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ORIGINAL PAPER

# Laccase treatment of recycled blue dyed paper: physical properties and fiber charge

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**Abstract** Recycled blue colored paper was treated with laccase under various combinations of physical and chemical parameters including enzyme concentration, temperature, oxygen, and reaction time. Laccase treatment of recycled dyed pulp increased acid group content, tear index, tensile index, and color removal in a dose-dependent manner. Lengthening the treatment time from 2 to 4 h was beneficial to acid group content (12% increase), dye removal, and tensile index but had a detrimental 8% decrease on the tear index. A higher reaction temperature (65 vs. 45 °C) had a beneficial effect on acid group content (+31%), and tensile index (+26%) and a slightly negative effect on tear index (−5%), but significantly reduced the ability of laccase to remove color. Comparison of reactions subjected to different levels of oxygen supplementation showed the greatest beneficial effect for laccase treatment with slow oxygen bubbling. The experimental results indicate that laccase treatment increases fiber carboxylic acid content and tensile strength, in addition to reducing the color of the enzyme treated paper.

**Keywords** Colored paper · Carboxylic acid · Mediator (TEMPO) · Recycling · Physical properties · Laccase · Biobleaching

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## Introduction

The extent of recycling of paper in North America has progressed from 33.5% in 1990 to 48% in 2002 [1]. Indeed, most commercial printing and writing grades of recycled paper contain 10–30% post-consumer waste. It is now well documented that recycling paper has less of an environmental footprint than virgin paper in terms of waste generation, woodland and fresh water demands [2]. Mixed office waste and colored papers are often an underutilized waste paper resource. A major difficulty in recycling these papers is decolorizing the dyes present in colored paper [3].

Laccase, a lignolytic enzyme, has been studied extensively in the past several years for biodelignification of wood pulps [4]. These studies have heightened the interest in using laccase for decolorizing dyes present in recycled paper [5] and textile wastewater [6]. Laccases are multicopper oxidases of wide specificity that carry out one electron oxidation of phenolic and related compounds and reduce oxygen to water [7]. To enable laccase to react with nonphenolic dyes such as C.I. Direct Yellow 11, the addition of a mediator is necessary [8]. Mediators have also been shown to be needed for laccase biobleaching of virgin pulps [9, 10].

Laccase oxidation of cellulose fibers has been reported to increase acid group content and improve virgin pulp properties [11]. Carboxylic acids groups are beneficial in the bonding of pulp fibers in paper and can increase the strength of the paper [12]. Laccase treatment has been shown to improve the strength of mechanical pulp by enhancement of the tear-tensile relationship [13]. Laccase-mediator treatment of high-kappa SW kraft pulps with 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid (ABTS) or phenothiazine-10-propionic acid (PPT) has been reported to beneficially improve wet-strength tensile strength

values [14]. Recent studies by Chandra and Ragauskas [15, 16] have also demonstrated the ability of laccase to graft select phenolic acid groups to SW kraft linerboard thereby increasing fiber charge and sheet strength properties. This study examines the potential to utilize the biobleaching properties of laccase to oxidatively remove color and improve the physical properties of a recycled paper.

## Materials and methods

### Recycled paper

Sheets of blue office paper, (*RepliCopy* blue) were immersed in distilled water (3 L) overnight and then transferred to a laboratory-scale repulper (Catep by Kadant Lamort, Vitry Le Francois) and pulped for 10 min. The resultant pulp was filtered and stored at 4 °C. Consistency (% solids) of the pulp slurry was 11.8%.

### Laccase activity

For these experiments, laccase from *Trametes villosa* was used. Laccase activity was measured by the oxidation of ABTS (2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) [17] in pH 4.5 acetate buffer using absorbance at 420 nm [18] Activity of enzyme preparation was 226 U/mL. One unit of laccase is defined as 1 μmol ABTS oxidized per minute.

