DECOLORIZATION OF INKJET INK AND DEINKING OF INKJET-PRINTED PAPER WITH LACCASE-MEDIATOR SYSTEM

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The emergence of novel high-speed inkjet printing technology has been hindered because of claims of poor deinkability of the printed product. Based on our results the decolorization of inkjet inks with the laccase-mediator system is a possible approach to improve the deinkability of inkjet-printed paper. The commercial *Myceliophtora thermophila* and *Trametes versicolor* laccases (1 U/mL) and a mediator compound acetosyringone (0.1 mM) decolorized water-soluble textile and inkjet ink dyes by up to 94% and aqueous dye-based inkjet inks by 40 to 98%. *M. thermophila* laccase decolorized magenta and black inks effectively even at pH 9.0. Acetosyringone was a better mediator compared to ABTS and violuric acid because of its high efficiency and low inherent color. The enzymatic decolorization of inkjet ink was also achieved in deinking experiments with inkjet-printed paper. A treatment with *M. thermophila* laccase (2 U/g of paper) and acetosyringone (0.02% of paper weight) improved ISO-brightness of the pulp by 5%.

Keywords: Enzymatic deinking; Laccase; Inkjet printing; Dye decolorization; Mediator

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INTRODUCTION

Inkjet printing is the most prominent digital printing technology for high-volume printing applications. With the emergence of the new versatile high-speed technology it has been forecasted that by the year 2012 inkjet printing will account for 7% of the overall printing market in terms of value and 4% in terms of volume, displacing analogue methods and competing with other digital technologies for many end-use applications (Smyth 2007).

The emergence of novel high-speed inkjet printing technology is hindered because of questions concerning the recyclability of the printed product. Deinking is a process performed on all recovered printed papers intended for graphic use, and its main unit operation is flotation, in which the ink particles are removed from the pulp with the aid of chemicals and the rising of small air bubbles. Water-based inkjet inks used in the high-volume printers contain hydrophilic dyes that that do not agglomerate and cannot be removed efficiently by flotation, which results in colored process waters and deteriorated brightness in the end product (Fischer 2006). Water-based inkjet inks have been determined to be non-deinkable and claimed to pose the biggest threat so far for the deinking industry by the International Association of Deinking Industry (INGEDE 2008).

Some approaches have been studied to improve the deinking of inkjet-printed paper. The approaches include incubation with the marine bacterium *Vibrio alginolyticus*

(Mohandass and Raghukumar 2005) and neutral or near-neutral secondary flotation (Süss and Grimmer 2004). Reductive bleaching with formaminide sulfinic acid has been found to be more effective towards inkjet dyes than oxidative bleaching (Hanecker and Strauss 2008). However, the incubation time required by the microbial treatment is long, there are additional process steps requiring investment in equipment, and a bleaching step is not always included in the deinking process. Ink removal by washing improves the elimination of small ink particles, but it always reduces yield, which cannot be compromised in the times of expensive raw materials (Süss and Grimmer 2004).

The oxidizing enzyme laccase (benzenediol:oxygen oxidoreductase EC 1.10.3.2), being capable of polymerizing and degrading various compounds through radical-mediated oxidation, has found use in the pulp and paper industry (Widsten and Kandelbauer 2008). Well-known applications include biobleaching of lignocellulosic pulps, mill water treatment, and grafting of compounds on lignin-rich pulps and surfaces. In the deinking industry, enzymes have been used to dislodge cross-linked non-aqueous inks from fiber or coating surfaces by the hydrolytic action of cellulases, xylanases, and amylases (Bajpai 1997).

The problem of color removal faced by the deinking industry concerns also the textile industry. Extensive research has been conducted for nearly two decades regarding biotechnology-based decolorization of textile industry effluents. Oxidizing enzymes such as laccase have been found to be able to degrade azo bonds, which are present within the chromophores in commonly used azo dyes. Also, other dye structures such as triarylmethane, indigoid, and anthraquinonic dyes have been decolorized enzymatically. Several mediator compounds have been found to improve the decolorization efficiency of laccase towards dyes by widening the substrate range and extending the degree of decolorization (Husain 2006).

The dyes used in inkjet inks are structurally similar to textile dyes that have been efficiently decolorized by laccase in previous studies. According to our best knowledge this is the first report on the enzymatic decolorization of inkjet inks or inkjet-printed paper. In this study the idea of decolorizing aqueous dye-based inkjet inks was investigated first by using the laccase-mediator system in decolorization of buffered inkjet and textile dye and inkjet ink solutions. Two commercial laccase products from different source organisms used in the study showed differences in their ability to achieve decolorization of the dyes. The effects of mediator choice, pH, and the presence of deinking chemicals on the decolorization efficiency of the more alkaline-tolerant laccase from *Myceliophtora thermophila* were evaluated, and based on the results the application seems to be feasible for industrial deinking environments. Finally, the possibility to improve inkjet-print deinking by laccase-mediator decolorization was demonstrated in laboratory scale deinking experiments.

