Biobleaching of high quality pulps with laccase-mediator system: Influence of treatment time and oxygen supply.

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Abstract

In this study we examined the influence of the treatment time and addition of oxygen on the efficiency of a laccase mediator system (L) applied to flax pulp at atmospheric pressure. The redox potential and the dissolved oxygen concentration during L tests are measured. After L stage, an alkaline extraction (E) is carried out. The pulp properties (kappa number, brightness and viscosity) and the effluents properties (color and COD) were measured in order to evaluate the environmental impact of this enzymatic treatment.

The biotreatment involves two distinct stages in both L and LE sequences; in the beginning the pulp exhibits a fast delignification and a slow viscosity decrease that is followed by slow delignification in the second. Pulp brightness changed differently during L stage and LE sequence. Initially brightness after the L stage decreased with respect to the initial pulp; then, it increased rapidly and eventually leveled off. After the LE sequence, brightness increased rapidly in the beginning and more gradually afterwards. The results show that supplying the medium with oxygen and increasing the oxygen concentration in it, influence the kinetics of the process.

Based on CIE L*a*b* color coordinates study, the enzyme treatment not only removes lignin, but also alters the structure of the pulp by causing the formation of chromophoric groups giving color. Such groups are removed in an E stage.

Keywords

Delignification, effluents, flax pulp, 1-hydroxybenzotriazole, laccase, optical properties, oxygen, reaction time, CIE L*a*b* coordinates, COD, color, viscosity, chromophore.

Introduction

The use of enzymes appears as a promising approach for clean bleaching process. In fact, xylanases have already demonstrated their effectiveness on the biobleaching procedures [1]. Some oxidative enzymes, such laccases, have provided an alternative to xylanases acting directly on lignin. However, this process is not fully developed due to the availability of efficient and stable in industrial conditions enzymes, the high cost and potential toxicity of the mediators and the long reaction times involved [2, 3]. Moreover, oxidative enzymes have scarcely been used to bleach non-wood pulp [4-8]; also, most kinetic studies on their effects have focused on the action of laccase mediator systems (LMS) in reactions with lignin model compounds for short times [9, 10]. In fact, studies on pulp delignification with LMS have largely failed to examine cellulose degradation via viscosity measurements; also, no COD or color data for the resulting effluents have seemingly been reported previously. Characterizing such effluents is important on account the ability to close circuits in TCF (Totally Chlorine Free) sequences.

Previous studies on the influence of the treatment time with a LMS on the extent of pulp delignification on reactions with lignin model compounds or eucalyptus kraft pulp revealed that the process involves two distinct stages [10, 11, 12]. In the initial stage, the pulp is rapidly delignified to a limiting kappa number similarly as in ozone-based treatments [13, 14]; in the final stage, delignification is slow but the oxygen uptake continues to be high, which suggests the presence of active chemical species in the system not reacting with residual lignin, but rather interacting with lignin fragments via side reactions [9, 10].

Some authors have found raising the oxygen pressure in the reactor to result in increased delignification of the pulp [12, 15, 16]. Also, using a pressurized reactor was found to result in

increased delignification and decreased viscosity in LP flax pulp relative to using the reactants in a flask [7, 8]. However, the influence of supplying the medium with oxygen or the dissolved oxygen concentration on flax pulp delignification and optical properties, as well as effluent properties, with a LMS has never to date been studied. An atmospheric delignification system would allow the application of LMS system in different points of the bleaching sequence without carrying important changes in the process.

The purpose of this work was to identify and quantify the variables influencing the kinetics of the process with a view to helping optimize industrial biobleaching treatments. Specifically, the performance of a laccase mediator treatment (L) in the biobleaching of flax pulp was examined and the influence of the treatment time and of supplying the medium with oxygen on various properties of the resulting pulp (kappa number, brightness and viscosity) and effluents (color and COD) was studied. The CIE L*a*b* color coordinates were also calculated. The redox potential of the system and the dissolved oxygen concentration were measured, which required designing appropriate equipment for biobleaching at atmospheric pressure.

