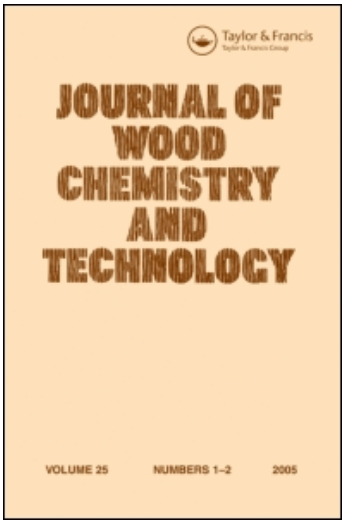

Journal of Wood Chemistry and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597282

Treatment of Douglas-Fir Heartwood Thermomechanical Pulp with Laccases: Effect of Treatment Conditions on Peroxide Bleaching

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To cite this Article Chandra, Richard P. , Saddler, John N. and Beatson, Rodger P.(2007) 'Treatment of Douglas-Fir Heartwood Thermomechanical Pulp with Laccases: Effect of Treatment Conditions on Peroxide Bleaching', Journal of Wood Chemistry and Technology, 27: 2, 73 — 82
To link to this Article: DOI: 10.1080/02773810701486709
URL: http://dx.doi.org/10.1080/02773810701486709

Journal of Wood Chemistry and Technology, 27: 73–82, 2007

ISSN 0277-3813 print/1532-2319 online
DOI: 10.1080/02773810701486709

Treatment of Douglas-Fir Heartwood Thermomechanical Pulp with Laccases: Effect of Treatment Conditions on Peroxide Bleaching

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Abstract: Douglas-fir heartwood thermomechanical pulp was treated with laccase enzymes at 25 and 50°C with and without added oxygen. The treated pulps were bleached with hydrogen peroxide at increasing alkali charges. Laccase treatments without added oxygen increased bleached brightness by 1.5–2.5 pts ISO, and decreased hydrogen peroxide consumption by 15–20%. The enzyme treatments were not enhanced when supplemented with oxygen. When the effectiveness of four different laccase enzymes was compared for the treatment of Douglas-fir heartwood thermomechanical pulp, there were no significant differences found in the performance among the enzymes. Possible explanations for the observed results are given.

Keywords: Douglas-fir, thermomechanical pulp, laccase, hydrogen peroxide, bleaching, oxygen, optimization, quinones

We thank Science Council of British Columbia and NSERC for their financial support. We also thank Dr. Claus Felby and Dr. Lisa Viikari for donation of laccase enzymes for this work and the Pulp and Paper Research Institute of Canada for Preparation of Mechanical Pulps.

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INTRODUCTION

Although Douglas-fir (*Pseudotsuga mensiezi*) wood chips have been used to produce kraft pulps, their poor brightness and bleachability preclude their use in the production of high yield mechanical pulps. The color formation in Douglas-fir mechanical pulps has been shown to be a result of the abundance of polyphenolic extractives present in the heartwood portion of the tree.[1,2] The most significant polyphenolic extractive has been identified as the leucochromophore dihydroquercetin (DHQ).[2] During aging of the tree and the subsequent mechanical pulping of the wood, polyphenolic compounds such as DHQ undergo autooxidation, condensation, and metal chelation, resulting in the formation of colored complexes in the pulp that are detrimental to pulp brightness and bleaching response.[3–5] Therefore, chemical or biological removal or alteration of the colored complexes and/or the extractives, would be an invaluable step toward production of a high brightness mechanical pulp from Douglas-fir. Previous chemical treatments include treatment of pulps with various solvents to remove extractives, the addition of chelating agents to prevent the formation of metal complexes and the addition of reducing agents to prevent the oxidation of phenolic extractives. Unfortunately these techniques have all had limited success in increasing pulp brightness and bleachability.[1–3] With the exception of our previous work,[6,7] biological methods remain an unexplored alternative for the brightness improvement of Douglas-fir mechanical pulps.

