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Obtaining biobleached eucalyptus cellulose fibres by using various enzyme combinations

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ABSTRACT

Various combinations of laccases, xylanase and cellulase were used to biobleach cellulose fibres from eucalyptus. The *Trametes villosa* and *Myceliophthora thermophila* laccases were used in combination with violuric acid (VA_{TvL} system) and methyl syringate (MeS_{MtL} system), respectively, as mediator. A dissimilar mode of action of the two systems was found: the VA_{TvL} treatment released both hexenuronic acids and lignin, whereas the MeS_{MtL} released lignin alone. Pulp properties were further improved by applying the mediator before the enzyme during treatment. Pulp properties comparable to those provided by industrial TCF sequences were obtained by inserting a xylanase pretreatment before VA_{TvL}, but no significant effect was observed after the cellulase pretreatment. As an added value, the resulting enzymatically bleached fibres possess a reduced hexenuronic acid content. The chemical oxygen demand of the effluents from each stage was also assessed.

1. Introduction

Environmental pressure to reduce or completely suppress the formation of highly toxic organochlorine compounds (AOX) during pulp bleaching processes has led to the development of new commercial pulp types obtained with ECF (Elemental Chlorine Free) and TCF (totally chlorine free) sequences (Roncero, Torres, Colom, & Vidal, 2000). Also, the use of biotechnology to bleach pulp has attracted enormous attention and provided very interesting results so far (Fillat & Roncero, 2010; Ibarra, Camarero, Romero, Martínez, & Martínez, 2006; Martínez, Ruiz-Dueñas, Martínez, del Río, & Gutiérrez, 2009; Valls et al., 2009). The use of enzymes such as xylanases has brought about great technological improvements; in fact, xylanases boost the bleaching effects of the chemical agents used in ECF (Valls et al., 2011; Valls, Gallardo, et al., 2010) and TCF sequences (Valls, Vidal, & Roncero, 2010a). Moreover, implementing xylanase treatments in pulp mills is simple and economically feasible (Fillat, Roncero, Bassa, & Sacón, 2010). Laccases are multicopper oxidases capable of oxidizing phenolic compounds. However, using laccase in the presence of redox mediators has strongly expanded its potential for degrading lignin and other aromatic compounds. Although much research has been conducted into the effectiveness of laccase–mediator systems for delignifying and bleaching various types of paper pulp (Fillat, Colom, & Vidal, 2010; Moldes & Vidal, 2011), several issues related to the availability of laccase, the high cost of synthetic mediators and the potential toxicity of their reaction products still need be solved before these systems can be implemented on the industrial scale. 1-Hydroxybenzotriazole (HBT) has proved highly efficient in bleaching different types of pulp; also, the application conditions for the laccase–HBT system have been optimized for eucalyptus and flax fibres (Fillat & Roncero, 2009a; Valls et al., 2010a). However, other synthetic mediators such as violuric acid (VA) or *N*-hydroxyacetanilide have recently been found to also hold great promise for this purpose (Moldes, Cadena, & Vidal, 2010; Monje et al., 2010; Oudia, Queiroz, & Simões, 2008; Valls, Colom, et al., 2010). In recent years, some lignin-derived phenols obtained from black liquor of the kraft process have been proposed as mediators for biobleaching or functionalising (Camarero et al., 2007; Gutiérrez et al., 2007). Also, their effectiveness on various types of pulp has been demonstrated (Aracri & Vidal, 2011; Aracri, Valls, & Vidal, 2012; Eugenio et al., 2010; Garcia-Ubasart et al., 2011).

One interesting property of enzymes in relation to biobleaching is their ability to remove hexenuronic acids (HexA) from fibres

Abbreviations: Cel, cellulase pretreatment stage; HexA, hexenuronic acids; L, laccase–mediator stage; K_{MtL}, laccase control treatment with the *Myceliophthora thermophila* laccase; K_{TvL}, laccase control treatment with the *Trametes villosa* laccase; KN_{lig}, kappa number produced by lignin; MeS, methyl syringate mediator; MeS_{MtL}, laccase–mediator treatment with the *Myceliophthora thermophila* laccase and methyl syringate as mediator; TCF, totally chlorine free; VA, violuric acid mediator; VA_{TvL}, laccase–mediator treatment with the *Trametes villosa* laccase; X, xylanase pretreatment stage.