EXPERIMENTAL

Materials

Eleven water-soluble dyes were purchased from Sigma-Aldrich, USA. The dyes Acid Blue 9, Acid Red 52, Acid Red 80, Acid Yellow 17, Direct Blue 86, and Reactive

Red 23 are used in inkjet inks (Blease et al. 2006; Lauw et al. 2000), and the dyes Reactive Black 5, Direct Blue 71, Acid Orange 52, Reactive Yellow 2, and Direct Red 28 have reportedly been decolorized by laccase or laccase-mediator treatment (Tauber 2005; Ciullini et al. 2008; Champagne and Ramsey 2005; Murugesan et al. 2007). Aqueous dye-based inkjet inks used in the tests were cyan, magenta, yellow, and black inks from the Kodak FV3000 ink series (USA).

Two laccase products were used in the experiments: Novozym 51003, from Novozymes, Denmark, and a solid enzyme formulate from Sigma-Aldrich, Germany. The source organisms of the laccases were *Myceliophtora thermophila* and *Trametes versicolor*, respectively.

The chemicals used were syring-aldazine, acetosyringone, and 2'-azino-biz-(3-ethylbenzothiazoline-6-sulfonic acid) from Sigma-Aldrich, Germany, sodium silicate, hydrogen peroxide, sodium hydroxide, oleic acid, and calcium chloride from Merck, Germany, violuric acid from Fluka, Germany, and sodium oleate from VWR, Belgium. The buffer chemicals citric acid, sodium phosphate, tris(hydroxymethyl)aminomethane, hydrochloric acid, glysine, and sodium hydroxide were analytical grade.

The paper used in the deinking experiments was KIP-77-10MS, a coated non-wood fine paper intended for inkjet printing from Kanzan Spezialpapiere GmbH, Germany. A four-color test image was printed on it with Kodak Versamark VX5000 inkjet press with a printed area of 67% and 110 mm^2/g.

Enzyme Activity Assays

The pH profiles of the two laccases were assayed with syringaldazine (4-hydroxy-3,5-dimethoxybenzaldehyde azine) as substrate within the pH range 4.5 to 7.5 in 50 mM citrate phosphate buffer at 23°C (modified from Lundell et al. 1990). Oxidation of syringaldazine was followed as an absorbance increase at 530 nm with Lambda 900 UV/VIS/NIR spectrophotometer (Perkin-Elmer, USA). *T. versicolor* laccase showed peak activity at pH 5, and the activity of *M. thermophila* laccase was stable until pH 7. The assay pH for both enzymes was chosen based on the pH profiles of the enzymes, and one unit (U) of laccase activity was defined as the amount of enzyme that catalyzed the formation of 1 µM of product per minute in 50 mM citrate phosphate buffer at pH 5.0 for *T. versicolor* laccase and pH 6.5 for *M. thermophila* laccase.

Decolorization Experiments

The decolorization experiments were carried out in a reaction mixture containing a suitable dilution of the dye or ink solution, 1 U/mL enzyme, and optionally 0.1 mM mediator compound and other deinking chemicals in a total volume of 1.5 mL 50 mM citrate phosphate buffer for pH range 5.0 to 6.5, 50 mM Tris-HCl buffer for pH range 7.0 to 9.0, or 50 mM Glys-NaOH buffer for pH range 9.5 to 10.0. The dye and ink concentrations were adjusted to give the absorbance of ca. 1.0 OD in the beginning of the experiment. Dye concentrations for this absorbance level were in the range of 6 to 190 mg/L.

The reaction mixture was incubated in 15 mL SuperClear® tubes (VWR, Belgium) on a rotary shaker (150 rpm) at room temperature for 24 h or in 2 mL microcentrifuge tubes on a 45°C hot plate for 1 h. Tubes were closed with a cap, and no

additonal oxygen was provided. Decolorization was determined by comparing the absorbance of the enzyme treated sample to the absorbance of the control sample (laccase inactivated by boiling for 10 min, no mediator) at the absorbance maximum of each dye. Negative decolorization value indicates that the enzyme or enzyme-mediator treated sample had a higher absorbance than the control sample. The absorbance of the samples was measured in Plastibrand PS disposable 1.5 mL cuvettes (Brand, Germany) in the wave length range of 350 to 750 nm.