### Laccase treatment conditions

Following literature conditions [19], recycled pulp [27.0 g oven dried (odg) of pulp] was mixed in a temperature-controlled Parr pressure reactor with 180 mL of 20.0 mM of acetate buffer pH 4.5 (4.0 mM final concentration) and 692.5 ml of distilled water (3% solids). To increase the concentration of oxygen, 1 MPa of oxygen pressure was applied to the sealed chamber. The standard experimental conditions consisted of 20.0 mL laccase solution, (167 U laccase/odg pulp), 1 MPa oxygen pressure, and continual stirring at 45 °C for 2 h. At the conclusion of the reaction, the pressure was released, the pulp was filtered and washed with distilled water (2 L). Test sheets were prepared according to TAPPI Standard Method T205 [20] and used to characterize physical properties.

### Study of reaction parameters

To optimize the laccase treatment, a series of reaction parameters were investigated including time (2 and 4 h), temperature, (45 and 65 °C), and enzyme amount (0, 20, 40 and 60 ml, corresponding to 0, 167.4, 334.8, and 502.2 U laccase/odg pulp). The effect of oxygen was varied: (a) constant 1 MPa $O_2$ pressure, (b) bubbling of $O_2$ at atmospheric pressure and (c) no oxygen supplementation.

### Laccase mediator treatment

A 1% charge of TEMPO (2,2,6,6-tetramethylpiperidine-1-oxyl, 10 mg TEMPO/odg pulp) was utilized with the remaining experimental conditions identical to the laccase only treatment.

### Strength testing of pulp testsheets

Testsheets prepared following TAPPI Standard Method T205 and dried under conditions as defined in TAPPI Standard Method 402, (75 F, 50% relative humidity). Tear and tensile strengths were determined according to TAPPI Standard Methods T 414 and T 494 [20].

### Color measurement

The CIE $L^*a^*b^*$ values of each testsheet was measured following TAPPI Standard Method T422 (20), using a Technibrite Micro TB-k (Technidyne Corp., New Albany, Indiana, USA). Experimental results represent the average of four testsheets.

### Calculation of dye removal index

The first step is the calculation of the geometric distance from the testsheet CIE $L^*a^*b^*$ location to the ideal bleach point where $a^* = b^* = 0$, and $L^* = 100$. The distance for the treated pulp testsheet is then subtracted from the distance of the matching no enzyme control. The difference is then divided by the starting distance (no enzyme control) to produce an index value (Dye Removal Index, DRI) that signifies the percentage of original color removed by the laccase treatment. The calculations were performed following Sharpe [21], using the equations below.

$$\text{Dye Removal Index (DRI)} = -100[\Delta R^2/R_1^2]$$

$$R^2 = \text{Distance from Ideal Bleach Point} = a^2 + b^2 + (100 - L)^2$$

$$\Delta R^2 = R_2^2 - R_1^2 = R^2 \text{ (for treated pulptestsheet)} - R^2 \text{ (for control testsheet)}$$

### Determination of carboxylic acid content

The carboxylic acid content of each treated pulp was determined following TAPPI Standard Method T237 [20]. Treated pulp (1.50 odg) was washed initially with nanopure water (2 L) and then stirred in a solution of 0.10 N HCl

(300.00 mL) for 1 h. The fibers were then filtered and washed with nanopure water (2 L). The washed pulp was transferred into 250.00 ml of 0.001 N NaCl aqueous solution which was the acidified with 1.50 ml of 0.10 N HCl. This solution was then titrated conductometrically with 0.05 N NaOH. Values were plotted and the calculations were performed as described in TAPPI T237 (20).

## Results and discussion

### Effect of laccase dosage

In this study, recycled blue dyed pulp was treated with varying amounts of laccase ranging from 0 to 502 U/odg pulp. The laccase dosage response with respect to dye removal and fibre charge is summarized in (Table 1). This data demonstrate the ability of laccase to decolorize the blue paper dye and its oxidative formation of fiber charge on the laccase treated fibers. A comparison of both the dye removal process and fiber charge development indicates that both benefit from high dosages of laccase, although the color removal appears to reach a limit at the highest laccase charge applied. As to be expected, the increased fiber charge was beneficial to the physical strength properties of paper treated with laccase (Table 2).