Laccase (benzenediol:O_2 oxidoreductase) is an oxidative enzyme produced by plants and fungi that catalyzes four one-electron oxidations of mostly phenolic compounds with a simultaneous four electron reduction of oxygen to water.[8–10] Laccase has been shown to possess the ability to actively degrade the residual lignin in chemical pulps when supplemented with a mediator compound.[11–13] The most frequently employed mediator compounds for laccase delignification studies of kraft pulps are ABTS (2,2-azino-bis-6-thiazoline-3-sulfonic acid) and HBT (1-hydroxybenzotriazole).[11–13] Recently, mediators, such as NHA (N-hydroxy-acetanilide) and violuric acid have emerged to further improve laccase-mediator delignification.[14] Laccase is a large enzyme (55–80 kDa) unable to penetrate pulp fibers.[9] Furthermore, laccase has been shown to oxidize phenolics in the absence of the mediator.[15] Therefore, the most likely functions of the mediator are to permeate the fiber/lignin matrix to degrade lignin, and extend the oxidative capabilities of laccase to non-phenolic compounds.[16,17] Although past work has shown that dihydroquercetin[18,19] could induce the production of laccase by *Trametes versicolor*, the use of laccases to improve the brightness and bleachability of Douglas-fir TMP has only recently been reported. In our recent work,[7] we found that the treatment of Douglas-fir heartwood TMP with the white-rot fungus *Trametes versicolor* was unsuccessful in increasing post-bleach brightness, however, treatment with laccase enzymes in the absence of a mediator resulted in an increase in bleached brightness of 3 pts ISO.[7] These results contrasted with previous findings observed during

laccase treatment of kraft pulp, where the presence of a mediator such as HBT or ABTS was essential for enhanced biological bleaching with laccase.[12,13,20,21]

The differences between the action of laccase on Douglas-fir heartwood TMP[7] and kraft pulp[12] presumably reflect quite different mechanisms. In kraft pulp bleaching, the laccase/mediator system enhances delignification, however, during laccase treatments of Douglas-fir heartwood TMP it appears that laccase aids in the removal and/or alteration of leucochromophores such as DHQ.[7] The observed capability of laccase to alter/degrade the polyphenolic extractives in Douglas-fir is consistent with the previously reported ability of laccase to react with phenolic lignin,[15] depolymerize high molecular weight lignosulfonates[22] and slightly depolymerize lignin.[23] In the case of mechanical pulps such as Douglas-fir heartwood TMP, extractives and much of the lignin are located at fiber surfaces (middle lamella lignin),[24] thus facilitating the access of laccase enzymes to potential reactants without the need for a mediator compound.

In the work reported here, hydrogen peroxide bleaching conditions after laccase treatments were adjusted to maximize the brightness gains. It was hypothesized that laccase-treated pulps may respond differently to hydrogen peroxide bleaching conditions compared to control pulps, therefore, alkalinity was varied during peroxide bleaching. Douglas-fir mechanical pulp was treated with four laccase enzymes to determine if different types of laccases could improve the bleaching effect during subsequent hydrogen peroxide bleaching.

MATERIALS AND METHODS

Pulp

Non-compression heartwood chips from a 129-year-old Douglas-fir tree were obtained from the University of British Columbia demonstration forest in Haney, B.C. Chips were screened and refined, to produce non-compression heartwood (NCH) thermomechanical pulp (TMP), at the Pulp and Paper Research Institute of Canada, Vancouver, B.C.

Laccase Enzymes

Laccases 1 and 2 were both obtained from Novo Nordisk (77 Perry Chapel Church Road Franklinton, N.C., USA). Laccase 1 was produced from *Trametes villosa* whereas laccase 2 was derived from *Aspergillus*. Crude (Laccase 3) and purified laccases (Laccase 4) derived from *Trametes hirsuta* were also tested. Treatments were performed at pH 5.0. Laccase 1 was used in all treatments except when otherwise specified.

Laccase Treatments

Samples (8.0 g OD) of Douglas-fir TMP were suspended in water at a 5% consistency and the pH adjusted to 5.0 with sodium hydroxide (NaOH). Laccase was added to the pulp samples at 5 U/gram pulp. Samples were incubated with shaking (200 rpm) for 2 h at 25 and 50°C with and without an oxygen sparge through the reaction vessel. Samples were boiled for 25 min to deactivate the enzyme. Control samples were treated identically to laccase-treated samples without the addition of laccase. All of the samples were bleached with hydrogen peroxide (see later).

