Enzymatic deinking of secondary fibers: Carbohydrate hydrolases versus laccase-mediator system

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Abbreviated title: Enzymatic deinking of secondary fibers

Abstract

The use of enzymes has been suggested as an environmentally-friendly alternative to complement conventional chemical deinking in the recycling of recovered paper. This study compares the use of carbohydrate hydrolases versus laccase-mediator system for deinking printed fibers from newspapers and magazines. For this purpose, two commercial enzyme preparations with endoglucanase and endoxylanase activities (Viscozyme Wheat from *Aspergillus oryzae* and Ultraflo L from *Humicola insolens*) and a commercial laccase (NS51002 from *Trametes villosa*), the latter in the presence of synthetic or natural (lignin-related) mediators, were evaluated. The enzymatic treatments were studied at laboratory scale, using a standard chemical deinking sequence consisting of pulping, alkaline deinking and peroxide bleaching stages. Then, handsheets were prepared and their brightness, residual ink concentration, and strength properties were measured. Among the different enzymatic treatments assayed, both carbohydrate hydrolases were found to deink the secondary fibers more efficiently. Brightness increased up to 3-4% ISO on newspaper fibers, being Ultraflo 20% more efficient in the ink removal. Up to 2.5% ISO brightness increase was obtained when magazine fibers were used, being Viscozyme 9% more efficient in the ink removal. As regards laccase-mediator system, alone or combined with carbohydrate hydrolases, it was ineffective deinking both newspaper and magazine fibers, resulting in pulps with worse brightness and residual ink concentration values. However, pulp deinking by laccase-mediator system was displayed when secondary fibers rich in lignin, i.e. printed cardboard, were used, obtaining up to 3% ISO brightness increase and lower residual ink concentrations.

Keywords: Cellulases; deinking; laccase-mediator system; recycling; secondary fibers; xylanases

1. Introduction

Recovered paper is an important source of raw material for the pulp and paper industry. Indeed, the utilization of these secondary fibers is increasing all over the world, being the deinking an important step in the recycling process for white grade papers. In the traditional deinking process, large quantities of chemicals are used (Shrinath *et al.* 1991), which makes the method expensive, environmentally damaging and increases the release of contaminants. In this context, enzymes could reduce the demand of chemicals and would also lower the process costs and the environmental impact (Thomas, 1994; Bajpai and Bajpai, 1998). These enzymes include cellulases, hemicellulases, pectinases, amylases, lipases, esterases and laccases (Call and Stritmatter, 1992; Gübitz *et al.* 1998; Zollner and Schroeder, 1998; Morkbak *et al.* 1999; Pleach *et al.* 2003; Pala *et al.* 2004).

Among the different enzymes assayed, the enzymatic deinking using carbohydrate hydrolases, with activities involved in cellulose and hemicellulose hydrolysis, has been widely demonstrated on different secondary fibers (Jeffries *et al.* 1994; Gübitz *et al.* 1998; Marques *et al.* 2003; Pleach *et al.* 2003; Vyas and Lachke, 2003; Pala *et al.* 2004; Lee *et al.* 2007; Zhang *et al.* 2008; Kuhad *et al.* 2010) being applied in several paper mills (Heise *et al.* 1996). The mechanism of deinking by these enzymes has not been completely elucidated, although some hypotheses have been described (Jeffries *et al.* 1994; Heise *et al.* 1996). The cellulose and hemicellulose hydrolysis on the surface of the fibers leads to a removal of small fibrils, phenomenon known as “peeling-off fibers”, which facilitates ink detachment from the surface. It has also been suggested that the alteration of the ink particles hydrophobicity because of the removal of small fibrils enhances their separation (fiber/ink separation) in the flotation/washing step.

The interest in the use of laccases, alone or in combination with carbohydrate hydrolases, for deinking secondary fibers has been greatly increased in the last years (Knutson and Ragauskas, 2004; Xu *et al.* 2004; Kapoor *et al.* 2007; Mohandass *et al.* 2008; Qinghua *et al.* 2009; Kuhad *et al.* 2010). Laccases in the presence of redox mediators, the so-called laccase-mediator system described 20 years ago (Bourbonnais and Paice, 1990), has been largely used to delignify, and bleach, different types of pulps (Bourbonnais and Paice, 1996; Sealey *et al.* 1999; Herpoël *et al.* 2002; Camarero *et al.* 2004a; Ibarra *et al.* 2006). This fact offers the possibility for deinking secondary fibers rich in lignin, such as fibers based on mechanical pulps (Bajpai and Bajpai, 1998). With the removal of lignin, the bonds between fiber and ink particles became loose, facilitating ink detachment. Moreover, successful transformation of recalcitrant dyes by laccase-mediator system has been recently described (Claus *et al.* 2002; Camarero *et al.* 2004b), which has supposed a new way for secondary fibers deinking by direct decolorization of the inks.

In the present study, two different strategies for enzymatic deinking of secondary fibers were evaluated: one using carbohydrate hydrolases for removing inks in an indirect way by peeling-off fibers; and other using the laccase-mediator system for removing inks in an indirect way by the removal of surface lignin or in a direct way by decolorizing the inks. For this purpose, different raw materials (secondary fibers from newspapers and magazines) were used. Brightness, residual ink concentration and strength properties of the handsheets formed from the resulting deinked pulps were tested.