### Effect of reaction time and temperature

Increasing the reaction time from 2 to 4 h at 1 MPa $O_2$, 45 °C enhanced color removal, and increased acid group content by 12%. Tensile strength was increased 28%, but tear strength decreased 8% (Fig. 1). Reaction temperature is also an important factor to consider because increased temperature can potentially increase both the rate of the desired enzymatic reaction and the rate of enzyme inactivation [22]. For this dyed recycled pulp, increasing the reaction temperature from 45 to 65 °C resulted in a large increase in acid group content (31%), but had a negative effect on pulp color removal. Laccase treatment at 65 °C (2 h, 167 U laccase/odg pulp) increased the tensile strength by 26% (Fig. 2).

**Table 1** Laccase dose on fibre acid groups and dye removal index employing 1 MPa oxygen pressure at 45 °C for 2 h

| Units of laccase used | Acid groups mM/100 odg | SD | % of dye removal index | SD |
|---|---|---|---|---|
| 0 | 6.21 | 0.16 | 0 | 0 |
| 167.4 | 6.50 | 0.16 | 32.86 | 0.11 |
| 334.8 | 6.76 | 0.17 | 58.41 | 0.13 |
| 502.2 | 8.09 | 0.20 | 57.27 | 0.12 |

**Table 2** Laccase dose on tensile and tear index of the recycled paper employing 1 MPa oxygen pressure at 45 °C for 2 h

| Units of laccase used | Tensile strength | % increase on tensile strength | SD | Tear index M2/g | % increase on tear index | SD |
|---|---|---|---|---|---|---|
| 0 | 28.37 | | 6.72 | 3.98 | | 0.34 |
| 167.4 | 41.06 | 44.73 | 3.59 | 4.26 | 7.04 | 0.41 |
| 334.8 | 47.00 | 65.67 | 2.73 | 4.15 | 4.27 | 0.36 |
| 502.2 | 53.59 | 75.78 | 3.38 | 4.53 | 13.82 | 0.33 |

### Effect of oxygen Supplementation

Oxygen is consumed during laccase treatment hence oxygen pressure is frequently applied to biobleaching reactions to maximize oxidative reactions [23]. The studies presented in Figs. 1 and 2 were performed with 1 MPa of oxygen pressure. The effect of oxygen pressure on laccase treatment of this dyed recycled paper was studied by comparing the effects from the 1 MPa $O_2$ reaction conditions with laccase treatment employing no oxygen supplementation

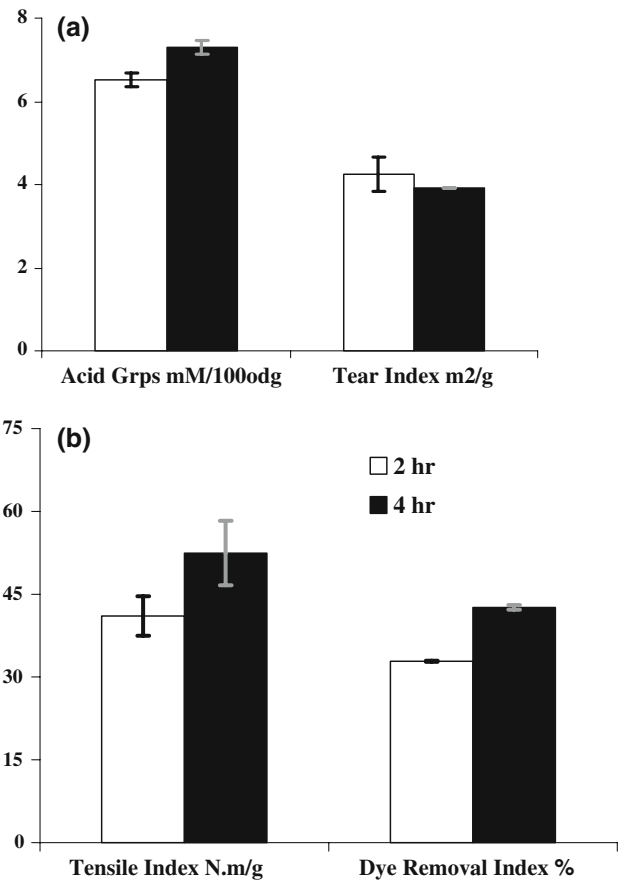


**Fig. 1** Increased incubation time in paper strength properties during biobleaching. Reaction conditions: 2 h, 45 °C, 167 U laccase/odg pulp