Experimental

Raw material

The unbleached flax pulp used was supplied by CELESA mill (Spain) and obtained by soda-anthraquinone (NaOH-AQ) chemical cooking. Before the bleaching study the pulp was acidified in order to remove residual liquor not eliminated by industrial washing and to bring the pulp to the pH required for the L stage. The properties of the washed pulp were 10.1 of kappa number, 35.5 %ISO of brightness and 952 $mL \cdot g^{-1}$ of viscosity.

Laccase-mediator treatment (L)

The enzyme used in this study was laccase from *Trametes villosa* available from Novozymes® (Ref. NS-51002; 0NN002; FS-002). Its initial activity was 47 $U \cdot mL^{-1}$ (one activity unit was taken to be the amount of enzyme needed to convert 1 mmol of the substrate ABTS per minute). The

mediator used was 1-hydroxybenzotriazole (HBT) from Fluka (Ref. 54802, grade: purum).

Treatments with the laccase mediator system were performed with 10 g of pulp at 1.5 %odp consistency in 50 mM sodium tartrate buffer (tartaric acid form Merck, Ref. 818531, for synthesis) at pH 4 containing the surfactant Tween 80 from Sigma (Ref. P1754) at atmospheric pressure. Tests were conducted in tall 1 L beakers at 30 ºC and open at the top (i.e. at atmospheric pressure). Fiber suspensions were used to measure redox potentials and dissolved oxygen concentrations. The L stage was applied in three different ways, namely: in the absence of gas (Ls), by bubbling air (Lair) and by bubbling oxygen (Lo) through the medium. The laccase dose was 25 $U \cdot g^{-1}$ and the HBT dose 2.6 %odp. The treatment time ranged from 0.5 to 30 h. Once treated, the pulp was filtered and residual liquor collected for subsequent analysis.

Alkaline extraction stage (E)

The L samples were subjected to an alkaline extraction stage in an Easydye AHIBA oscillating individual reactor from Datacolor. Treatments were done on 5 g of pulp at 5 %odp consistency. The operating conditions were: 1.5 %odp NaOH from Panreac (Ref. 211687, QP), 90 ºC and 120 min.

Pulp and effluent properties

The pulp samples from L and LE sequences were characterized for brightness, kappa number and viscosity according to the applicable ISO standards, ISO 2470, ISO 302 and ISO 5351-1, respectively. Determinations also included CIE L*a*b* color coordinates. The effluents from the L stage were characterized for color and COD in accordance with ASTM D1252-00 and ASTM D1209-00, respectively. All chemicals reagents were of analytical grade from Panreac and Merck.

Results and discussion

Dissolved oxygen concentration

The variation of the dissolved oxygen concentration during the tests involving the addition of air

(Lair) and no gas (Ls) was measured (data not shown). The oxygen concentration in the Lo test exceeded 20 ppm, which was beyond the measuring range of our oxygen meter. The oxygen concentration in Ls was 7 ppm prior to addition of the reagents, but immediately started to fall and reached 4 ppm after 40 min of treatment; beyond that point, the oxygen concentration rose to 5.5 ppm at 5 h and 6.7 ppm at 24 h. The initial oxygen concentration in Lair was 7.5 ppm; after the reagents were added, it decreased to 7 ppm, where it levelled off. Then, it rose to 8.5 ppm at 4 h and leveled off until the end of the test (9 h). The more oxygen is supplied to a medium, the higher is the dissolved oxygen concentration to be expected. The oxygen concentration measured during the treatments was found to depend on the way the gas was supplied and changed with time. Thus, in Ls and Lair, the oxygen concentration decreased from its initial value over the first few minutes of reaction and then increased to a constant level that was reached after 4 h of treatment.

Variation of the kappa number

No significant differences between treatments were observed during the first 3 h in kappa number after L stage (Fig. 1). At longer times, however, delignification was higher by 3 to 11 % with Lair than with no gas (Ls). The differences between Lair and Lo were less substantial and in favour of the latter. The smallest kappa number obtained with L stage was 6.0, which corresponded to 41 % delignification and was provided by Lo at the longest treatment time studied (30 h).