2. Materials and Methods

2.1 Recovered paper

Secondary fibers from newspaper and magazines were used. Prior to the treatments, the dried raw materials were shredded and maintained in distilled water for 24 h. In addition, secondary fibers rich in lignin, i.e. printed cardboard, were also used to evaluate the laccase-mediator system.

2.2 Enzymes and mediators

Carbohydrate hydrolases used in this work, Viscozyme Wheat (from *Aspergillus oryzae*) and Ultraflo L (from *Humicola insolens*) were obtained from Novozymes. Their activities involved in cellulose hydrolysis (exoglucanase, E.C.3.2.1.91; endoglucanase, E.C.3.2.1.4; and β-glucosidase, E.C.3.2.1.21) were studied on: Avicel (Merck) for total cellulose activity (exoglucanase); and the specific substrates carboxymethylcellulose (Serva) and *p*-nitrophenyl glucoside (Sigma) for endoglucanase and β-glucosidase activities, respectively (Wood and Bhat, 1988). Their activities involved in hemicellulose hydrolysis (endoxylanase, EC 3.2.1.8; and β-xylosidase, EC 3.2.1.37) were studied on birch xylan (Sigma) and *p*-nitropheyl xyloside (Sigma) for endoxylanase and β-xylosidase activities, respectively (Wood and Bhat, 1988). Their activities involved in starch hydrolysis (α-amylase, E.C. 3.2.1.1; and glucoamylase, E.C.3.2.1.3) were studied on soluble starch (Calbiochem) and *p*-nitrophenyl glucoside (Sigma) for α-amylase and glucoamylase activities, respectively (Wood and Bhat, 1988). The endoglucanase, exoglucanase, endoxylanase and α-amylase activities, determined in 50 mM sodium acetate buffer (pH 5) at 50 ºC, were followed by the release of reducing sugars estimated as glucose or xylose at 540 nm (Nelson 1944). The β-glucosidase, β-xylosidase and glucoamylase activities, determined in 50 mM sodium acetate buffer (pH 5) at 50 ºC, were followed by the release of *p*-nitrophenol (ε_{412} 15 200 $M^{-1}cm^{-1}$). Their optimum pH was investigated on the different substrates in 100 mM citrate-phosphate-borate buffer (pH range from 3 to 8) at 24 ºC, determining the activities as described above.

A Novozymes commercial laccase NS51002 (from *Trametes villosa*) was used. Laccase activity was determined by measuring the oxidation of 5 mM 2,2’-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) buffered with 100 mM sodium acetate (pH 5) at 24 ºC. Formation of the ABTS cation radical was monitored (ε_{436} 29 300 $M^{-1}cm^{-1}$).

Proteins were determined according to Bradford method (1976), using bovine albumin as standard and Bio-Rad kit assay.

One unit of enzyme activity was defined as the amount of enzyme that transforms 1 μmol of substrate per minute. All spectrophotometric measurements were carried out on a Shimadzu UV–vis 160.

The compounds used as redox mediators 1-hydroxybenzotriazole (HBT), violuric acid (VIO), sinapic acid (SIN), ferulic acid (FER) and *p*-coumaric acid (PCO) were purchased from Sigma.

2.3 Deinking pulp sequences

A standard (industrial type) deinking sequence P−D−B (Fig. 1) was performed in laboratory scale including: (1) pulping stage (P) carried out in a lab disintegrator ENJO-D-33.73/D at 3% consistency, room temperature and 3000 rev/min, according to the ISO standard 5263-1:2004; (2) alkaline deinking stage (D) using 1.5% NaOH, 3% $Na_2Si_2O_3$ and 0.06% of soap for 60 min at 60ºC and 5% consistency; and (3) alkaline peroxide bleaching stage (B) using 3% H_2O_2 and 1.5% NaOH for 120 min at 90 ºC and 5% consistency (above percentages referred to dry weight pulp). To facilitate the separation of ink particles detached from the fibers (fiber/ink separation), the pulp samples were exhaustively washed with distilled water through a 200-mesh wire.

Enzymatic treatments were assayed at the standard deinking sequence by: (1) incorporating a carbohydrate hydrolase stage (C), using Viscozyme or Ultraflo (sequence P−C−D−B); (2) incorporating a laccase-mediator (L) stage (sequence P−L−D−B); and (3) incorporating a carbohydrate hydrolase stage, using Viscozyme or Ultraflo, followed by a laccase-HBT stage (P−C−L−D−B) (Fig. 1).