Deinking Trials

The deinking procedure followed the INGEDE method no. 11 (INGEDE 2007a) with minor modifications. The deinking solution contained 0.1% NaOH, 1.8% sodium silicate, 0.7% H_2O_2, and 0.8% sodium oleate. A 100 g (d.w.) sample of aged and torn inkjet-printed paper was pulped for 20 min at 45 °C in a N50 pulper (Hobart, Germany) with the deinking solution (consistency 15%). The pulp was then diluted with pre-heated water to 5% consistency and mixed for 60 min at 45 °C in the presence of 2 or 10 U/g *Myceliophthora thermophila* laccase and 0.02% or 0.04% of acetosyringone per paper weight. Reference sample was prepared without laccase and mediator. After the incubation the pulp was diluted to 4% consistency and disintegrated at 3000 rpm for 1 min in a Lorentzen & Wettre disintegrator (Sweden). Flotation of 60 g pulp was conducted in a flotation cell 50990 (Outokumpu, Finland) at 0.8% consistency for 10 min with 1600 rpm mixing and 6 L/ min air supply. During the flotation the formed froth was skimmed off manually to a collecting vessel.

Filter pads and handsheets were prepared from pulp before flotation (undeinked pulp) and pulp after flotation (deinked pulp) from LMS-treated and reference samples according to INGEDE method 1 (INGEDE 2007b) as closely as possible. The pulp samples were diluted with tap water to 0.4% consistency, and 535 mL of the sample was filtered in a 110 mm diameter Büchner funnel through filter paper. Two parallel filter pads were made from each sample. The filter pads were stacked with pressing paper and pressed at 2.9 bar for 1 min and dried in a tunnel drier at 23 ºC over night. Handsheets were prepared with recirculated white water according to standard EN ISO 5269-3:08 with the target grammage of 45 g/m^2. Filtrates from preparing the deinked filter pads were filtered again through a cellulose nitrate membrane filter (pore size 0.45 µm) in a 50 mm diameter Büchner funnel. Reference filter and filtrate were made by filtering 100 mL of tap water.

The main deinkability analysis followed the INGEDE Method 2 (INGEDE 2007c), except that the dirt particle measurement was not performed. ISO brightness and luminosity according to ISO 2470:99, CIELAB color coordinates according to ISO 5631:00, and effective residual ink concentration (ERIC) according to TAPPI T567 pm-97 were analyzed from the filter pads and handsheets.

Strength properties including tensile index, stretch, tensile energy absorption index, and tensile stiffness index were measured from the sheets according to the modified standard ISO 1924-2:94. The luminosity of the membrane filters was measured from the top side of the filter, and the absorbance spectrum of the filtrates was determined spectrophotometrically.

RESULTS AND DISCUSSION

Decolorization of Dyes

Decolorization experiments were conducted to evaluate the color removal effect of two commercial laccase products and a mediator compound acetosyringone on eleven dyes and four inkjet inks (Table 1). Several dyes were decolorized effectively by over 80% with laccase-mediator treatment.

Table 1. Decolorization (%) of Dyes and Inkjet Inks with 1 U/mL Laccase and 0.1 mM Acetosyringone in 50 mM Citrate Phosphate Buffer in 24h Treatment at Room Temperature

	pH 6.5			pH 5.0		
Dye/Ink (type, peak wave length)	***M. thermophila* laccase**	**Acetosyringone**	***M. ther.* + Ac.**	***T. versicolor* laccase**	**Acetosyringone**	***T. ver.* + Ac.**
Reactive Black 5 (disazo, 596 nm)	1	18	94	19	11	84
Direct Blue 71 (trisazo, 580 nm)	-2	13	94	83	9	92
Acid Orange 52 (monoazo, 463 nm)	27	83	93	84	9	93
Direct Red 28 (disazo, 488 nm)	4	11	91	n.a.	n.a.	n.a.
Acid Yellow 17 (monoazo, 400 nm)	0	62	81	12	4	63
Reactive Yellow 2 (monoazo, 405 nm)	1	13	37	-7	17	3
Reactive Red 23 (monoazo, 522 nm)	-1	1	24	5	8	28
Direct Blue 86 (copper phthalocyanine, 615 nm)	3	4	12	91	-3	91
Acid Red 52 (xanthene, 565 nm)	5	3	5	17	1	17
Acid Blue 9 (triarylmethane, 629 nm)	1	2	5	57	4	56
Acid Red 80 (anthrapyridone, 531 nm)	2	-1	0	2	-2	-4
Magenta (519 nm)	21	92	96	82	2	93
Black (656 nm)	5	6	57	49	2	64
Yellow (403 nm)	1	1	40	0	0	25
Cyan (618 nm)	1	4	7	78	-7	70
n.a. = not analysed						

The structural class that was decolorized the best was the azo dyes, and five out of the seven azo-dyes tested were decolorized at least by 81% by one of the two laccases when mediator was present in the reaction mixture. In addition *T. versicolor* laccase was able to decolorize other dye classes, especially the phthalocyanine dye Direct Blue 86. On the other hand, *M. thermophila* laccase could decolorize only azo dyes by over 15%. Negative decolorization values indicate that the treatment increased the absorbance of the sample in certain cases. The increase in those cases is most likely caused by experimental error.