Laccase Assays

Laccases were assayed spectrophotometrically according to Bourbonnais and Paice[25] at 420 nm in 0.1 M sodium acetate buffer (pH 5.0) and 0.5 mM ABTS (2,2-azino-bis-6-thiazoline-3-sulfonic acid). The oxidation of ABTS was monitored by determining the increase in A_{420} [$\epsilon_{420} = 3.6 \times 10^4$ M^{-1} cm^{-1}). One unit (U) was equivalent to 1 μmol ABTS oxidized per min.

Bleaching

All pulp samples were washed with water at 1% consistency (fines recycled) and pH adjusted to 5.5 with NaOH and hydrochloric acid prior to chelation. Chelation was performed at 2% consistency with 0.3% ethylenediaminetetraacetic acid (EDTA) for 30 min at 50°C. After a 1% water wash (fines recycled), pulps were bleached with 6.0% hydrogen peroxide, at alkaline charges of 2.0, 3.0, 4.0, and 5.0% NaOH for 3 h at 80°C. Sodium silicate (8.0% $NaSiO_3$) and magnesium sulfate (0.05% $MgSO_4$) were added as peroxide stabilizers. After bleaching, pulps were washed at 1% consistency (fines recycled) and handsheets were made for brightness measurement according TAPPI test method T-452.

RESULTS AND DISCUSSION

In an effort to improve the bleaching of Douglas-fir heartwood TMP and to obtain a better understanding of the interactions between laccases and wood components, Douglas-fir TMP was treated with various laccase enzymes at different temperatures and amounts of available oxygen prior to bleaching with hydrogen peroxide. The effects of the treatments on pulp brightness, brightness after peroxide bleaching and peroxide consumption were monitored. Laccase treatments were performed at 50°C and 25°C. Although 50°C is known to be optimal for the activity of laccases employed in this study, our previous work had shown that incubation at 25°C provided a

Table 1. The effect of laccase treatment on the brightness of Douglas-fir heartwood TMP[a]

	ISO brightness		L*		a*		b*	
Pulp treatment	25°C	50°C	25°C	50°C	25°C	50°C	25°C	50°C
Control	40.1	39.8	80.5	79.3	2.9	3.6	18.6	19.3
Laccase	34.8	32.3	76.9	75.5	4.8	5.4	20.4	21.4
Control + O_2	41.1	40.0	80.7	79.9	3.5	3.8	18.9	19.4
Laccase + O_2	36.4	33.2	78.3	75.9	4.7	5.1	20.6	20.6

[a]Original D.fir heartwood TMP: ISO Brightness: 41.1, L*:81.41, a*:2.53, b*:19.45.

compromise between laccase activity and possible thermal darkening of the TMP. As oxygen is essential for the catalytic action of laccase,[16] an increase in the supply of oxygen was expected to improve the ability of laccase to act upon the chromophores in the pulp. Therefore, several treatments were supplemented with additional oxygen in the reaction vessel while others were limited to the oxygen in the air of the reaction vessels.

The brightness of the TMP decreased with laccase treatment at both 25 and 50°C, by 5 to 7 pts ISO when compared to controls (Table 1). This was most likely due to the oxidation of lignin and extractives (such as DHQ) to colored quinone groups on the pulp surface. The possibility of additional oxidation within TMP fibers cannot be excluded. The formation of stable low molecular weight phenoxy radicals from colloidal lignin in solution has been reported previously during the treatment of beech wood fibers and TMP with laccase enzymes.[26–28] It has been proposed that these phenoxy radicals may act as mediators between the laccase enzyme and the lignin/extractives in the fiber–lignin matrix.[28] In the case of Douglas-fir TMP, there may be two possible explanations for the results of laccase treatment. There is either sufficient accessible reactive material at the fiber surface for laccase treatments to result in the observed effects in the absence of an added mediator, or polyphenolic extractives and/or low molecular weight lignin could be acting as mediators between the laccase and the lignin/extractives distributed throughout the fiber.

The brightness values of the control pulps treated at 25°C without enzyme, were slightly higher than the values obtained at 50°C (Table 1). The a* and b* values of the pulps increased with laccase treatment, especially at 50°C, indicating the formation of red and yellow colors, respectively (Table 1). It is probable that the increases in a* and b* were due to enzymatic and thermal polymerization and/or oxidation of lignin or other leucochromophores such as dihydroquercetin (DHQ), to colored quinone structures. As the polymerization of dihydroquercetin has been implicated in the formation of Douglas-fir brownstain, it is probable that DHQ is also a precursor to color formation in the Douglas-fir heartwood TMP.[2,29,30]

The addition of oxygen during enzyme treatment increased the brightness of all the pulps compared to their counterparts without added oxygen by approximately 1 pt ISO (Table 1). Hydrogen peroxide bleaching of laccase treated Douglas-fir TMP is discussed in the following sections.