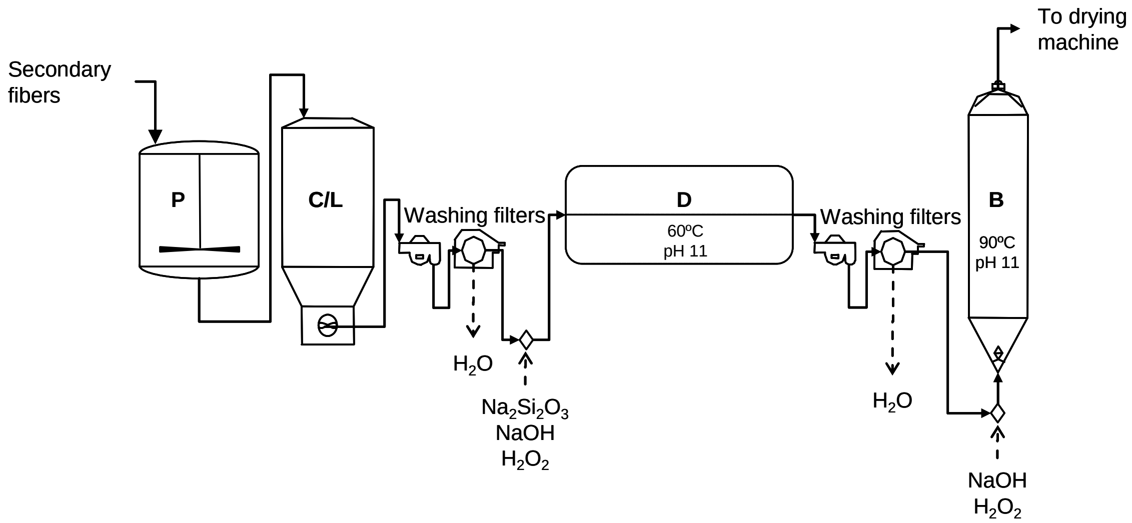

Fig 1. Scheme of a standard deinking sequence including: (1) pulping stage (P); (2) alkaline deinking stage (D); and (3) alkaline peroxide bleaching stage (B). Enzymatic treatments were assayed in laboratory scale using this standard sequence by: (a) incorporating a carbohydrate hydrolase (C) stage (P−C−D−B); (b) incorporating a laccase-mediator (L) stage (P−L−D−B); and (c) incorporating a carbohydrate hydrolase stage followed by laccase-mediator (C−L) stage (P−C−L−D−B). In order to facilitate the removal of ink particles detached from secondary fibers (fiber/ink separation), the pulp samples were exhaustively washed with distilled water through a 200-mesh filter.

Viscozyme and Ultraflo treatments were carried out in duplicate with 10 g (dry weight) of pulp at 3% consistency in 50 mM sodium tartrate buffer (pH 4) or sodium citrate buffer (pH 7) respectively. Two enzymatic dosages, 10 or 30 U/g dry weight pulp, were tested. The treatments were carried out in flasks, at 160 rev/min and 50 ºC, for 1 h. Laccase-mediator treatments were carried out was carried out in duplicate with 10 g (dry weight) of pulp at 3% consistency in 50 mM sodium tartrate buffer (pH 4), using 20 U/g dry weight pulp of laccase and 0.5% (w/w) (referred to dry weight pulp) of different mediators. Tween 80 (0.05% w/v) was added as surfactant. The treatments were carried out in flask under O_2 atmosphere (continuous bubbling) at 160 rev/min and 50 ºC, for 5 h. I all cases, pulps were also treated under identical conditions without enzymes or mediators.

2.4 Optical and mechanical properties measurements of the handsheets

Three handsheets per tested sequence with a grammage of 60 g/m^2 were prepared using a Rapid-Kothen sheet former according to ISO 5269/2. The handsheets were characterized measuring the tensile index by an extensometer according to ISO 1924, and the tear index by Elmendorf equipment according to ISO 6383 and ISO 1974.

Brightness (% ISO) and residual ink concentration (ppm) of the handsheets were measured by a Datacolor Elrepho 2000 spectrophotometer. The ink removal index was calculated using the following equation:

$$\text{Ink removal index (\%)} = (C_{\text{control pulp}} - C_{\text{enzymes deinked pulp}}) / C_{\text{control pulp}}$$

where C is the residual ink concentration.

3. Results and discussion

3.1 Deinking using carbohydrate hydrolases

3.1.1 Optical properties

Fig. 2 shows the changes of brightness and residual ink concentration in deinked pulps from newspaper and magazine fibers after a carbohydrate hydrolase containing deinking sequence (P−C−D−B), using Viscozyme or Ultraflo, and their corresponding control sequences. In general, control deinked pulp from newspaper fibers showed optical properties slightly better than those of deinked pulp from magazine fibers (Fig. 2); obtaining brightness values higher and residual ink concentrations lower. Moreover, control deinking sequence at pH 7 (optimum pH for endoglucanase and endoxylanase activities determined in Ultraflo preparation) resulted in slightly higher brightness (1.5-2% ISO brightness higher) and lower residual ink concentration (130-150 units residual ink concentration lower) than obtained when the control deinking sequence was carried out at pH 4 (optimum pH for endoglucanase and endoxylanase activities determined in Viscozyme preparation). Conventional deinking process employs sodium hydroxide in combination with a range of other chemicals (Shrinath *et al.* 1991). High pH values favor fibers swelling, increasing their flexibility and consequently facilitating the detachment of the adhered ink (Wielen *et al.* 1999). Furthermore, it may also act directly on the printed ink film and weaken its structure, leading to fragmentation (Shrinath *et al.* 1991). For these reasons, the slight alkaline pH could explain the better optical properties for control deinked pulps obtained at pH 7.