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(Cadena et al., 2011; Valls, Vidal, & Roncero, 2010b; Valls, Vidal, et al., 2010). HexA are formed from methylglucuronic acid present in xylans during kraft cooking (Jiang, Bouchard, & Berry, 2006) and eucalyptus pulp contains large amounts of these compounds (Cadena, Vidal, & Torres, 2010). The HexA content of fibres is important as these acids can adversely affect pulp bleachability by increasing reagent consumption and facilitating brightness reversion; also, they increase the kappa number of pulp (Cadena, Vidal, & Torres, 2010; Li & Gellerstedt, 1998).

The aim of this work was to obtain modified cellulose fibres from eucalyptus with a complete TCF biobleaching sequence ($LQPoPW_A$). Two different laccase–mediator systems (*viz. Trametes villosa* laccase with violuric acid and *Myceliophthora thermophila* laccase with methyl syringate) were used for comparison and the effects of xylanase or cellulase pretreatments on the results assessed. Although the focus was placed on the removal of HexA and lignin by the different enzyme systems, cellulose integrity and effluent COD were also examined.

2. Materials and methods

2.1. Raw material

The raw material used was oxygen-delignified eucalypt kraft pulp (*Eucalyptus globulus*) produced by the Torraspapel S.A mill in Zaragoza, Spain. Before treatment, the pulp was washed with a buffer solution consisting of 50 mM Tris–HCl buffer at pH 7 in the laboratory at room temperature for 30 min. The initial kappa number and brightness of the pulp after washing were 7.3 and 50.2% ISO, respectively.

2.2. Xylanase pretreatment (X stage)

The enzyme used in the X stage was a commercial xylanase provided by NOVOZYMES® (Pulpzyme HC). Enzymatic treatment was conducted in polyethylene bags that were placed in a laboratory water bath. The X stage involved using 3 U g^{-1} odp (oven-dried pulp) xylanase at 10% consistency and Tris–HCl buffer at pH 7 at 50 °C for 2 h, after which the treatment liquors were recovered to measure their final pH. The resulting pulp was washed with decalcified water three times and distilled water once (Valls, Gallardo, et al., 2010).

2.3. Cellulase pretreatment (Cel stage)

The cellulase used was an endoglucanase Cel9B from *Paenibacillus barcinonensis* supplied by the University of Barcelona (Chiriac et al., 2010). Enzyme treatments were conducted in polyethylene bags that were placed in a laboratory water bath; the pulp, at 10% consistency, was treated with 50 mM acetate buffer at pH 5.5 for 1 h, at 55 °C. The Cel9B rate used was 60 IU carboxymethyl cellulase activity g^{-1} odp. The resulting pulp was washed with decalcified water three times and distilled water once.

2.4. Laccase–mediator treatment (L stage)

High-redox potential laccase from the basidiomycete *T. villosa* (TvL) (NS-51002) and low-redox potential laccase from the ascomycete *M. thermophila* (MtL) were used. TvL was used in combination with the synthetic mediator violuric acid (VA_{TvL}) and MtL with the natural phenol methyl syringate (MeS_{MtL}). Both laccases and MeS were supplied by NOVOZYMES®, and VA was obtained from Sigma–Aldrich. Tests were performed by using an amount of 140 g of pulp at 5% consistency in 50 mM sodium tartrate buffer at pH 4 for TvL treatments and in 50 mM sodium phosphate buffer at pH 7 for MtL treatments. Different pHs were used since the MtL is more stable and works efficiently at higher pH (Ibarra, Romero, Martínez, Martínez, & Camarero, 2006). All treatments were carried out in a pressurized reactor (0.6 MPa) at 50 °C at 60 rpm for 4 h, using 20 U g^{-1} odp laccase and a proportion of 1.5% (w/w) of mediator. A few drops of 0.05% (w/v) of the surfactant Tween 20 were also added. Control pulp samples were processed in the absence of mediator (K_{TvL} and K_{MtL}). Laccase activity was monitored by measuring ABTS (2,2′-azinobis(3-ethylbenzthiazoline-6-sulphonic acid)) oxidation at 436 nm (ε_{436} = 29,300 M^{-1} cm^{-1}), at pH 5 and 25 °C. One unit of laccase was defined as the amount of enzyme oxidizing 1 μmol of substrate min^{-1}. The L stage was always followed by thorough washing of the pulp.