In LE sequence, the kappa number decreased with respect to the initial pulp from the beginning. The effect was similar to that of the L stage alone: rapid delignification during the first 5 h of treatment, by up to 40 % within the first 30 min (Fig. 1), and then slower delignification to a limiting kappa number. The smallest kappa number obtained with LE sequence was 3.1, which corresponded to 70 % delignification and was provided by Lo at 30 h (the longest treatment time). The delignification was stronger throughout LairE and LoE than with LsE, which contradicts the results obtained after L. The limiting kappa number, 4.5, was obtained after 30 h in LsE and only 9 h in LoE. The differences in delignification efficiency between LairE and LoE were less significant than those of LsE with LairE and LoE at long treatment times; however, delignification was slightly more marked with LoE. Oudia et al. (2008) reported delignification increased from 38.9 % with air

to 47.9 % with oxygen at 11 bar on eucalyptus kraft pulp and there was a significantly different effect of oxygen under pressure from the atmospheric air environment until they reached 5 bar.

The fact that a limiting kappa number was reached beyond which increasing the treatment time resulted in no further delignification, was previously observed by other authors [7, 9] and reflects that lignin in the pulp can only be partially removed in a single enzyme-based stage. This may be the result of the enzyme losing some activity at the end of the treatment [17, 18], HBT being oxidized to its inactive form [19, 20], highly reactive oxidized lignin fragments accumulating in the medium or the chemical reagents diffusing in the pulp fibers.

The presence of increased amounts of oxygen (4 to 7 ppm) during the laccase mediator treatment resulted in more efficient oxidation of lignin in the pulp and hence in kappa numbers smaller by 1.2 units in the Lair and Lo treatments than with no oxygen added. Also, the presence of increased amounts of oxygen resulted in a slightly increased initial slope in the curve reflecting the decrease in kappa number with the treatment time in Lo with respect to Lair. The limiting kappa number was virtually identical with both treatments; as a result, increasing the oxygen concentration in the medium above 7 ppm accelerated delignification of the pulp, albeit only slightly. The fact that an increased amount of oxygen in the medium reduced the limiting kappa number (and the amount of residual lignin in the pulp) suggests that such a number must be closely related to physical factors governing the diffusion of reactants in the pulp.

The primary effect of alkaline extractions in industrial bleaching processes is dissolving lignin by ionizing acid groups formed in it in previous stages. As can be seen from Fig. 1, the E stage resulted in increased delignification with respect to L. The curves representing the variation of the kappa number with time were similarly shaped for all treatments, both after L and after LE.

Application of an E stage to the initial pulp (in the absence of L) reduced the kappa number by 1.8 units. Therefore, a fraction of lignin present in the initial pulp was dissolved during the alkaline extraction stage. If the L stage was performed, then the kappa number after LE exceeded that obtained after L in 1.8 units throughout the treatment (Fig. 1). Therefore, the L stage alters lignin in

such a way that the polymer becomes insoluble at pH 4 during L, and also at neutral pH in the subsequent washings, but is dissolved in the alkaline extraction stage. The laccase mediator system changes the ratio between lignin syringylpropanoid (S) to guaiacylpropanoid (G) units (S/G ratio) [7] and causes the formation of carbonyl and carboxyl groups in lignin [21] which increases the hydrophilicity of lignin and facilitates its dissolution in a subsequent alkaline stage ; this entails using an alkaline extraction stage after L in order to extract the modified lignin produced by the enzyme system. The LoE sequence was that reducing the kappa number to the greatest extent at short treatment times of L in the LE sequence (Fig. 1); therefore, the presence of increased amounts of oxygen in the medium increases the ability of the laccase mediator system to modify lignin in the pulp to a greater extent than it increases the ability of the enzyme treatment to remove the polymer.