The best decolorization overall (84 to 94%) was obtained with the azo dyes Reactive Black 5, Direct Blue 71, Acid Orange 52, and Direct Red 28, which contain electron-donating –OH, -NH_2, and -NR_2 groups in the *ortho* or *para* position to the azo bonds. Somewhat lower decolorization (24 to 81%) was obtained with azo dyes Acid Yellow 17, Reactive Yellow 2, and Reactive Red 23, which contain electron-withdrawing Cl-groups, a heterocyclic pyrazole ring, or a chelated metal atom. The only dye not decolorized at all was the anthrapyridone dye Acid Red 80. The acetosyringone mediator alone did not decolorize dyes to a great extent (decolorization <18%) with two exceptions: Acid Orange 52 and Acid Yellow 17, which were decolorized by 83% and 62%, respectively, at pH 6.5. The oxidized mediator was slightly colored in the concentration used (0.1 mM), increasing the absorbance of the samples at short wave-lengths (350 to 450 nm), which was taken into account when interpreting the results. The spectra of all samples were inspected, and no new prominent peaks were found after the laccase-mediator treatment.

In general, the decolorization results of the commercial dyes were in accordance with the literature. The well-reported positive effect of electron-donating groups on enzymatic decolorization was supported also by our results. Reactive Black 5 is one of the most studied dyes for enzymatic decolorization. It has been reported several times to be effectively decolorized only in the presence of a mediator (Claus et al. 2002; Camarero et al. 2005; Murugesan et al. 2007) or by fungal culture supernatants (Abadulla et al. 2000; Nyanhongo et al. 2002) that may contain natural metabolite mediators (Eggert et al. 1996). Accordingly in this study Reactive Black 5 could not be oxidized effectively (decolorization < 20%) without a mediator with either of the laccases. Decolorization of azo dyes Direct Blue 71 and Acid Orange 52 has been obtained without a mediator also earlier with *T. versicolor* laccase (Nyanhongo et al. 2002; Tauber et al. 2005). Phthalocyanine-type dyes, which are often found to be recalcitrant to enzymatic or microbial decolorization (Camarero et al. 2005; Šušla et al. 2007), were effectively decolorized by *T. versicolor* laccase in this study.

The lack of decolorization of Acid Red 80 could be explained by its high degree of oxidation. The conjugated structure with two carbonyl groups has only few electrons left to be donated. Suprisingly chlorine-containing azo dye Reactive Yellow 2 was decolorized better by *M. thermophila* laccase than *T. versicolor* laccase. A possible explanation for the better reactivity of low redox potential laccase towards a dye with an electron-withdrawing group may lie in the relationship of the pKa of the dye, the pH, and the reaction mechanism.

Decolorization of Inkjet Inks

All of the inkjet inks responded to at least one of the tested laccase or laccase-mediator treatments with decolorization from 40% to 96% (Table 1). Magenta ink was very efficiently decolorized with laccase-acetosyringone treatment, showing almost complete absence of color in the treated sample with both the laccases.

Differences between the decolorization ability of the two laccase enzymes and their dependence on the mediator were observed. Without mediator *T. versicolor* laccase was capable of decolorizing magenta, black, and cyan inks, while *M. thermophila* laccase had only a slight effect on magenta ink. Mediator increased the decolorization efficiency of especially *M. thermophila* laccase, but also *T. versicolor* laccase needed mediator addition to be able to decolorize yellow ink. When the mediator was present, *T. versicolor* laccase decolorized black and especially cyan inks better than *M. thermophila* laccase, whereas the opposite result was obtained with magenta and yellow inks. Duplicated experiments with *M. thermophila* laccase showed maximum difference of 6% units in the decolorization results.

Magenta ink, as well as the azo dyes Acid Orange 52 and Acid Yellow 17, showed decolorization with only acetosyringone without laccase at pH 6.5 but not at pH 5. Claus et al. (2002) have reported the same phenomenon with HBT on azo dye Acid Red 28 at pH 5.9. Results do not provide a basis for speculation on the cause of the decolorization with only acetosyringone, but it can be concluded that the reaction is pH-dependent.