2.5. Complete bleaching sequence

Different biobleaching sequences were performed with or without enzymatic pretreatment: $XLQPoPW_A$, $CelLQPoPW_A$ and $LQPoPW_A$. The chelant stage (Q) was performed with 0.3% odp DTPA (diethylenetriaminepentaacetic acid), at 10% consistency, 85 °C and pH 5–6 (adjusted with H_2SO_4 1 N) during 1 h at 30 rpm. The pressurized peroxide bleaching stage (Po) was carried out with 1.5% odp NaOH, 2% odp H_2O_2, 0.5% odp Na_2SiO_3, and 0.2% odp $MgSO_4$ at 10% consistency, 105 °C and 0.6 MPa O_2 during 2 h at 30 rpm. The peroxide bleaching stage (P) was carried out with 1.5% odp NaOH, 1% odp H_2O_2, 0.5% odp Na_2SiO_3, and 0.2% odp $MgSO_4$ at 10% consistency, 98 °C during 2 h at 30 rpm. In order to neutralize the high pH of the pulp avoiding undesirable ageing of pulp, after the biobleaching sequence the pulp was washed in acidic medium (W_A) at pH 2 (distilled water adjusted with H_2SO_4 1 N) at 2.5% consistency during 30 min (Aracri & Vidal, 2011; Fillat, Roncero, & Vidal, 2011).

2.6. Pulp properties

Treated pulp samples were characterized in terms of kappa number, and brightness according to ISO 302 and ISO 2470-1, respectively. The kappa number was measured two times and brightness four in order to calculate a standard deviation, which was found to be 0.1 for both properties. Viscosity of bleached fibres was performed by duplicate according to ISO-5351-1. The hexenuronic acid (HexA) content was determined according to Chai, Zhu, and Li (2001). An estimate of the actual lignin content of the pulp was obtained by determining the kappa number due to lignin (KN_{lig}). This involved measuring kappa number following removal of HexA by acid hydrolysis with mercury acetate and efficient washing with distilled water. From these values, the actual degree of delignification was calculated.

Bleached pulps were also characterized by quantitative acid hydrolysis with 72% sulfuric acid (TAPPI T13m method) and the resulting hydrolysates were analyzed for monosaccharides (glucose coming from cellulose, and xylose coming from hemicelluloses) by HPLC (Valls, Gallardo, et al., 2010).

2.7. Effluent properties

The chemical oxygen demand (COD) was calculated in accordance with ASTM D1252-00.

3. Results and discussion

In this work, we assessed the removal of lignin and hexenuronic acids during $LQPoPW_A$, $XLQPoPW_A$ and $CelLQPoPW_A$. TCF biobleaching sequences involving two different commercial laccases, one from *T. villosa* that was used in combination with the synthetic mediator violuric acid (VA) and the other from *M. thermophila* that was applied with the inexpensive natural phenol

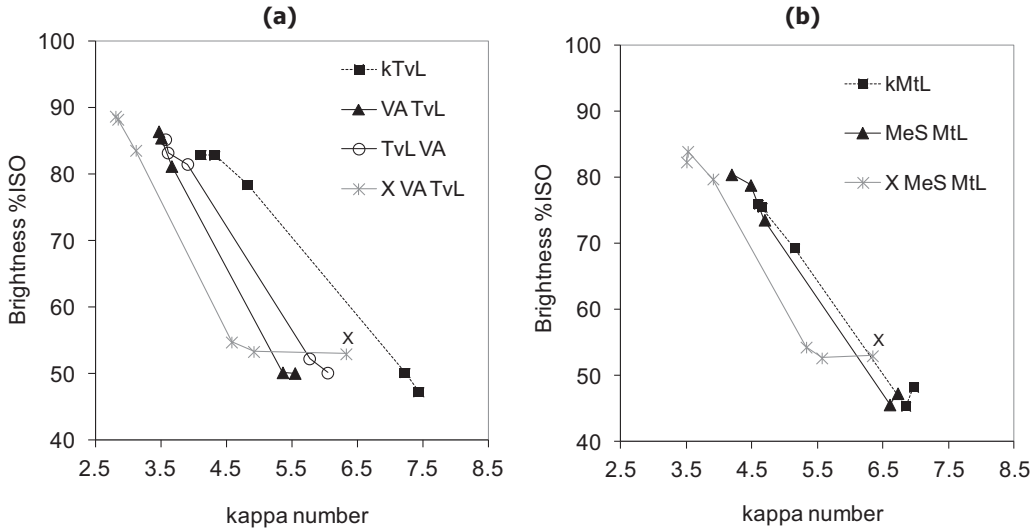

Fig. 1. Evolution of kappa number *vs.* brightness during the different stages of the LQPoPW$_A$ and XLQPoPW$_A$ bleaching sequence with the *Trametes villosa* (a) or *Myceliphthora thermophila* (b) laccases. Effect of control treatment with laccase and without mediator (K_{TvL} and K_{MtL}); effect of the addition of VA mediator before or after the laccase (VA_{TvL} or TvL_{VA}); effect of the addition of MeS mediator (MeS_{MtL}) and effect of a xylanase pretreatment stage (XVA_{TvL} and $XMeS_{MtL}$).

methyl syringate (MeS). Cellulose integrity in the resulting fibres was also analyzed and effluent COD measured.

