-extracts, the O₂ consumption ceased completely at some point during the experimental time frame. The O₂ uptake corresponding to the oxidation of the directly laccase-accessible groups was calculated from the plot of O₂ concentration vs time by extrapolation of the linear region to the concentration axis.

The O₂ consumption of the MWL (50 mg) was determined mostly as above using a YSI 5100 Dissolved Oxygen Meter and a YSI 5010 BOD Probe. However, since the plots of O₂ concentration vs time were nearly linear, the O₂ uptake was read from the amount of O₂ consumed after 16 min.

**Solid state ¹³C CP/MAS NMR spectra**

The solid state ¹³C NMR cross polarization/magic angle spinning (CP/MAS) spectra were recorded at room temperature using cross polarization and magic angle spinning at 7 kHz on a Chemagnetics CMX Infinity 270 MHz spectrometer operating at 67.9 MHz for carbon. The cross-polarization contact time was 2 ms, data acquisition time 24 ms and the pulse delay 2 s. The rf power levels were 60 kHz. The number of transients was ~20000 for water-extracts and ~5000 for other samples. A line width of 40 Hz was used for processing the spectra. The peaks were referenced to TMS using hexamethyldisilane (methyl peak at 7.3 ppm) as a secondary reference. The samples were air-dry portions of ~200 mg of whole fiber or ~50 mg of fiber water-extract with a moisture content of about 5%.

Variations in the proportion of β-O-4 etherified syringyl units of the lignin component of the samples were estimated by monitoring intensity changes of syringyl C-4 across 151 ppm. The degree of etherification was expressed as the proportion of etherified syringyl C-4 (155–151 ppm) of the total C-4 signal strength (155–145 ppm) neglecting signals from guaiacyl units (Leary et al. 1986; Leary, Newman 1992).

**Results and discussion**

**Assignment of radicals formed on laccase treatment**

The solid state ESR spectra of untreated and laccase-treated fibers consisted of one unresolved signal and differed from one another only by virtue of their intensities and slightly different g-values, ranging from 2.0035 to 2.0044. The g-value is a measure of the local magnetic field experienced by an electron and it is used to characterize the
position of a resonance (Hon 1983). A typical solid state spectrum, that of the laccase-treated sample B-196, is given in Fig. 1. The g-values were similar to those reported for phenoxyl radicals (Hon 1983), the expected oxidation product, but unambiguous assignment to phenoxyl radicals was not possible due to the absence of hyperfine structure. Further proof of the formation of phenoxyl radicals was therefore sought by monitoring radical formation during laccase treatment of water-extract from B-196. The solution ESR spectra obtained (Figs. 1 and 2) provide information about the nature of at least part of the radicals that were formed. The ESR spectrum of the laccase-treated fiber water-extract in Fig. 1, recorded after 5 min of laccase treatment, consists of 7 peaks with a coupling constant of about 1.5 gauss. The 7-peak framework of the spectrum corresponds to the strong coupling of a syringyl radical to the six methoxyl protons of the syringyl units. Treatment with phenol-oxidizing enzymes of syringyl-type lignin model compounds (Caldwell, Steelink 1969) or the aqueous suspension liquid of beech fibers (Felby et al. 1997b) has yielded similar spectra. In light of these results, it is evident that syringyl radicals were formed as a result of the laccase treatments, whereas the fate of phenolic guaiacyl units remains unclear. The fact that guaiacyl radicals were not detected could be due to the different redox potentials of syringyl and guaiacyl units, which is why phenolic guaiacyl units are only oxidized after the oxidation of syringyl units is complete. An alternative explanation is that if guaiacyl radicals were formed, they escaped detection because of rapid recombination reactions involving guaiacyl units unsubstituted at the C-5 position.

**Stability of fiber radicals**

To study the effect of temperature and atmospheric oxygen on the stability of fiber radicals and to elucidate the mechanism of radical decay, radicals in fibers treated with M. thermophila laccase were quantified. The fibers were then aged at different temperatures under the effect of atmospheric oxygen or in vacuo. As shown in Fig. 3, the radicals were more stable in vacuo than at ambient conditions, which may be due to the absence of atmospheric oxygen or a somewhat lower temperature in vacuo. However, the plot of aging time against reciprocal radical concentration (Fig. 3, insert) indicates that radical decay was essentially a second-order reaction (probably involving coupling and disproportionation of fiber radicals), and thus should not have been affected by atmospheric oxygen, which was present in excess. Heat treatment of fibers at 50°C or 100°C strongly increased the rate of radical decay (Fig. 4), which again followed second-order kinetics (Fig. 5). It is also seen that radicals in different fibers had different stabilities at 100°C. The decay of radi-

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**Fig. 1.** ESR spectra of laccase-treated fibers. a) Typical spectrum of solid state fibers (sample B-196, M. thermophila laccase). b) Spectrum of water-extract from untreated sample B-196 run in solution after 5 min from the start of treatment with M. thermophila laccase.