The absorbance spectra of the inkjet ink samples before and after treatment with *T. versicolor* and *M. thermophila* laccases are presented in Fig. 1. For cyan, yellow and magenta inks the decrease of absorbance at the maximum wavelength of the ink was not accompanied by a significant increase in absorbance elsewhere in the spectrum, especially if the increased absorbance of the oxidized mediator in short wavelengths was taken into account. For example, the double peak of cyan retained its form, but the sample treated with *T. versicolor* laccase had smaller peak area. The spectrum of the magenta ink treated with *T. versicolor* laccase retained the form of a peak, while the *T. versicolor* laccase-mediator treated sample showed no sign of the peak. Black ink showed a distinctive increase in the absorbance at short wavelengths, which was more prominent than that caused by the oxidized mediator.

According to our knowledge this is the first report on the use of laccase for decolorization of inkjet inks. Varying degrees of decolorization of different textile and inkjet dyes were obtained in this study using two laccases with different origin. All four inkjet inks were susceptible to laccase decolorization with acetosyringone mediator and all except yellow ink also without the mediator.

Compared to *M. thermophila* laccase the *T. versicolor* laccase decolorized more dyes and inks effectively alone and benefitted less from the addition of the acetosyringone mediator. This was expected, because it is known that *T. versicolor* laccase has a higher redox potential than *M. thermophila* laccase, 775-785 mV and 450-480 mV respectively (Solomon et al. 1996).