The main difference of both laccases was its redox potential. The basidiomycete fungi *T. villosa* produces a high-redox potential laccase whereas the ascomycete *M. thermophila* produces a commercially available laccase with a low-redox potential. Due to its low redox potential, the MtL is unable to oxidize high redox potential mediators like HBT (Fillat, Prieto, Camarero, Martínez, & Martínez, 2012; Ibarra, Romero, et al., 2006). Since a successful use of syringyl-type phenolics as mediators of *M. thermophila* laccase has been reported (Babot et al., 2011; Fillat et al., 2011) the MtL was applied with methyl syringate.

3.1. Effects on kappa number and brightness

We assessed the effect of adding the mediator before (VA_{TvL}) or after (TvL_{VA}) the enzyme, and also the effect of a xylanase pretreatment stage (XVA_{TvL}), during the treatments with the TvL–VA combination (Fig. 1a). The control sequence with laccase but no mediator (K_{TvL}) reduced the kappa number and increased brightness slightly during the chelating stage (Q)—which was intended to remove metals potentially inactivating the subsequent alkaline stage with hydrogen peroxide (Po). Brightness significantly increased (28%ISO) and the kappa number decreased (2.4 units) during the hydrogen pressurized stage (Po). Both pulp properties were also improved, albeit to a lesser extent than with Po (4.4%ISO and 0.5 units, respectively), during the second P stage. Finally, the final acid wash (W_A), which was intended to preserve the optical properties of the pulp until analysis, had little effect.

The presence of VA in the L stage introduced significant differences. Also, the laccase–mediator system performed better if the mediator was added before the enzyme (VA_{TvL} sequence). If the laccase was added before the mediator (TvL_{VA}) the enzyme could start oxidizing the pulp lignin diminishing the subsequent oxidation and effect of the mediator. The enzymatic stage VA_{TvL} reduced the kappa number and increased brightness by 2 units and 3%ISO, respectively, with respect to the control. The biobleaching sequence (LQPoPW$_A$) with this enzymatic stage reduced the kappa number to a lesser extent (0.6 units) and improved brightness 3.5%ISO with respect to the control sequence. As can be seen from Fig. 1a, the xylanase pretreatment had a strongly favourable effect. In fact, the final bleached fibres obtained with the two enzyme treatments (XLQPoP sequence) had smaller kappa numbers and higher brightness (0.7 units smaller and 2.3%ISO higher, respectively) than the enzymatically bleached fibres in the absence of xylanase.

The previous sequence was also performed with MtL as enzyme and MeS as mediator (Fig. 1b). The enzyme stage using the mediator MeS (MeS_{MtL}), reduced the kappa number slightly (0.2 units) and decreased brightness relative to the control treatment (K_{MTL}). The effects peaked at the end of the bleaching sequence, where the kappa number was 0.5 units smaller and brightness 5%ISO higher with the MeS_{MtL} treatment. A similar effect of this laccase–mediator system was recently observed in unbleached eucalyptus and flax fibres (Babot et al., 2011; Fillat et al., 2011). However, a complete bleaching sequence with MtL and MeS had never previously been applied to oxygen-delignified eucalyptus pulp. The beneficial effect of xylanase on fibres was also apparent: the kappa number was reduced by 0.7 units and brightness increased by 1.9%ISO with $XMeS_{MtL}$ relative to MeS_{MtL}.

3.2. Effects on hexenuronic acids (HexA) and lignin

The hexenuronic acid content was measured both after the laccase enzyme stage and in bleached fibres (Fig. 2). HexA decreased during the control treatments with both laccases (K_{TvL} and K_{MtL}). This was a result of the acid application conditions used (Valls et al., 2010b) and also of the effect of the laccases on these compounds (Aracri & Vidal, 2011; Fillat et al., 2011). A further reduction of HexA was obtained by including VA in the laccase treatment (20% with respect to the initial pulp), which testifies to the efficiency of the VA_{TvL} system in removing these compounds. However, no further HexA were removed by the MeS_{MtL} system relative to K_{MtL}.