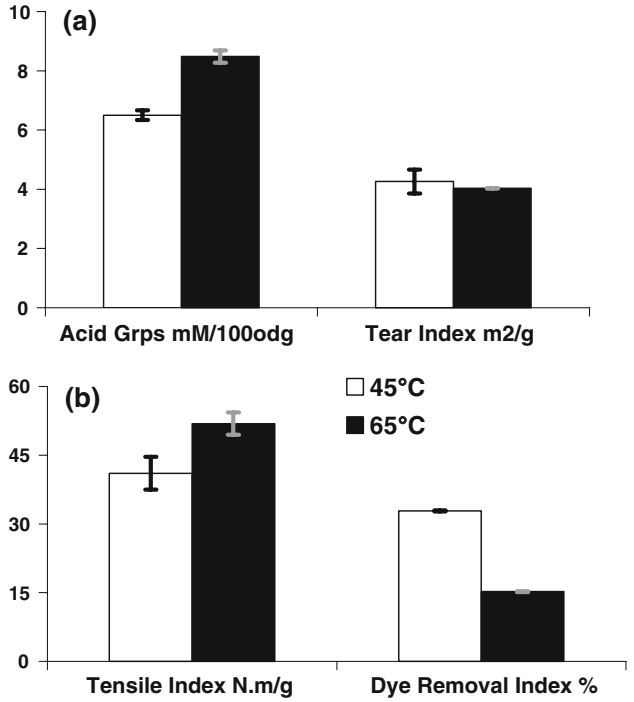


**Fig. 2** Increased temperature in paper strength properties during biobleaching Reaction conditions: 2 h, 45 °C, 167 U laccase/odg pulp

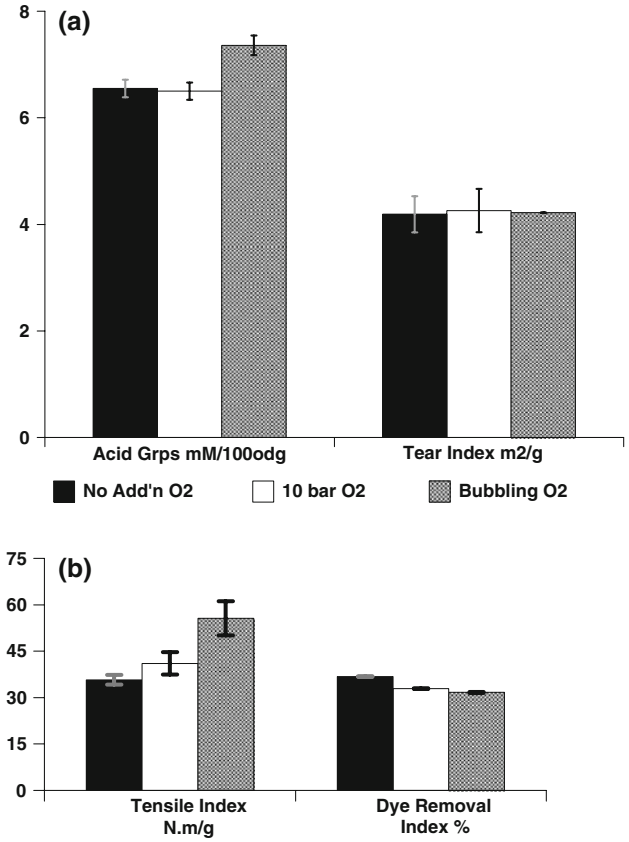


**Fig. 3** Effect of oxygen supplementation during laccase biobleaching recycled blue paper on **a** fiber *acid group* content and *tear index* and **b** *tensile index* and *dye removal*. Reaction conditions: 2 h, 45 °C, 167 U laccase/odg pulp