Peroxide Bleaching

To determine if there were any differences in the bleachability of laccase-treated pulps compared to controls, the laccase treated TMP was bleached with hydrogen peroxide (6%) for 3 h at 80°C while varying the sodium hydroxide charge. For laccase pre-treatments without added oxygen (Figure 1a), a 3% sodium hydroxide concentration resulted in the highest bleached brightness for both the enzyme treated and control pulps at both 25 and 50°C. At an alkali charge of 3%, the bleached brightness of the pulp treated with laccase at 25°C was 56.7% ISO whereas the corresponding control only reached a brightness value of 54.9% ISO (Figure 1a). Similar to our previous results,[7] the brightness of the pulp after treatment with laccase at 25°C was 34.8% ISO compared to 40.1% ISO for the corresponding control (Table 1). Despite an initial darkening of the pulp after laccase treatment, the laccase treated pulp (25°C) gained 21.9 pts ISO upon

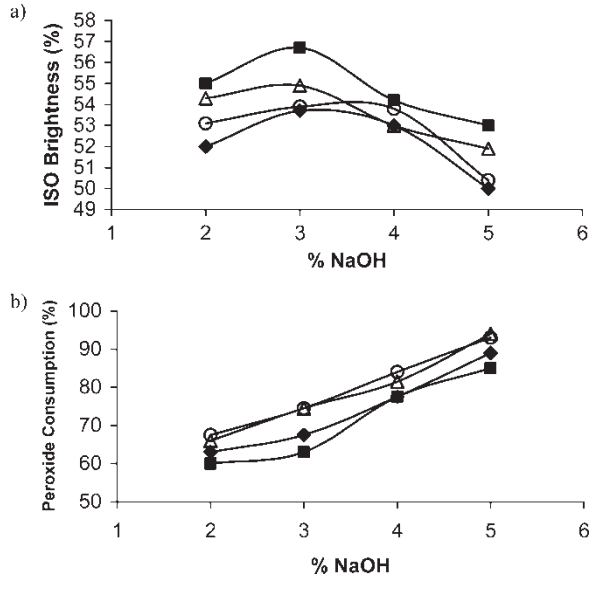

Figure 1. Brightness and hydrogen peroxide consumption for bleaching (6% H_2O_2, 80°C, 3 h) of laccase-treated Douglas-fir TMP at various alkali charges. (-△-) Control 25°C; (-■-) Laccase 25°C, (-○-) Control 50°C; (-◆-) Laccase 50°C.

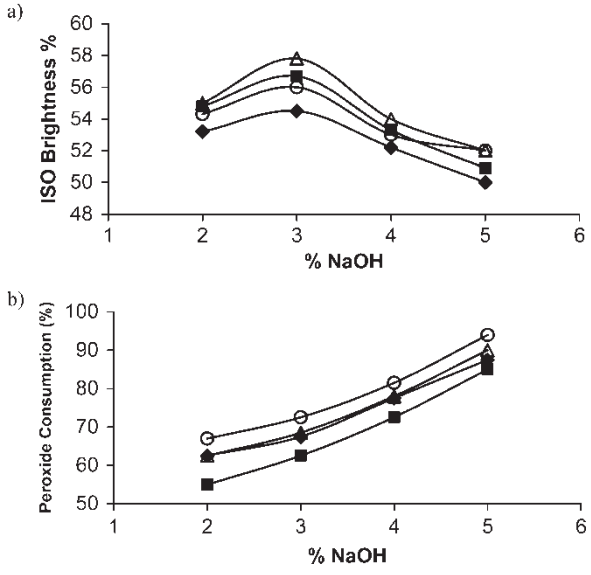

Figure 2. Brightness and hydrogen peroxide consumption for bleaching (6% H_2O_2, 80°C, 3 h) of laccase treated Douglas-fir TMP at various alkali charges in the presence of added oxygen. (-△-) Control 25°C; (-■-) Laccase 25°C; (-○-) Control 50°C; (-◆-) Laccase 50°C.

bleaching, compared to only 14.8 pts ISO for the control (Figure 1a). Concurrent with this high brightness gain, a decrease in peroxide consumption from 74% to 63% was also observed (Figure 1b). Although a similar trend was also obtained for treatments at 50°C, the initial pulp darkening was greater at 50°C than at 25°C and the final bleached brightness was slightly less than observed with the corresponding control. It appears that the chromophoric materials formed on reaction of the pulp with laccase at lower temperature (25°C), are more readily bleached by alkaline hydrogen peroxide.