**Fig. 2.** Solution ESR spectra of water-extract from untreated sample B-196 recorded before and during treatment with M. thermophila laccase.

**Fig. 3.** Stability at ambient conditions and in vacuo of radicals in sample B-188 treated with M. thermophila laccase. Insert: reciprocal radical concentration as a function of time, indicating that radical decay followed second-order kinetics.

**Fig. 4.** Decay of radicals in fibers treated with M. thermophila laccase on heat treatment.
no radicals at all were formed in the non-defibrated sample B-Wood, the radical concentration of the other samples was strongly increased by the laccase treatments. The radicals present in the untreated samples were probably formed by photo- and mechanochemical reactions e.g. during defibration.

Comparison of aspen samples A-171 and A-188 or birch samples B-171, B-188 and B-196 respectively shows that the number of persistent radicals increased with an increase in refining temperature. The formation of persistent radicals also depended on the wood species: comparison of radical formation in samples A-171, B-171 and E-171 or in samples A-188 and B-188, respectively, shows that the birch and eucalypt fibers behaved rather similarly, whereas the laccase-treated aspen fibers contained clearly less radicals than the corresponding birch and eucalypt fibers. Similar results regarding the effect of wood species and defibration temperature were obtained with all of the laccases used.

As laccases catalyze the oxidation of phenolic hydroxyl (PhOH) groups to phenoxy radicals by molecular oxygen (O₂), it is possible to monitor radical formation by measuring the O₂ uptake during laccase treatment. The O₂ uptakes during laccase treatments (Fig. 8) indicated that an approximately equal number of radicals was formed in samples A-171, B-171 and E-171. The same is true for samples B-188 and A-188. It seems, therefore, that the aspen samples were as reactive toward laccase as the other samples but the radicals in the aspen samples were less persistent than those in the other samples. Radicals in sample A-188 also exhibited much lower thermostability than those in sample B-188 (Fig. 4). The MWL, representative of native lignin, consumed less O₂ than the fibers. This is probably due to their water-insolubility, agglomerated physical state and low PhOH content.

The quantification of persistent radicals formed and O₂ consumption measurements both proved to be suitable methods for evaluation of fiber laccase-reactivity, as there was a fairly good agreement between the results obtained with the two methods. Both methods clearly showed that the laccase-reactivity of fibers increased with an increase in defibration temperature.

**Effect of time of laccase-treatment on radical concentration**

To study the effect of treatment time on the formation of persistent radicals, fibers were treated with T. hirsuta laccase for 10–120 min (Fig. 9) and the persistent radicals
Firstly, although the same amount of laccase in terms of activity was used for each experiment, the determination of laccase activity against ABTS may not have provided a truthful picture of laccase activity against lignin and other phenolic substrates present. Secondly, although these results show that the pH of the reaction medium plays an important role in the formation (or stability) of radicals, the actual pH on the fiber surface, at which laccase-accessible reactive groups are largely located, may be different from the pH of the buffer solution.

**Location of laccase-reactive groups in fibers**

The fibers were water-extracted and the reactivity of the water-extracts, unextracted fibers and water-extracted fibers were compared in order to locate the laccase-reactive groups. The reactivities of the unextracted and water-extracted fibers were compared by measuring the number of persistent radicals formed (Fig. 11), given by the difference between the radical concentration of the laccase-treated and untreated fibers, as well as by their O₂ consumption (Fig. 12). In view of the rapid decay of the radicals formed in the fiber water-extracts (Fig. 6), the reactivities of the water-extracts were determined by O₂ consumption measurements only. These clearly show that the water-extracts are far more reactive toward laccase than the unextracted and water-extracted fibers. Comparison of the unextracted and water-extracted fibers also shows that the extraction drastically decreases both the number of persistent radicals formed in the fibers and their O₂ uptake. A good agreement exists between the number of persistent radicals formed in the unextracted and water-extracted fibers and their O₂ uptake.