Substantial HexA removal (30%) was observed during the xylanase enzyme stage by effect of the elimination of xylans (Valls, Vidal, et al., 2010). As a result, xylanase-pretreated fibres (XAV_{TvL} and $XMeS_{MtL}$) contained less HexA (10 μmol g^{-1} odp less than with the AV_{TvL} and MeS_{MtL} treatments).

The final bleached fibres contained large amounts of HexA (20–30 μmol g^{-1} odp HexA) since the QPoPW$_A$ treatments removed HexA only slightly (15%). In fact, this is one of the main problems encountered in using TCF bleaching sequences to replace existing ECF sequences; thus, TCF reagents have no effect on HexA, whereas chlorine dioxide efficiently removes them from fibres (Ventorim, Colodette, Gomes, & da Silva, 2008). HexA accounted

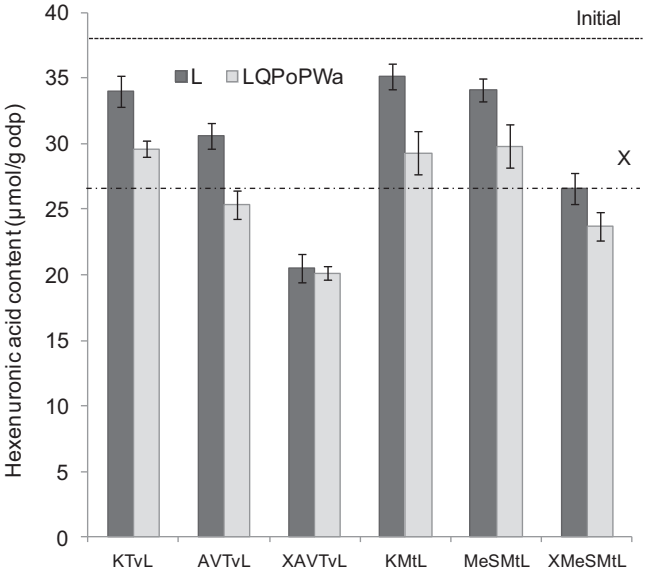

Fig. 2. Hexenuronic acid content of fibres after L stage and in bleached eucalypt fibres treated with different enzymatic treatments.

for 30–50% of the kappa number after the L stage, and up 65–90% in the bleached fibres. Bleached pulp with a high HexA content may undergo brightness reversion as a result of the instability of double bonds in the acids (Sevastyanova, Li, & Gellerstedt, 2006). As recently shown, however, the phenomenon can be lessened by a laccase–mediator treatment for bleached pulp (Cadena et al., 2010a).

The substantial contribution of HexA to the kappa number led us to determine the fraction due to lignin (KN_{lig}, Fig. 3) in order to assess the actual delignifying efficiency of the treatments. The K_{TvL} treatment resulted in 18% delignification and the addition of VA increased it to 40%. The control treatment with MtL (K_{MtL}) caused only slight delignification (4%) that increased with the addition of MeS—to a similar level as with VA_{TvL}, 40%. Bleached fibres were delignified by about 80% with VA_{TvL} and 90% with MeS_{MtL}. Therefore, based on the actual degree of fibre delignification, the MeS_{MtL}

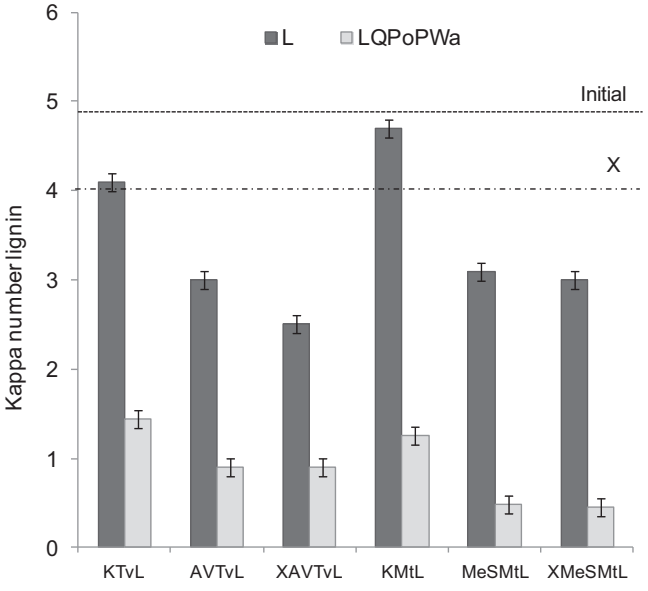

Fig. 3. Kappa number produced by lignin (KN_{lig}) of fibres after L stage and in bleached eucalypt fibres treated with different enzymatic treatments.