or with oxygen enrichment achieved by bubbling oxygen at ambient atmosphere pressure (Fig. 3). The acid group content was very similar for the unsupplemented reaction and the 1 MPa oxygen treated pulps, but oxygen bubbling increased fiber acid group content by 13% as summarized in oxygen aeration, had little effect on tear index values but did improve tensile strength with respect to no oxygen supplementation. Oxygen aeration had a negative effect on color removal for this recycled pulp. The results obtained from continuous bubbling may be attributed to greater laccase activity in the presence of oxygen bubbles [24].

## Effect of mediator addition

As has been well documented in laccase biobleaching of chemical pulps, the oxidative removal of lignin benefits from the addition of a chemical mediator [25, 26]. Several mediators have reported in the literature and the most commonly studied mediators have *N*-hydroxyl structures including violuric acid. In terms of removing color from dyes pulps, redox potentials are important and the choice of mediator influences the extent of color removal [27].

TEMPO (2,2,6,6-tetramethylpiperidine-1-oxyl) was selected as laccase mediator for this study of recycled blue dyed pulp, based on a previous report of laccase-TEMPO treatment increasing carbonyl products [28]. This mediator has been shown to be an oxidative agent for selective oxidation of 6-hydroxy groups of cellulose [29]. TEMPO

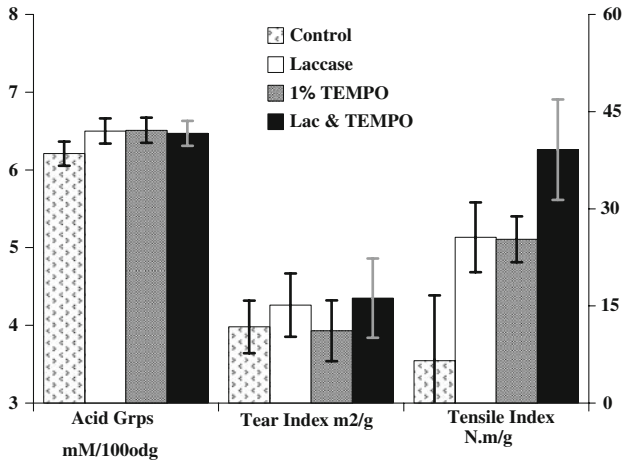


**Fig. 4** Effect of 1% TEMPO as laccase mediator for biobleaching recycled blue paper

has also been applied as a laccase mediator in the oxidation of phenols [30] and alcohols [31].

In our experiments, the results of treatments with laccase, laccase with TEMPO, and TEMPO alone were

compared with a control treatment (standard conditions of pH 4.5, 1 MPa oxygen pressure, 45 °C, 2 h reaction). Little change was observed in acid group content or tear index values (Fig. 4). Oxidative treatment did appear to increase tensile strength and the combination of TEMPO and laccase had a beneficial effect.

## Conclusions

Laccase treatment of recycled blue dyed pulp increased color removal, acid group content, and tensile index in a dose-dependent manner. Lengthening the treatment time from 2 to 4 h was beneficial with respect to acid group content, dye removal, and tensile index, but had a detrimental effect on the tear index. Performing the laccase treatment at a higher temperature, 65 versus 45 °C, had a positive effect on tensile index and acid group content, and a slightly negative effect on tear index, but significantly reduced the ability of laccase to remove color. Changes in aeration of the reaction had slight effect on color removal and tear index. Pressurizing the reaction with 1 MPa of oxygen resulted in higher tensile index. Bubbling oxygen though the reaction had the most positive effect on both tensile index and acid group content. Possible synergism between laccase and TEMPO was observed for tensile strength. The experimental results presented here show that laccase treatment increases fiber acid group content, tensile strength and in some cases tear index values, in addition to reducing the color of a recycled dyed pulp. The production of recycled paper from dyed/printed paper, especially mixed office waste involves several unit operations including deinking and bleaching. This paper provides an innovative biobleaching application for the later operation. [32].

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