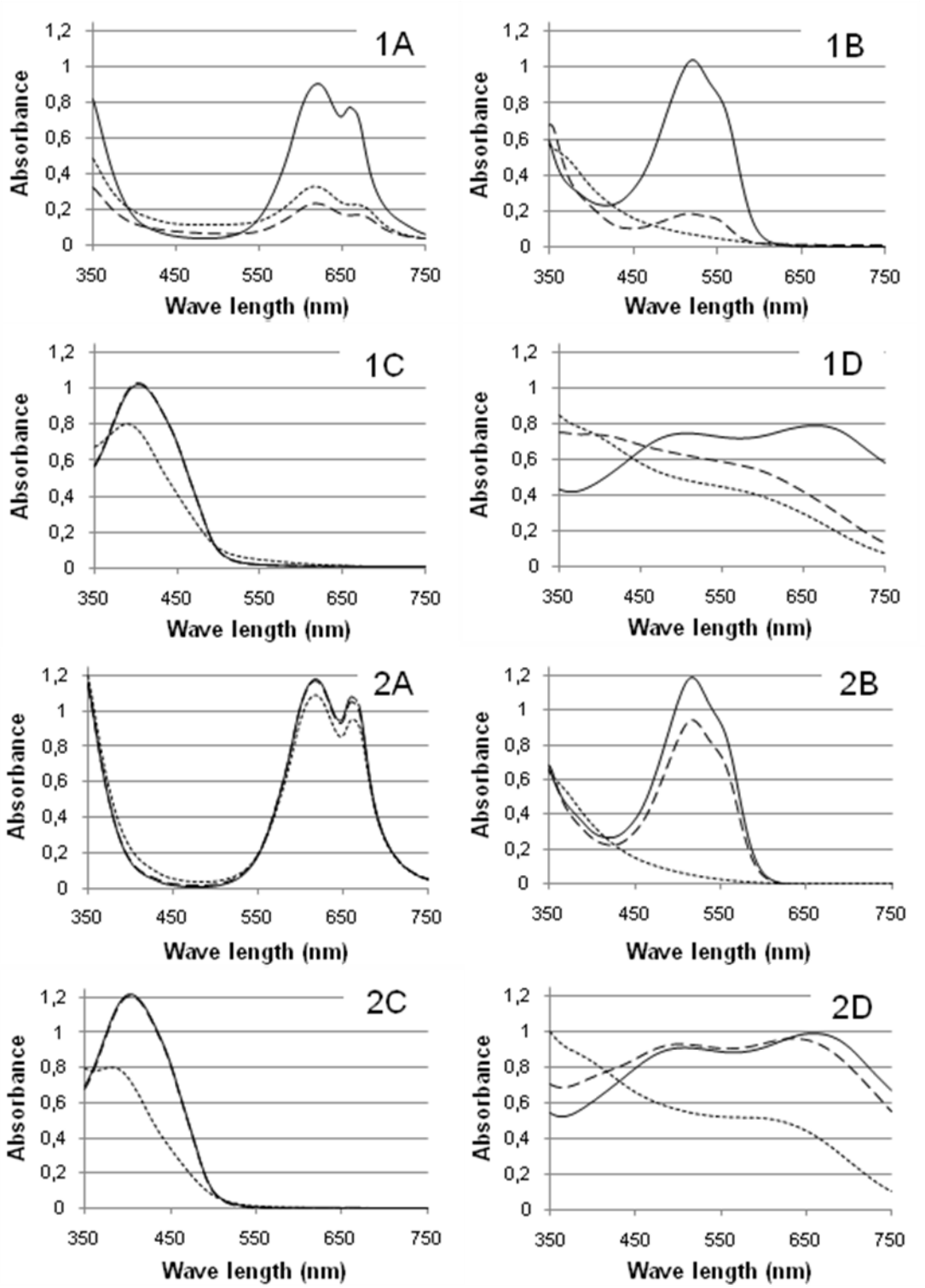

Fig. 1. Absorbance spectra of cyan (A), magenta (B), yellow (C), and black (D) inkjet inks treated with 1 U/mL *T. versicolor* (1) or *M. thermophila* (2) laccase and 0.1 mM acetosyringone in 50 mM citrate phosphate buffer pH 6.5 or pH 5.0 after 24 h treatment at room temperature. Control — , laccase - - - -, laccase and acetosyringone · · · ·.

The small increase in the absorbance of the enzyme-mediator treated samples in the shorter wavelength area observed in this study has been observed also by others (Claus et al. 2002; Michniewicz et al. 2008). The slight increase in the orange-yellow shades probably derived from the color of the oxidized mediator and could be decreased by decreasing the concentration of acetosyringone in the test.

The overall lower absorbance of the enzyme-treated cyan, magenta, and yellow inks suggests that the chromophores of the dye molecules in the inks were removed and no new ones were formed. However, the increase in absorbance in the lower region of the spectrum of enzyme treated black ink suggests that the chromophores in the dye molecules were not completely removed but were transformed to other structures absorbing at short wavelengths. No precipitation was formed by enzymatic action in the decolorization experiments, suggesting that the dyes and inks were indeed degraded instead of being polymerized by laccase. Ciullini et al. (2008) report extensive precipitation, but the dye concentrations were in most cases 2 to 50 times higher than those used in this study.

The Effect of Mediators on Decolorization by *M. thermophila* Laccase

In addition to acetosyringone, the effects of two other mediators, ABTS and violuric acid, on the decolorization of inkjet inks were studied (Table 2). Out of the three mediators tested, acetosyringone proved to be on the whole the most effective mediator for *M. thermophila* laccase under these conditions. Violuric acid and ABTS were able to enhance the decolorization of magenta and black inks, but to a lesser extent. Violuric acid did not have any effect on cyan and yellow ink decolorization, whereas ABTS caused a bias to the decolorization results of these two inks because of the strong green color of the oxidized mediator.

The acetosyringone mediator generally improved the decolorization of dyes and inks. An interesting exception to this was the decrease of decolorization of cyan ink with *T. versicolor* laccase when mediator was applied (Fig. 1). This suggests that acetosyringone might have behaved as a competitive substrate for laccase, thus decreasing the oxidation of the dye. Reyes et al. (1999) observed a similar decrease in decolorization efficiency with HBT for dyes Reactive Blue 198 and Acid Blue 185.

The beneficial effect of acetosyringone to dye decolorization by laccase has been reported by others as well. Acetosyringone alongside with a very similar compound syringaldehyde, was determined to be the best mediator out of 44 different compounds in a screening experiment by Camarero et al. (2005).

Table 2. Decolorization (%) of Inkjet Inks with 1 U/mL *M. thermophila* Laccase (L) and 0.1 mM Mediator Compounds Acetosyringone (A), 2,2'-azino-biz-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and Violuric acid (VA) in 50 mM Citrate Phosphate Buffer pH 6.5 in 24 h Treatment at Room Temperature

Inkjet ink	A	ABTS	VA	L+A	L+ABTS	L+VA
Cyan	4	3	-1	7	-35	0
Magenta	92	0	-1	96	64	48
Yellow	1	-1	-5	40	-102	-2
Black	4	11	7	57	23	13

The Effect of pH on Decolorization by *M. thermophila* Laccase

The decolorization of inks in alkaline conditions was studied with *M. thermophila* laccase due to its relatively broad pH range. The laccase-mediator decolorization of magenta and black ink occurred within a wide pH range (Fig. 2). By contrast, the decolorization of yellow ink seemed to have an optimum in the acidic range, as did the decolorization of cyan ink, although its decolorization by *M. thermophila* was at a low level under all conditions. Variations in pH alone did not appear to shift the peak position or change the absorbance of the control samples.