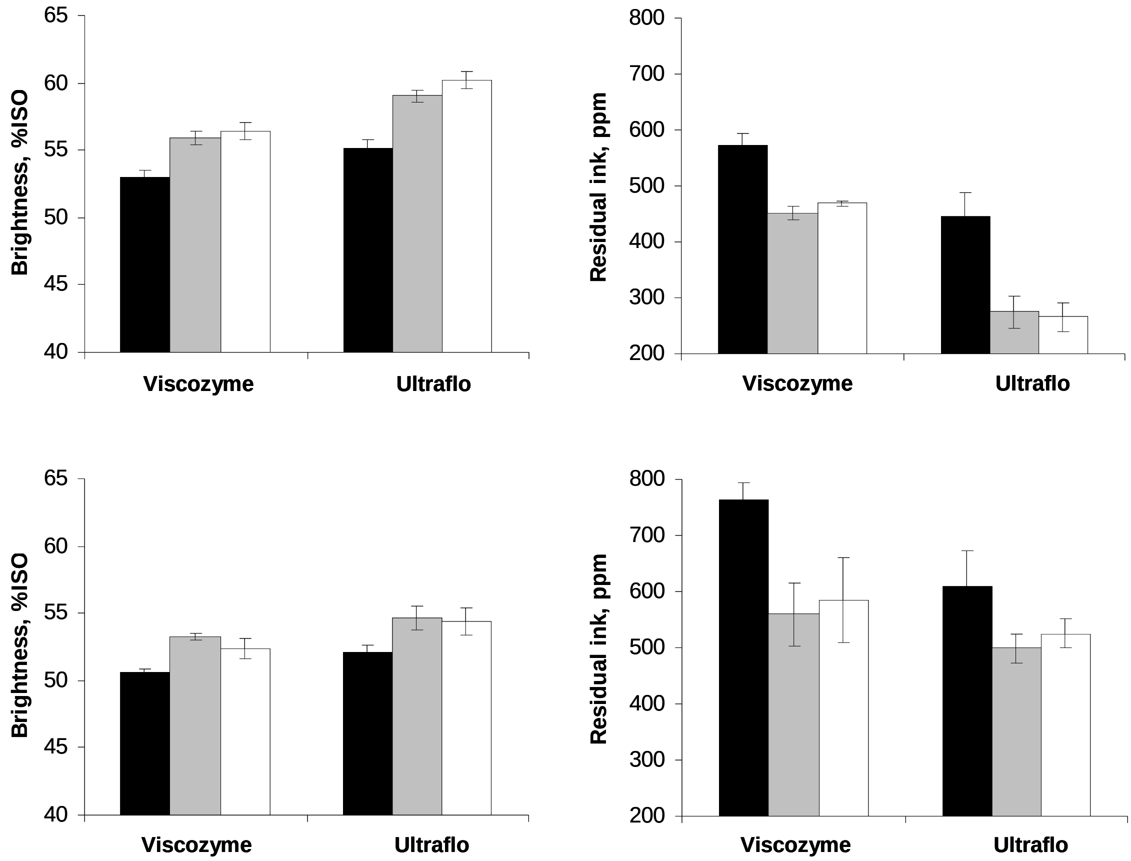

Fig 2. ISO brightness (left) and residual ink concentration (right) of deinked pulps from newspaper (top) and magazine (bottom) fibers after a carbohydrate hydrolase-containing deinking sequence (P−C−D−B). Enzyme dosages: 0 (black bars), 10 (gray bars), and 30 U/g (white bars) dry weight pulp. Mean values and 95% confidence limits are shown.

Using 10 U/g dry weight pulp of both carbohydrate hydrolases the brightness of deinked pulp from newspaper fibers increased 3-4 units, reaching 56% ISO and 59% ISO brightness with Viscozyme and Ultraflo, respectively (Fig. 2). Moreover, Ultraflo was 20% more efficient than Viscozyme in the ink removal, i.e. ink detachment, (Fig. 3). By contrast, an increment of 2.5 units was obtained when magazine fibers were used, reaching 53% ISO and 55% ISO brightness with Viscozyme and Ultraflo, respectively (Fig. 2). In this case, Viscozyme was 9% more efficient than Ultraflo in the ink removal (Fig. 3). As mentioned above, the enzymatic deinking using carbohydrate hydrolases has been successfully demonstrated (Jeffries *et al.* 1994; Gübitz *et al.* 1998; Marques *et al*. 2003; Pleach *et al*. 2003; Vyas and Lachke, 2003; Pala *et al.* 2004; Lee *et al.* 2007; Zhang *et al.* 2008; Kuhad *et al.* 2010). Among the different carbohydrate hydrolase activities, endoglucanase and/or endoxylanase activities seems to play an important role in the deinking efficiency, although it is difficult to know the contribution of each one to the deinking process. Some authors support the idea that the main contribution is given by endoglucanases (Gübitz *et al.* 1998; Vyas and Lachke, 2003), which attack the less ordered cellulose between and on the surface of the fibrils, leading to fiber wall swelling and loosening of short fibers, and consequently dislodging inks. Other authors consider that endoxylanases provide the main activity (Berlin *et al*. 1997), promoting ink detachment due to hemicellulose hydrolysis on the fibers surface. In any case, the combined action of the two activities has shown better deinking efficiencies (Gübitz *et al.* 1998; Lee *et al.* 2007). In this sense, Viscozyme Wheat (containing 17 U/mg endoglucanase activity estimated from glucose release from carboxymethylcellulose and 65 U/mg endoxylanase activity estimated from xylose release from birch xylan, Table 1) and Ultraflo L (containing 23 U/mg endoglucanase activity estimated from glucose release from carboxymethylcellulose and 61 U/mg endoxylanase activity estimated from xylose release from birch xylan, Table 1) showed mainly both endoglucanase and endoxylanase activities, explaining their effectiveness deinking secondary fibers from newspapers and magazines. In addition, Viscozyme also showed a slight glucoamylase activity (1 mU/mg), which could explain its better efficiency in the ink removal of magazine fibers (with a hypothetical higher starch amount than newspaper fibers). Increment of dosage from 10 to 30 U/g dry weight pulp of both enzymes did not enhance significantly the optical properties (Fig. 2 and 3).