Variation of brightness

After L stage, brightness initially decreased with respect to the initial pulp, then rose after 2.5 h of treatment and, finally, leveled off at a value exceeding that for the initial pulp after 3 h (Fig. 2). No appreciable differences in brightness between Ls, Lair and Lo were observed in the first 5 h of treatment. After 16 h, however, Lo resulted in the highest brightness values, followed by Lair. The brightness differences between the Ls and Lair treatments, and those between the Lair and Lo post-treatments were in the region of 2 %ISO units. Although the kappa numbers obtained with Lair and were similar, the Lo pulp had higher brightness.

After LE sequence, brightness increased relative to the initial pulp from the beginning of the treatment (Fig. 2). Thus, it rose rapidly during the first 5 h, peaked at 16 h of treatment and then leveled of until the end (30 h). As with the kappa number, the processes exhibited two distinct stages. In the first (0 to 5 h), brightness increased rapidly (by about 10 %ISO units); in the second, brightness rose in a more gradual manner to its peak value. Similarly to the L stage, brightness differences between LsE, LairE and LoE at the beginning of the treatment were insubstantial; at long times, however, LoE provided the highest brightness levels, followed by LairE. In any case, differences in brightness between LE sequences only became apparent after 4 h and exceeded

those observed between the L stages. The differences between the LsE and LairE treatments, and those between the LairE and LoE post-treatments, ranged from 2 to 5 %ISO units.

As can be seen from Fig. 2, the E stage increased pulp brightness relative to L. Brightness varied with the treatment time in L and LE. Thus, the LsE, LairE and LoE sequences increased it to 38, 41 and 51 %ISO, respectively, which are 12, 14 and 17 %ISO units higher than the values for the respective L stages. As noted earlier, the brightness differences between the initial pulp and E pulp was 5 %ISO units. Also, pulp brightness after LE was 5 %ISO higher than after L throughout the treatment; therefore, the L stage must alter the lignin structure in such a way that the subsequent E stage raises its brightness.

According to some authors, laccase-mediator systems cause a loss of brightness in the initial pulp that is offset during the subsequent alkaline extraction [22, 23]. However, previous studies on flax pulp predicted no decrease in brightness after L [7]. Based on our results, whether brightness increases or decreases with respect to the initial pulp, it depends on the particular treatment time. The initial decrease in brightness may have resulted from the formation of chromophoric groups during the oxidation of lignin. In fact, the polymer was previously found to undergo oxidative degradation of its side chains [24], and also to form quinones during the reaction of the laccase-HBT system with lignin model compounds [25]; in addition, lignin isolated from pulp previously treated with a laccase-HBT system has been found to exhibit increased FTIR signals for carbonyl groups [21, 25, 26]. The structurally modified lignin remaining in the pulp is removed by the subsequent alkaline treatment hence the increased brightness observed after E.

Variation of color

The above-described potential formation of chromophoric groups during the enzyme treatment was studied by determining the CIE L*a*b* color coordinates of the pulp samples and obtaining the amount of chromophores after the L stage and the LE sequence (Fig. 3 and 4).

The CIE L*a*b* color space is a three-dimensional space based on opposite colors. The L*

coordinate (lightness) defines the amount of light present in a given color, in our case, whether the pulp was lighter or darker. A positive a* coordinate is indicative of red color and a negative one of green color, whereas a positive b* coordinate indicates yellow and a negative one blue. The a* and b* coordinates were positive in all treatments; therefore, an increase in a* or b* represented an increase in red or yellow, respectively. The L*, a*, b* coordinates for the initial pulp were 73, 1.6, and 13, respectively; and those for the E initial pulp 77, 1.7 and 14 respectively. Since the a* and b* coordinates were similar in both, the alkaline extraction stage caused no color change in the pulp. However, it increased L*, as a result of the removal of lignin from the pulp suggested by the reduction in kappa number with respect to the initial pulp. As can be seen from Fig. 3, the a* and b* coordinates for the pulp after the L stage exceeded those for the initial pulp throughout the treatment; as a result, the pulp acquired a stronger color, particularly during the first hour of treatment. The a* and b* coordinates after the LE sequence were smaller than those after the L stage; therefore, the alkaline extraction removed color acquired during L stage. a* coordinate was smaller in the LE sequence than in the initial pulp at any treatment time; on the other hand, b* coordinate at the beginning of the treatment was somewhat greater than in the initial pulp. b* coordinate remained constant virtually throughout L; on the other hand, a* coordinate decreased with increasing treatment time (Fig. 4). Overall, a* and b* coordinates decreased with increasing time in the LE sequence. Both coordinates initially decreased rapidly, leveled off after 5 h and increased slightly by the end of the treatment.