**Effect of laccase type and pH on radical concentration**

The effect of pH and laccase type on radical concentration was studied by treating sample B-188 with different laccases at different pH levels. In terms of the concentration of radicals in laccase-treated fibers (Fig. 10), the effectiveness of the laccase treatment depended on both the laccase used and the pH of the buffer solution. The pH optima of the catalytic activities of the laccases from various sources are known to vary within a wide pH range (Yaropolov et al. 1994). In the present study, this is shown by the variance in persistent radical formation at various pH levels between the laccases used (Fig. 10). Four of the laccases had a pH optimum of about 6.5 with an abrupt drop occurring at pH 7, whereas two of them had higher pH optima.

It is also seen that with some laccases more persistent radicals are formed at all the pH levels used than with other laccases. This may be at least partly due to different physical dimensions of the laccases, causing the laccase accessibility of the PhOH groups on the fiber surface to vary between different laccases. However, on interpretation of the results, two facts must be taken into account.

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**Fig. 10. Effect of pH and laccase type on radical formation in sample B-188.**

**Fig. 11. Formation of persistent radicals in unextracted and water-extracted fibers on treatment with M. thermophila laccase.**

**Fig. 12. O₂ consumption of unextracted fibers, fiber water-extracts and water-extracted fibers on treatment with T. hirsuta laccase.**
Despite the high \( O_2 \) uptake of the water-extracts, even less persistent radicals were formed in them (results not shown) than in the water-extracted fibers. The rapid decay of the phenoxy radicals by second-order reactions in solution (half-life of about 4 min) is probably due to their small size and high mobility, resulting in a high collision rate between the radicals. It has been reported that electron transfer takes place between phenoxy radicals present on the fiber surface and in the water suspension, and PhOH groups of inner fiber domains inaccessible to the bulky laccase molecule (Barsberg et al. 1997; Felby et al. 1997b). As phenoxy radicals are continuously regenerated and reoxidized, a steady radical concentration remains in the material present in the suspension liquid as long as it is in contact with the insoluble fiber fraction containing unoxidized PhOH groups. Such a mechanism would also account for the low number of persistent radicals formed in the water-extracted fibers, lacking laccase-accessible substrates needed for the electron transfer reactions. These substrates are found in the water-extracts, which contain 17–27% of lignin, lignin-lignolicomplexes, ligan
d nanos or other aromatic substances rich in PhOH groups.

The amount of water-extractable aromatic substances in the unextracted fibers increases with an increase in defibration temperature for birch and aspen fibers, respectively (Table 2). This largely accounts for the corresponding increase in their laccase-reactivity. The CP/MAS NMR spectra of laccase-treated and untreated fibers and fiber water-extracts (Fig. 13), discussed in more detail below, show that the PhOH groups of the water-extract were oxidized much more extensively than the PhOH groups of the unextracted fibers.

In unextracted and water-extracted fibers only the PhOH groups on the fiber surface are directly accessible to laccase. As a consequence, the \( O_2 \) uptake of the unextracted and water-extracted fibers per mass unit is much lower than that of the water-extracts (Fig. 12). In spite of this, the water-extracted fibers account for 30–50% of the \( O_2 \) uptake of the unextracted fibers (Fig. 14) because of their large proportion (76–94%) of the unextracted fibers. The influence of a possible synergistic effect of water-extract and water-extracted fibers which are both present in the unextracted fibers is not included in these results. As shown by Fig. 15, the \( O_2 \) consumption calculated for the two fiber fractions recombined to the same proportions present in the unextracted fibers is mostly lower than that of the corresponding unextracted fibers. Some synergistic benefit, due e.g. to electron transfer from water suspension to inner fiber domains as discussed earlier, may thus be derived from the simultaneous presence of both fractions. An extension of laccase

**Table 2.** Effect of PhOH and lignin contents on the laccase-reactivity of fiber water-extracts, and the distribution of reactive material between water-extracts and water-extracted fibers as determined by \( O_2 \) uptake with \( T. hirsuta \) laccase.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Characteristics of water-extracts</th>
<th>( O_2 ) uptake of water-extract per PhOH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% of fiber</td>
<td>Water-extractable aromatic substances, % of water-extract</td>
</tr>
<tr>
<td>A-171</td>
<td>8.0</td>
<td>27.2</td>
</tr>
<tr>
<td>A-188</td>
<td>20.0</td>
<td>25.3</td>
</tr>
<tr>
<td>B-171</td>
<td>8.5</td>
<td>17.5</td>
</tr>
<tr>
<td>B-188</td>
<td>19.8</td>
<td>15.8</td>
</tr>
<tr>
<td>B-196</td>
<td>23.2</td>
<td>18.2</td>
</tr>
<tr>
<td>E-171</td>
<td>5.9</td>
<td>20.7</td>
</tr>
<tr>
<td>Average</td>
<td></td>
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<tr>
<td>Theoretical</td>
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</table>

1) Data from Widing et al. (2002b).
2) Corresponding to a one-electron oxidation whereby one molecule of \( O_2 \) oxidizes 4 PhOH groups.
activity to inner fiber domains not directly accessible to laccase (Barsberg et al. 1997; Felby et al. 1997b) in the unextracted samples by soluble/colloidal lignin-related fiber fragments is a plausible explanation for this.