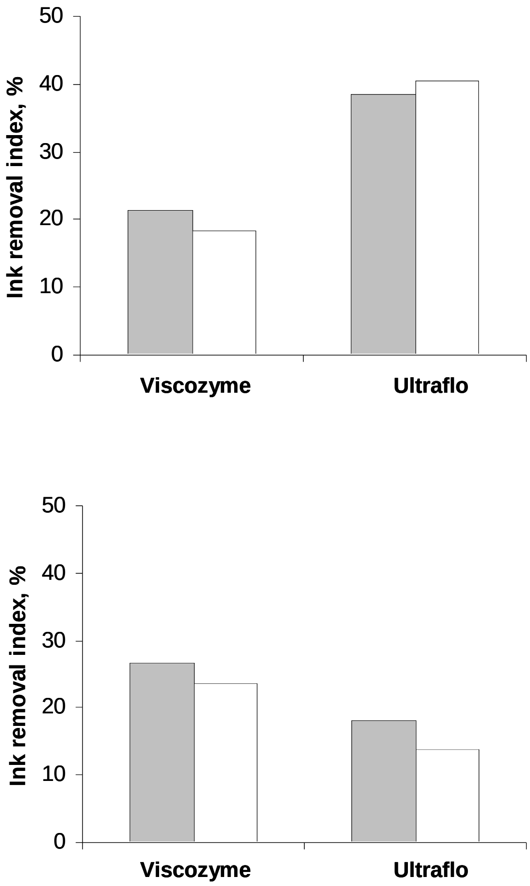

Fig 3. Ink removal indices of deinked pulps from newspaper (top) and magazine (bottom) fibers after a carbohydrate hydrolase-containing deinking sequence (P−C−D−B). Enzyme dosages: 10 (gray bars), and 30 U/g (white bars) dry weight pulp.

Table 1
Summary of different enzymatic activities detected in both Viscozyme Wheat and Ultraflo L preparations.

	Viscozyme		Ultraflo	
Type of enzyme activity	U/ml	U/mg	U/ml	U/mg
Cellulase				
exoglucanase	135	4	90	13
endoglucanase	520	17	160	23
β-glucosidase	5500[1]	177[2]	7630[1]	1090[2]
Hemicellulase				
endoxylanase	2000	65	430	60
β-xylosidase	21000[1]	680[2]	2630[1]	380[2]
Amylase				
α-amylase	nd	nd	nd	nd
glucoamylase	25[1]	1[2]	nd	nd

[1]Activity expressed as mU/ml; [2]activity expressed as mU/mg; protein concentration (mg/ml) according to Bradford method: 31 and 7 mg/ml for Viscozyme Wheat and Ultraflo L preparations, respectively; nd, not detected.

3.1.2 Mechanical properties of the handsheets

Table 2 shows the mechanical properties (tensile and tear indexes) of the handsheets from deinked pulps from newspaper and magazine fibers, after a carbohydrate hydrolase containing deinking sequence (P−C−D−B), using Viscozyme or Ultraflo, and their corresponding control sequences. The integration of both carbohydrate hydrolases resulted in strength properties practically unaltered, being even slightly improved, especially using 10U/g dry weight pulp of Viscozyme on newspaper, or scarcely worsened using Ultraflo. In contrast to use of multicomponent carbohydrate hydrolases, which often have a detrimental effect on paper properties (Suurnakki *et al*. 2000), several studies sustain the idea that the use of monocomponent carbohydrate hydrolases, endoglucanases and/or endoxylanases, maintains or even enhances the strength properties (Gübitz *et al.* 1998; Marques *et al*. 2003; Vyas and Lachke, 2003). Our results support this assumption. The losing of small fibrils from the surface fibers by the action of monocomponent enzymes employed improves the interfibrillar bonding and consequently enhances the strength properties (Vyas and Lachke, 2003).

Table 2
Mechanical properties of the handsheets from deinked pulp from newspaper and magazine fibers after a carbohydrate hydrolase containing deinking sequence (P−C−D−B) with two different enzyme dosages (10 and 30 U/g dry weight) and the corresponding control without enzyme (C).