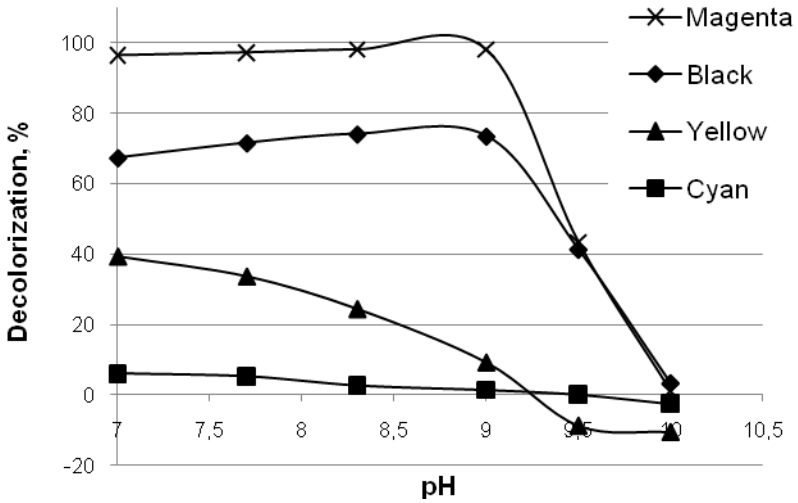

Fig. 2. The effect of pH to the decolorization of inkjet inks with 1 U/mL *M. thermophila* laccase and 0.1 mM acetosyringone in 50 mM citrate phosphate buffer at pH 6.5, 50 mM Tris-HCl buffer at pH 7-9 and 50 mM Glys-NaOH buffer at pH 9.5 to 10 during 1 h treatment at 45 ºC.

The yellow ink colorants behaved differently in terms of the effect of pH and deinking chemicals when compared to black and magenta inks. According to Xu (1997), a bell shaped pH profile is typical for phenolic substrates, for which the decolorization begins with the transfer of a proton. For some compounds such as ABTS, in which proton transfer is not involved in the oxidation, the pH activity profile is monotonic, because pH affects the system only through OH-inhibition rather than through changes in redox potential. Although the shape of the whole curve was not tested for the inks, it seems that the decolorization of yellow ink colorant could have a different route, possibly without a proton transfer, than that of magenta and black ink.

The observed high alkaline-tolerance of the *M. thermophila* laccase product is very beneficial for many applications in the pulp and paper industry. However, the steady efficiency even until pH 9.0 is quite surprising, because oxidation efficiency of beech TMP fibers and syringaldazine decreases significantly in the pH range 6 to 7.5 according to the enzyme product sheet from Novozymes. Thus it seems that the dyes in magenta and black inks are very good substrates for the *M. thermophila* laccase-acetosyringone system.

The Effect of Deinking Chemicals on Decolorization

The effect of common deinking chemicals on ink decolorization by laccase-mediator treatment is presented in Table 3. The concentrations of sodium silicate and sodium oleate in the experiment corresponded to the concentrations used during the enzymatic treatment in the subsequent deinking experiments. Only the decolorization of yellow ink responded noticeably to the addition of the deinking chemicals sodium oleate and sodium silicate, while the decolorization of other inks was not inhibited. Hydrogen peroxide affected the decolorization of yellow ink the most, but also the decolorization of black ink was inhibited by H_2O_2.

Table 3. Decolorization (%) of Inkjet Inks with 1 U/mL *M. thermophila* Laccase, 0.1 mM Acetosyringone and Common Deinking Chemicals (0.9 g/l Sodium Silicate, 0.43 g/l Sodium Oleate and H_2O_2) in 50 mM Tris-HCl Buffer pH 8.5 in 1 h Treatment at 45 ºC.

	Magenta	Black	Yellow	Cyan
No deinking chemicals	98	73	18	4
Sodium silicate	97	73	13	3
Sodium oleate	93	73	7	1
Sodium silicate and sodium oleate	94	72	5	2
100 ppm H_2O_2	99	66	6	2
500 ppm H_2O_2	99	55	1	5

Laccase-Mediator Deinking of Inkjet-Printed Paper

In this first experiment with inkjet-printed paper the laccase-mediator treatment visibly changed the color of the waste paper pulp during the first minutes of the treatment. The red and grey hues of the liquid phase disappeared, and the pulp became colored light blue to green. This visually observed decolorization was measured using the INGEDE method no.11 deinkability test. The enzymatic decolorization of the ink increased the Y-value or the luminosity, the main parameter of the test determining the overall brightness of the deinked filter pad or handsheet. Low enzyme dosage (2 U/g, 0.02% mediator) increased the Y-value of filter pads by 4% before deinking and by 7% after deinking, while ISO brightness was increased by 2% before deinking and by 5% after deinking (Fig. 3). The ISO brightness and luminosity of handsheets prepared with recirculated white water was increased by 4% and 5%, respectively, compared to the reference (data not shown). High enzyme and mediator dosage (10 U/g, 0.04% mediator) increased the luminosity and brightness slightly more for filter pads and handsheets, especially for pulp before deinking. The CIELAB color coordinate a* dropped from 0.7 (reference) to -0.6 (low dosage) and -1.0 (high dosage), and coordinate b* rose from -6.3 to -5.2 (low dosage) and -4.9 (high dosage) in the filter pads after deinking indicating color removal during the process.