The presence of added oxygen during the enzyme pre-treatment increased the brightness after peroxide bleaching by approximately 1 pt ISO (Figure 2a). These results were consistent with the 1 pt brightness gain obtained when the pre-treatment was conducted with supplemental oxygen (Table 1). However, when compared to the controls, laccase in the presence of oxygen did not improve the final bleached brightness of the pulps. In fact, decreases in the order of 1 pt ISO were obtained. Laccase treatments with added oxygen resulted in lower peroxide consumption compared to treatments performed without added oxygen, especially at 25°C (Figure 2b). Although treatments with laccase alone and oxygen alone improved hydrogen peroxide bleached brightness, their actions were not synergistic.

Thus far, the results have indicated that brightness could be enhanced by treatment at 25°C with Laccase 1 derived from *Trametes villosa* in the absence

Table 2. The effects of peroxide bleaching on Douglas-fir heartwood TMP treated with five different laccases

	Brightness (%ISO)		
Enzyme treatment	Enzyme treated	Peroxide bleached	% H_2O_2- consumed
Original heartwood TMP	41.1	57.8 ± 1.0	75.1 ± 3
Control (no enzyme)	40.1 ± 0.2	57.6 ± 1.0	75.6 ± 4
Laccase 1	35.0 ± 0.2	58.2 ± 0.8	64.4 ± 8
Laccase 2	35.8 ± 0.2	58.0 ± 0.2	66.6 ± 3
Laccase 3	34.6 ± 0.2	57.4 ± 0.4	65.2 ± 6
Laccase 4	35.5 ± 0.5	57.6 ± 0.7	68.2 ± 6

of supplemental oxygen. Three more laccases were investigated. One was derived from *Aspergillus* (Laccase 2); the others were a crude (Laccase 3) and purified form (Laccase 4) of laccase derived from *Trametes hirsuta*. The laccases from *Trametes villosa* have been reported to improve the surface of beech mechanical pulp fibers.[26,27] The laccase derived from *Trametes hirsuta* has been shown to react with phenols in kraft pulp.[23] The activity of the latter with phenolics indicate that it was a viable candidate for modifying the polyphenolic compounds responsible for the low brightness of Douglas-fir heartwood TMP. The optimum pH for all the laccases used in this work was pH 5. For this series of experiments, laccase treatments were performed at 25°C and bleaching was conducted using 6% hydrogen peroxide at 80°C for 3 h at an alkali charge of 3%.

As was observed earlier during treatment with Laccase 1 (Table 1), all of the unbleached brightness values decreased after treatment with laccases (Table 2). This brightness loss was recovered after bleaching with a decrease in hydrogen peroxide consumption compared to control pulps. The effects of the enzyme treatments were similar for all the laccases.

CONCLUSIONS

It was apparent that treatment of Douglas-fir heartwood TMP with laccase enzymes at 25°C or 50°C resulted in decreased brightness, especially at 50°C. However, after subsequent peroxide bleaching the brightness drop could be recovered to give similar or higher final brightness values with a reduction in hydrogen peroxide consumption. Laccase enzymes may be acting on the pulp by oxidizing the leucochromophores on the fiber surface. Alternatively, laccase enzymes may be using a mediator mechanism where lignin or extractives in the reaction liquor act as the mediator compounds. The increased bleachability of the pulps may be due to the formation of

quinone structures, which are more amenable to hydrogen peroxide bleaching. The optimum alkaline charges for hydrogen peroxide did not change after laccase treatment. The addition of oxygen to the laccase treatments further reduced the peroxide consumption during hydrogen peroxide bleaching; however, with the addition of oxygen, laccase treatments slightly decreased final pulp brightness. These results indicate that laccase and oxygen treatment do not act synergistically in increasing pulp brightness. No significant differences were found in the efficacy of the laccase enzymes from different sources.

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