	Newspaper						Magazine					
	Viscozyme			Ultraflo			Viscozyme			Ultraflo		
	C[a]	10	30	C[a]	10	30	C[a]	10	30	C[a]	10	30
Tensile index (Nm/g)	20.1	26.8	21.8	25.5	23.8	23.2	27.8	28.2	30.3	29.8	29.5	28.6
Tear index (mNm^2/g)	2.35	2.35	2.35	2.15	2.35	2.15	2.75	3.10	3.10	2.60	2.35	2.35

[a]Secondary fibers treated under the same enzymatic conditions (1 h, 50 ºC at pH 4 for Viscozyme or pH 7 for Ultraflo) but without enzymes.

3.2 Laccase-mediator deinking

3.2.1 Optical properties

Fig. 4 shows the changes of brightness and residual ink concentration in deinked pulps from newspaper and magazine fibers after a laccase-mediator containing deinking sequence (P−L−D−B), and the corresponding control deinking sequence. Compared to carbohydrate hydrolases, no significant differences were found when the laccase-mediator system, using 0.5% w/w of different synthetic (HBT and VIO) and natural (SIN, FER and PCO) mediators, was integrated in the deinking sequence. In fact, deinked pulps with slightly lower brightness and different colour tones were obtained depending of the mediator used. No improvement of the deinked pulp properties was observed when mediator concentrations were increased (1.5% and 3% w/w) (data not shown). These results disagree with those reported recently (Knutson and Ragauskas, 2004; Xu *et al.* 2004; Kapoor *et al.* 2007; Mohandass *et al*. 2008; Qinghua *et al.* 2009; Kuhad *et al.* 2010) describing the capacity of laccases and laccase-mediator systems (using different synthetic mediators) for deinking of secondary fibers. Moreover, some of these studies have also displayed the enzymatic synergistic deinking combining cellulases or hemicellulases with laccase-mediator system (Kapoor *et al.* 2007; Qinghua *et al.* 2009). In this sense, enzymatic deinking sequences containing a carbohydrate hydrolase stage, Ultraflo or Viscozyme, followed by laccase-HBT stage (P−C−L−D−B) were tested on both newspaper and magazine fibers. None synergistic effect was observed between Ultraflo/Viscozyme and laccase-HBT system (Table 3). Even, the enhanced optical properties attained after the carbohydrate hydrolase containing deinking sequences (P−C−D−B) were negatively affected when the laccase-HBT system was integrated after the carbohydrate hydrolase stage (P−C−L−D−B), as shown in Table 3.

Table 3

Optical properties of deinked pulps from newspaper and magazine fibers after a carbohydrate hydrolase followed by LMS-containing deinking sequence (P−C−L−D−B), compared to carbohydrate hydrolase-containing (P−C−D−B) and laccase-mediator-containing (P−L−D−B) deinking sequences.

	Newspaper		Magazine	
	Brightness (% ISO)	Residual ink (ppm)	Brightness (% ISO)	Residual ink (ppm)
Control[a] *	53±0.02	545±53.5	51±0.2	665±13
P−C−D−B[a]	55.8±0.03	445±9.5	53.5±0.4	480±16
P−L−D−B	52.6±0.25	570±5.5	50.3±0.8	680±16
P−C−L−D−B[a]	51.4±0.05	600±53.5	49.7±0.6	680±52
Control[b] *	54.5±0.20	470±12.5	52.1±0.4	615±40
P−C−D−B[b]	58.3±0.35	340±35	55±0.5	470±19
P−L−D−B	52.8±0.40	560±15	51.5±0.9	620±26
P−C−L−D−B[b]	52.3±0.30	575±28	51±0.45	640±31

P, pulping stage; D, alkaline deinking stage; B, alkaline bleaching stage; C, carbohydrate hydrolase stage using 10 U/g dry weigh pulp of [a] Viscozyme or [b] Ultraflo; L, laccase-mediator stage using 0.5% of 1-hydroxybenzotriazole (HBT) as mediator.* Secondary fibers treated under the same enzymatic conditions (1 h, 50 ºC at pH 4 for Viscozyme or pH 7 for Ultraflo, and 5 h, 50 ºC at pH 4 for laccase-HBT) without enzymes and mediator.