ERIC (effective residual ink concentration) decreased during flotation for the all samples. ERIC of the filter pads from deinked enzyme treated samples was lower (19 to 20 ppm) compared to the reference (28 ppm). Enzyme treatment did not cause any major changes to paper strength properties, i.e. tensile index, stretch, tensile energy absorption index, or tensile stiffness index (data not shown). The luminosity of the cellulose nitrate

membrane filter increased from 91.7% to 92.8% (low dosage) and 93.8% (high dosage), while no increase in the filtrate absorbance was found. Pulp yield in flotation was 74 to 78%, and pH before the enzymatic treatment was 7.7 to 8.2.

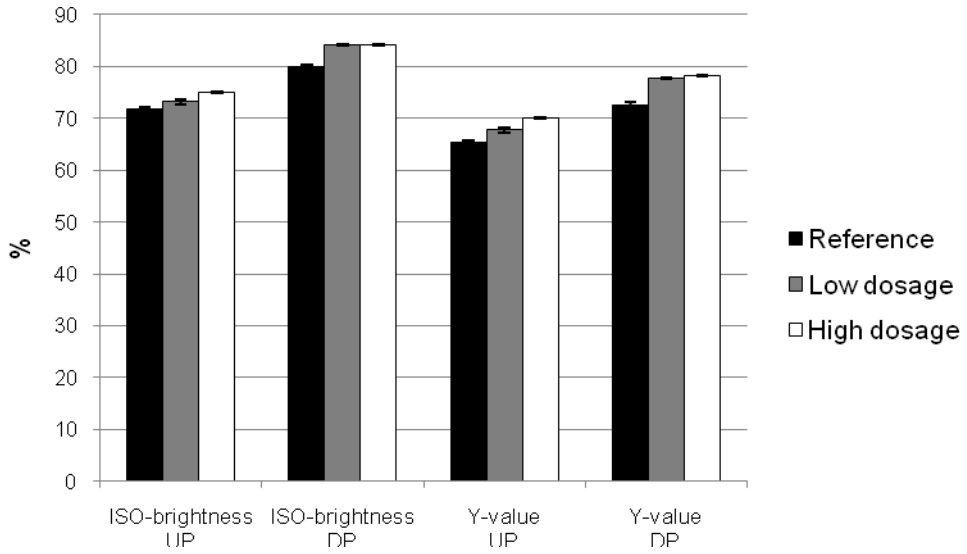

Fig. 3. ISO brightness and Y-value (luminosity) of filter pads from enzymatic deinking trials with *M. thermophila* laccase (low dosage 2 U/g, high dosage 10 U/g) and acetosyringone (low dosage 0.02%, high dosage 0.04%) and four-color inkjet-printed Kanzan paper before (UP) and after (DP) deinking (error shown as 5% confidence level)

Laccase has been used in deinking in a few studies for improving ink detachment from the fiber surface through delignification (Xu et al. 2007, 2009) and for biobleaching of colored waste papers (Li et al. 2000; Knutsson and Ragauskas 2004), but to our knowledge not in decolorization of printing ink. In addition to the improvements in ISO brightness and ERIC value, the positive effect of the laccase-mediator treatment could be visually detected in the color of the sheets and filter pads between the enzyme-treated and reference samples. The analysis of the luminosity of the membrane filter and the absorbance of the filtrate prove that the dyes were not merely transferred to the filtrate but in fact decolorized. Thus it can be concluded that laccase-acetosyringone treatment improved the optical properties of four-color inkjet-printed wood-free paper. It seems that a major part of the decolorization took place during the treatment before deinking as expected, but during flotation there was also a slight improvement in the optical properties. In this study the positive effect of the enzymatic treatment on optical properties was not accompanied by any major changes in paper strength properties.

CONCLUSIONS

1. Laccase products from *T. versicolor* and *M. thermophila* decolorize dyes used in textile industry and inkjet inks as well as inkjet inks as such.

2. Acetosyringone is a better mediator in terms of efficiency and low inherent color than ABTS or violuric acid for this application.
3. The tolerance of *M. thermophila* laccase to common deinking chemicals and alkaline conditions shows potential for use in industrial deinking processes.
4. All but one ink were decolorized by the high redox potential laccase from *T. versicolor* alone, which suggests that expensive mediators might not be needed.
5. Laccase-mediator decolorization of inkjet inks improves the deinking of inkjet-printed paper.

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