Table 1
Viscosity (mL g^{-1}) and glucose and xylose percentages of different eucalypt fibres bleached according to the $LQPoPW_A$ sequence with different enzymatic treatments.

	Viscosity (mL g^{-1})	Glucose (%)	Xylose (%)
Initial	936 ± 20	76.1 ± 0.6	14.2 ± 0.4
X	1033 ± 20	85.4 ± 0.2	13.4 ± 0.0
K_{TvL}	570 ± 40	78.9 ± 0.4	14.5 ± 0.1
VA_{TvL}	563 ± 30	81.5 ± 0.6	15.6 ± 0.3
XVA_{TvL}	401 ± 20	85.4 ± 0.4	12.8 ± 0.1
K_{MtL}	552 ± 50	85.0 ± 0.1	15.4 ± 0.2
MeS_{MtL}	505 ± 1	85.5 ± 1.1	15.6 ± 0.0
$XMeS_{MtL}$	457 ± 16	81.7 ± 0.42	9.5 ± 0.0

system is seemingly more efficient than the VA_{TvL} system—the effect, however, did not reflect in the total kappa number, which can be ascribed to HexA being released by VA_{TvL} but not by MeS_{MtL}. Thus, the VA_{TvL} system released both HexA and lignin, whereas the MeS_{MtL} system only removed lignin. Consequently, the MeS_{MtL} treatment removed greater amount of lignin.

The effect of a xylanase pretreatment on KN_{lig} was only appreciable after the enzyme stage in the XAV_{TvL} sequence; on the other hand, the pretreatment had no effect on delignification in bleached fibres. Therefore, the xylanase pretreatment boosted the removal of HexA without affecting lignin in the fibres, as previously found by Valls et al. (2010b).

3.3. Effects on fibre polysaccharides

The effects of the treatments on fibre polysaccharides in bleached fibres were also examined (Table 1). Cellulose integrity was assessed *via* viscosity measurements. Viscosity in bleached fibres was reduced as the likely result of the formation of radicals during the hydrogen peroxide stages and their reaction with carbohydrates (Ferrer, Rosal, Valls, Roncero, & Rodríguez, 2011). A similar reduction in viscosity was previously observed in flax and sisal fibres (Aracri & Vidal, 2011; Fillat et al., 2011). However, viscosity was not significantly affected by the presence of the mediator in the laccase treatments. The X pretreatment increased pulp viscosity by effect of the removal of low-molecular weight xylans (Roncero, Colom, & Vidal, 2003). By contrast, the laccase–mediator systems used in conjunction with a xylanase pretreatment caused a slight reduction in viscosity. This is an unusual occurrence with xylanase pretreatments, but can be ascribed to easier penetration of the reagent after xylans are removed from fibre surfaces.

Bleached fibres contained more glucose than the initial pulp, probably as a result of the lower lignin content of the fibres (Table 1); however, using a mediator in the laccase control treatments had no significant effect on the glucose content of the fibres. The xylanase pretreatment also reduced the proportion of xylose, which confirms that the enzyme acts on xylans and causes their hydrolysis. Moreover, the xylanase pretreatment boosted the removal of xylans during the L stage by facilitating access of the enzyme and reagents after xylans were removed from fibre surfaces.

3.4. Effects on the chemical oxygen demand (COD) of the effluents

COD in the effluents from the different bleaching stages was also examined (Table 2). In the control sequence, COD increased mainly during the enzyme stage (K_{TvL}), the Q stage having little effect on this property. Both peroxide stages had a similar effect on COD, which, however, was unaffected by the presence of the mediator (VA_{TvL} sequence). Therefore, effluent COD after the enzyme stage was the result of the presence of sodium tartrate buffer and the commercial enzyme preparation, specifically, by the additives used to maintain its activity (Fillat & Roncero, 2009b). COD was strongly

Table 2
COD (kg O_2/T pulp) produced during the different stages of the bleaching sequence XLQPoP.