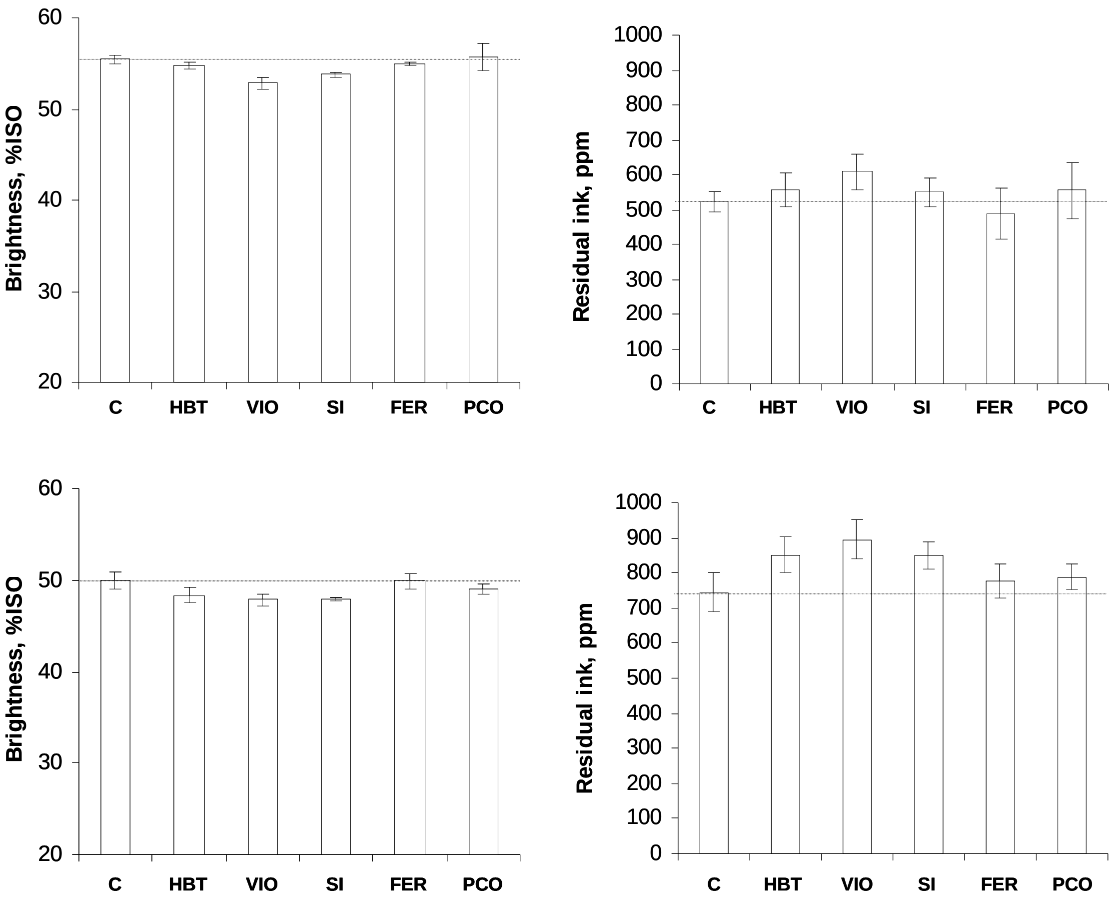

Fig 4. ISO brightness (left) and residual ink concentration (right) of deinked pulps from newspaper (top) and magazine (bottom) fibers after a laccase-mediator-containing deinking sequence (P−L−D−B) using 0.5% (referred to pulp dry weight) HBT (1-hydroxybenzotriazole), VIO (violuric acid), SI (sinapic acid), FER (ferulic acid) and PCO (*p*-coumaric acid). C (control pulp) treated under identical conditions but without enzyme and mediators. Mean values and 95% confidence limits are shown.

The secondary fibers used in this study, i.e., newspapers and magazines, with a scarce residual lignin content on which act the laccase-mediator system could explain the deinking inefficiency of this system. However, when a secondary fiber rich in lignin, such as printed cardboard, was assayed in a deinking P−L−D−B sequence, using HBT as mediator, the optical properties were enhanced (Fig. 5). Using 1.5% w/w of mediator the brightness of deinked pulp increased 3 units, reaching 39% ISO brightness, and the ink residual concentration was reduced 200 units. These results demonstrate the connection between delignification and deinking process when using the laccase-mediator system. With the removal of surface lignin from printed cardboard by laccase-mediator system, the bindings between fiber and ink particles attached of the fiber became loose, facilitating ink detachment (Bajpai and Bajpai, 1998). No improvement of the optical properties was observed when HBT concentration was increased to 3% w/w.

Further work is necessary to proceed towards the applicability of the laccase-mediator system for fiber deinking. In addition to the secondary fibers types and mediators used, other parameters could have importance in the treatment, such as the nature of different, hardly degradable, inks employed in the printing of these fiber types.

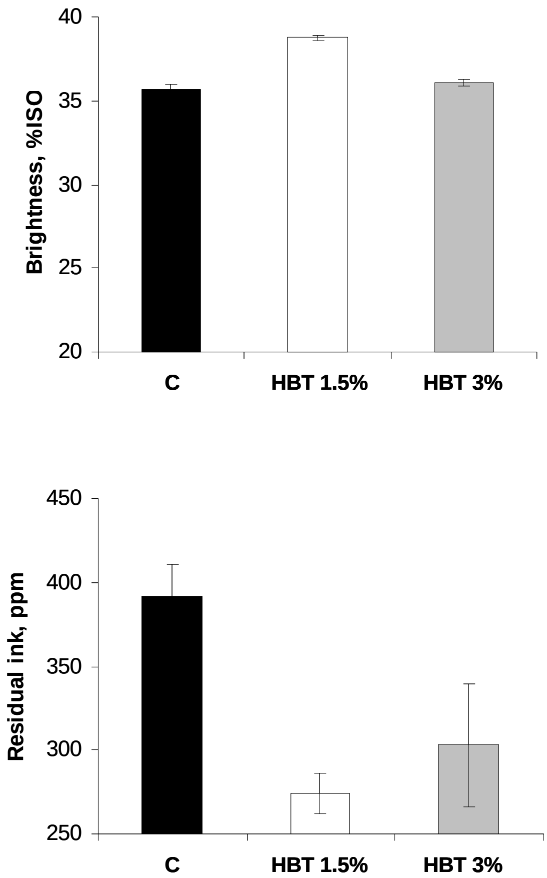

Fig 5. ISO brightness (left) and residual ink concentration (right) of deinked pulps from printed cardboard after a laccase-mediator-containing deinking sequence (P−L−D−B) using 1.5% and 3% (referred to pulp dry weight) HBT (1-hydroxybenzotriazole). C (control pulp) treated under identical conditions but without enzyme and mediator. Mean values and 95% confidence limits are shown.