The stoichiometry of laccase-catalyzed oxidation of PhOH groups to phenoxy radicals was studied by calculating the O$_2$ uptake per PhOH group of fiber water-extract (Table 2). An average of 0.51 molecules of O$_2$ was consumed per PhOH group corresponding to a transfer of two electrons. Simple phenols have been found to display similar oxidation stoichiometry on laccase treatment (Xu 1996). However, the laccase-catalyzed one-electron oxidation of phenols to phenoxy radicals with concomitant reduction of O$_2$ to water involves the transfer of four electrons. Several explanations can be envisaged for this discrepancy. For example, some of the oxygen intermediates generated in the multielectron oxidation process may react with other solutes before reacting with PhOH groups. An example of such a reaction is coupling of a phenoxy and a superoxide anion radical to give a peroxy intermediate, which may decompose to yield a quinonoid structure. Quinonoid structures are known to be present in laccase-treated lignins (Wariishi et al. 1987). A change in the laccase's redox potential in the course of the treatment could also affect the laccase-oxygen interaction (Yaropolov et al. 1994). Moreover, formation of new PhOH groups during laccase treatment from originally etherified moieties of phenolic β-O-4 structures has occasionally been reported. This would result in a higher than expected O$_2$ uptake. Higuchi (1989) and Wariishi et al. (1987) report that the laccase-catalyzed oxidation of syringylglycerol-β-guaiacyl ether yields guaiacol. According to Martinez-Ingo and Kurek (1997), the action of laccase on alkali wheat straw lignin, which contains β-O-4 structures of mainly guaiacyl and syringyl types, causes the oxidation of not only PhOH groups originally present but also those formed from non-phenolic β-O-4 linked syringyl units.

$^{13}$C CP/MAS NMR spectra of untreated and laccase-treated samples

The $^{13}$C CP/MAS NMR spectra (Fig. 13) of the unextracted sample B-196 recorded before and after laccase treatment are similar. By contrast, the spectra of the water-extract from sample B-171 run before and after laccase treatment (M. thermophila laccase) show that the intensity of the peak at 148 ppm assigned mainly to C-3 and C-5 of phenolic syringyl units and C-3 of guaiacyl units (Nimz 1974; Leary, Newman 1992) was strongly reduced as a result of the laccase treatment, probably due to oxidation of PhOH groups of syringyl units. The peak at 153 ppm, which in the spectrum of the untreated sample is assigned mainly to C-3 and C-5 of β-O-4 etherified syringyl units and C-4 of etherified guaiacyl units, increased in intensity. This may result from a formation of etherified structures as coupling products of phenoxy radicals. However, as laccase-catalyzed oxidation of lignin involves benzylic oxidation (Higuchi 1983), a downfield shift of signals from C-4 caused by formation of α-carbonyl groups may contribute to the signal strength near 153 ppm (Nimz 1974). The absence of signals attributable to α-carbonyl groups (190-200 ppm) in the lignin side-chains does not provide conclusive evidence to the contrary because the α-carbonyls may have long relaxation times. The peak at about 180 ppm, overlapping with the large carboxyl peak originating mainly from hemicellulose acetyl groups and seen only in the spectrum of the laccase-treated sample, may arise from carbonyl groups in quinonoid structures formed via phenoxy radicals.

**Conclusions**

The main findings of this work were as follows:

- The laccase reactivity of hardwood fibers, defined as the number of phenoxy radicals in the fibers after a laccase treatment or as oxygen consumption during the treatment, increased with an increase in defibration temperature in the range 171–196°C. Smaller differences between the reactivity of fibers from different wood species defibrated at the same temperature could also be observed.

- Most of the laccase reactive material could be removed from the fibers by water extraction.

- The number of radicals formed depended significantly on the laccase type and pH used, while extending the treatment time from 10 min up to 120 min had only a minor effect in this respect.

- The phenoxy radicals detected in lyophilized fibers were quite stable at ambient conditions, but decayed rapidly by coupling or other second-order reactions when the fibers were subjected to heat. The stability of the radicals formed varied between wood species.

**Literature**


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