	X	L	Q	Po	P	Accumulated
K_{TvL}	–	124.9 ± 2.2	7.4 ± 0.1	22.0 ± 0.5	23.9 ± 1.7	178.2
VA_{TvL}		102.9 ± 2.6	4.6 ± 0.7	20.9 ± 1.6	22.6 ± 1.3	151.0
XVA_{TvL}	200.2 ± 9.6	101.4 ± 3.6	2.8 ± 0.5	21.8 ± 1.4	21.7 ± 0.2	347.9
K_{MtL}	–	26.5 ± 3.5	3.5 ± 0.5	23.2 ± 1.0	18.7 ± 1.6	71.9
MeS_{MtL}	–	40.7 ± 2.4	4.3 ± 1.5	24.3 ± 2.4	20.0 ± 1.3	89.3
$XMeS_{MtL}$	200.2 ± 9.6	48.4 ± 3.1	4.0 ± 0.4	22.2 ± 4.0	21.7 ± 0.6	296.5

increased by the XVA_{TvL} sequence owing to the xylanase, which produced 60% of all cumulative COD. This result can also be ascribed to the buffer, and also to substantial carbohydrate degradation and lignin removal during this stage, which testifies to the efficiency of the enzyme. Recently, it has been shown that xylan solubilized by a xylanase treatment diminishes pulp yield (Fillat, Roncero, Sacón, & Bassa, 2012). The sequences using MtL raised effluent COD less markedly by effect of the lesser effect of the enzyme stage on this property, and a result of the presence of sodium phosphate buffer. However, COD was increased by the presence of MeS, its degradation products and those of lignin (Aracri & Vidal, 2011; Fillat et al., 2011). We can therefore partially ascribe the high COD values produced by the enzyme to the buffer used (Fillat, Colom, et al., 2010), which can always be replaced with one contributing less markedly to effluent COD.

3.5. Effect of a cellulase pretreatment stage (Cel)

Like xylanases, cellulases are hydrolytic enzymes and have proved effective in altering fibre morphology. In fact, they are known to act on the surface and inner layers of cellulose fibres in an efficient enough manner to allow the production of special paper with reduced energy consumption (Cadena, Chiriac, et al., 2010). Enzymatic effects on fibre morphology can lead to improved fibre–fibre bonding in the refining process and hence to increased fibre cohesion in the final paper. In this work, the effects of inserting a pretreatment stage with cellulase in the TCF $CelLQPoPW_A$ sequence using the VA_{TvL} system on various pulp properties were assessed and compared with those of $LQPoPW_A$ and $XLQPoPW_A$ sequences. The cellulase pretreatment failed to reduce the kappa number and diminished brightness at the end of the sequence relative to the absence of pretreatment (Fig. 4). However, it reduced the amount of HexA after L and in the bleached fibres (Table 3), probably by reducing that of xylose. Cel9B is an endoglucanase and may have also removed some xylans with HexA during the hydrolysis of glucose. No significant effects on pulp lignin, viscosity or glucose content were observed in relation to the sequence without pretreatment. As can be seen from Fig. 4 and Table 3, xylanase performed better than cellulase.

In contrast with the hydrolytic enzyme xylanase, the hydrolytic enzyme cellulase was not efficient in boosting bleaching; however, it decreased the HexA content of the fibres.

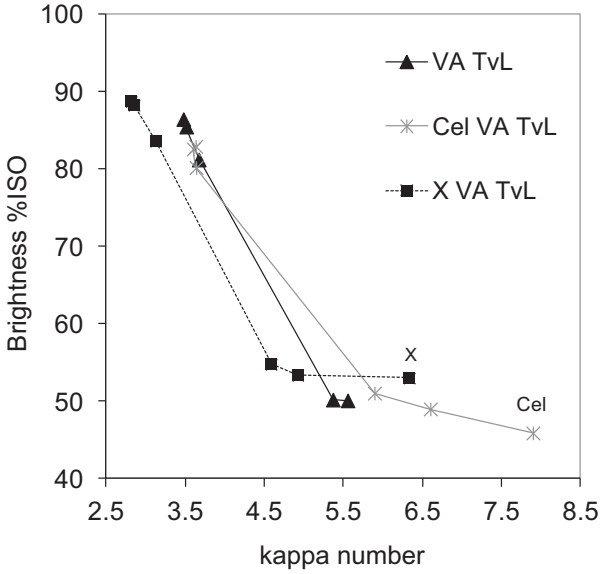

Fig. 4. Effect of cellulase pretreatment ($CelVA_{TvL}$) in the evolution of kappa number *vs.* brightness during the different stages of the $LQPoPW_A$ bleaching sequence using the *Trametes villosa* laccase (TvL) and violuric acid (VA). Comparison between the same sequence without enzymatic pretreatment (VA_{TvL}) and with a pretreatment stage with xylanase (XVA_{TvL}).