3.2.2 Mechanical properties of the handsheets

Table 4 shows the mechanical properties (tensile and tear indexes) of the handsheets from deinked pulps from newspaper and magazine fibers after a laccase-mediator containing deinking sequence (P−L−D−B), and the corresponding control deinking sequence. The effect of the laccase-mediator system on these properties differed depending of the secondary fiber type and mediator used. The laccase-HBT system did not affect substantially the strength properties of the resulting deinked pulps from both newspaper and magazine fibers. However, contradictory results were observed with the other mediators used. On the one hand, significant increments of tensile and tear indexes for deinked pulps of newspaper fibers; on the other hand, important drops for deinked pulps of magazine fibers. Few studies have reported the effect of laccase-mediator system on physic-mechanical properties of fibers with scarce residual lignin content. Mohandass *et al*. (2008) described an improvement of tensile index, with a little increment of tear index, in recycled blue office paper after laccase-TEMPO treatment. In the same way, Cadena *et al*. (2010) reported increments of both indexes in ECF (elementally chlorine-free) and TCF (totally chlorine-free) bleached *Eucalyptus globulus* pulps after laccase-HBT treatment. These positive effects have been ascribed to alterations in electrokinetic properties. Changes on pulp surface charge, due to reduction of the content in ionizable groups (carboxylic and hexenuronic acids) by the laccase-mediator system, improve the physic-mechanical properties by increasing the number of bonds between fibrils with both reduced ionic charge and repulsive forces. This assumption could be accepted to explain the beneficial effects observed for the strength properties of newspaper fibers after the laccase-mediator system in the presence of violuric, sinapic, ferulic and *p*-coumaric acids. However, other reasons should be explored to explain the negative effects observed in magazine fibers.

Printed cardboard (rich in lignin) treated with laccase-HBT resulted in deinked pulps with tensile index increased up to 4% and tear index slightly enhanced (Table 4). Similar results have been often described on unbleached chemical pulps with high lignin content (Lund and Felby, 2001; Camarero *et al*. 2004a; Saparrat *et al*. 2008). They suggested that both the releasing lignin and chemically modified lignin, i.e. more hydrophilic, by the laccase-HBT system might lead to an increase in the formation of interfibrillar bonds and consequently an improvement of strength properties.

Table 4
Mechanical properties of the handsheets from deinked pulp from newspaper and magazine fibers and printed cardboard after a laccase-mediator-containing deinking sequence (P−L−D−B).

	Newspaper						Magazine						Printed cardboard	
	C[a]	HBT	VIO	SI	FER	PCO	C[a]	HBT	VIO	SI	FER	PCO	C[a]	HBT
Tensile index (Nm/g)	24.7	25.6	31.6	35	32.2	36.2	22.1	21.5	19.9	17.4	14.5	13.3	28.6	32.5
Tear index (mNm^2/g)	2.55	2.35	3.15	3.00	3.00	3.55	2.35	2.35	2.10	1.70	1.15	1.75	3.15	3.25

[a]Secondary fibers treated under the same enzymatic conditions (5 h, 50 ºC at pH 4) without laccase and mediators; HBT ,1-hydroxybenzotriazole; VIO, violuric acid; SI, sinapic acid; FER, ferulic acid; and PCO, *p*-coumaric acid.

4. Conclusion

According to this study, the effectiveness of enzymatic deinking depends to a large extent on both the different enzymatic activities selected and the secondary fiber types employed. In this sense, a great variability in the results of properties tested, i.e. brightness, residual ink concentration and strength properties, was obtained after evaluating two enzymatic deinking strategies on different types of secondary fibers. Carbohydrate hydrolases, mainly containing endoglucanase and endoxylanase activities, enhanced the optical properties of resulting deinked pulps from both newspaper and magazine secondary fibers, maintaining or slightly improving the strength properties. By contrast, the laccase-mediator system was ineffective deinking both secondary fibers, resulting in deinked pulps with worse brightness and residual ink concentration values. In addition, both significant increments and drops of physic-mechanical properties were attained in function of fibers types and mediators used. Pulp deinking by laccase-mediator system was displayed when secondary fibers rich in lignin, i.e. printed cardboard, were used, obtaining both enhanced optical and strength properties. Nevertheless, in addition to the different enzymatic activities selected and secondary fiber types used, other factors such as printing methods, ink types, and fiber/ink separation process should be taking into account in order to proceed towards the applicability of enzymatic deinking processes.

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