3.6. Comparison of biobleached fibres with commercial bleached fibres

Table 4 compares the final properties of the bleached fibres obtained with our treatments with those of fibres bleached by using industrial TCF and ECF sequences. As can be seen, the brightness values provided by xylanase in combination with the VA_{TvL} system were similar to those of the industrial fibres: 89%ISO. The other enzyme treatments provided also high brightness (80–86%ISO). As can also be seen from the table, the main shortcoming of TCF bleached fibres is their high content in HexA, which are inefficiently removed during peroxide and oxygen stages relative to chlorine dioxide in ECF sequences (Cadena et al., 2010b; Valls, Gallardo, et al., 2010). Therefore, the large kappa number of the TCF industrial fibres (5.0) was due mainly to HexA. It should be noted that all enzymatically treated fibres had a smaller kappa number than the

Table 3
Pulp properties after L stage or in bleached fibres after a cellulase pretreatment stage ($CelVA_{TvL}$). Comparison with the pulp properties obtained with a xylanase pretreatment stage (XVA_{TvL}) or without pre-treatment (VA_{TvL}).

	Stage	$CelVA_{TvL}$	XVA_{TvL}	VA_{TvL}
HexA (μmol g^{-1} odp)	L	26.0 ± 1.2	20.5 ± 1.1	30.6 ± 1
	LQPoP	22.2 ± 0.5	20.1 ± 0.5	25.3 ± 1.1
KN_{lig}	L	3.4 ± 0.1	2.5 ± 0.1	3 ± 0.1
	LQPoP	0.6 ± 0.1	0.9 ± 0.1	0.9 ± 0.1
Viscosity (mL g^{-1})	LQPoP	512 ± 20	401 ± 20	563 ± 30
Glucose (%)	LQPoP	84.51 ± 0.06	85.37 ± 0.45	81.5 ± 0.60
Xylose (%)	LQPoP	14.54 ± 0.01	12.82 ± 0.07	15.61 ± 0.3

Table 4
Final pulp properties of the bleached fibres according to the LQPoP or XLQPoP TCF enzymatic sequences and comparison of the same properties of fibres treated according to TCF and ECF industrial sequences (Cadena et al., 2010b).

	Brightness (%ISO)	Kappa number	Kappa number lignin	HexA (μmol g^{-1} odp)
VA_{TvL}	86.5 ± 0.1	3.5 ± 0.1	0.9 ± 0.1	25.3 ± 1.1
XVA_{TvL}	88.7 ± 0.1	2.8 ± 0.1	0.9 ± 0.1	20.1 ± 0.5
MeS_{MtL}	80.5 ± 0.1	4.2 ± 0.1	0.5 ± 0.1	29.8 ± 1.6
$XMeS_{MtL}$	82.3 ± 0.1	3.5 ± 0.1	0.5 ± 0.1	23.7 ± 1.1
Industrial TCF	89.0 ± 0.1	5.0 ± 0.1	1.1 ± 0.1	35.4 ± 1.1
Industrial ECF	89.5 ± 0.1	0.5 ± 0.1	–	0.2 ± 0.1

industrial TCF fibres. This can be ascribed to the lower HexA content of all enzymatically treated fibres. Whereas the pulp samples treated with *T. villosa* laccase exhibited no significant differences in lignin content from the industrial fibres, those treated with *M. thermophila* laccase had lower contents. Based on their low HexA contents, all enzymatically bleached fibres can be expected to be less prone to brightness reversion—which constitutes an added value for these fibres. In fact the HexA content was 45% lower with the $XTvL_{VA}$ sequence than with the industrial TCF sequence.

4. Conclusions

Two oxidoreductases (laccases) and two hydrolases (xylanase and cellulase) were applied to eucalyptus pulp in order to obtain biobleached cellulose fibres with a complete TCF sequence. The two laccases, from *T. villosa* (TvL) and *M. thermophila* (MtL), where used in combination with violuric acid (VA) and methyl syringate (MeS), respectively, as mediator. The two systems were found to act differently on fibres. Thus, the VA_{TvL} system released both lignin and hexenuronic acids from fibres, thereby increasing brightness during the $LQPoPW_A$ sequence; on the other hand, the MeS_{MtL} system only removed lignin, which increased its effective delignification capacity. Using the mediator before the laccase during the treatments was found to increase the efficiency of the system. Concerning hydrolases, a significant bleach boosting effect was obtained with the xylanase pretreatment stage, but no improvement was observed with cellulases. However, both hydrolases (but, especially, xylanase) removed HexA from the fibres. Fibre polysaccharides where not significantly affected by the enzyme treatments and effluent COD was increased mainly by the buffer used in the treatments. Using enzymes including xylanase in combination with the VA_{TvL} system provided bleached pulp similar in properties to TCF bleached fibres and the added value of containing smaller amounts of hexenuronic acids.

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