The k/s index is a measure of the amount of chromophores present in pulp. As can be seen from Fig. 5, treatment time of 1 h in Lo resulted in increased amounts of chromophoric groups relative to the initial pulp. After 3 h, however, such amounts were smaller than in the initial pulp. The pulp samples provided by the LE sequence exhibited less chromophores than did the E initial pulp. The color changes determined from the color coordinates and spectral curves were visually apparent in the paper sheets obtained. Based on the previous results, the enzyme treatment not only removes lignin, but also alters its structure with the formation of chromophoric groups that are eliminated in the subsequent alkaline extraction stage.

Variation of viscosity

Oxidation of the pulp during the bleaching process can lead to the formation of carbonyl groups in the carbohydrate network of cellulose. Such groups are degraded in a subsequent alkaline stage and the viscosity of the pulp reduced as a result. The variation of viscosity with time was similar in the three treatments, both after L and after LE (data not shown). The viscosity decreased slightly with respect to the initial pulp during the first 4 h of treatment, after which it fell by more than 100 $mL \cdot g^{-1}$ for a further 9 h and then leveled off until the end of the treatment, where it decreased slightly. The addition of oxygen had virtually no influence here.

Viscosity differences between L and LE pulps were small during the first 3 h of treatment (in the region of 30 $mL \cdot g^{-1}$) and increased with time (50 $mL \cdot g^{-1}$ after 5 h and to 100 $mL \cdot g^{-1}$ in some cases). The L stage can have two effects on the pulp, namely: direct degradation of cellulose and alteration of functional groups in it leading to easier degradation in a subsequent alkaline stage (E). Because viscosity measurements are made in an alkaline medium, the viscosity after L was a measure of both degrading effects. The E stage was performed in a hot, strongly alkaline medium, which may have resulted in modified cellulose being more markedly degraded than during the viscosity measurements. As a result, the viscosity differences between L and LE may have resulted from degradation of the cellulose modified by L.

Relationship between the properties of L pulp and LE pulp

A decrease in kappa number was correlated with an increased brightness after both L and LE (Fig. 6). Although, in general, a decrease in kappa number and an increase in brightness reduce pulp viscosity, no clear-cut correlation between the two pairs of properties could be established owing to the high dispersion of the results (Fig. 7). The viscosity initially decreased slowly while the pulp was very rapidly delignified (Fig. 1). Once the delignification rate started to decrease, the viscosity decreased very rapidly until both pulp properties reached a steady state. Once the amount of lignin was reduced to a limiting kappa number, the system ceased to degrade the polymer and oxidized cellulose up to a limiting viscosity value; at that point, pulp degradation

stopped, but the system possibly continued to oxidize dissolved compounds in the effluents.

The kappa number obtained after the LE sequence differed insignificantly between LoE and LairE (Fig. 1), whereas brightness was higher with the former treatment (Fig. 2). Such increased brightness was not the result of the presence of a smaller amount of lignin in the pulp, but rather of the lignin in LoE pulp containing fewer chromophoric groups than that in LairE pulp by effect of its being oxidized to a different extent. Increasing the amount of oxygen supplied above a given level resulted in no further delignification, but altered chromophoric groups in residual lignin present in the pulp.

Color and COD in the effluents from L stage

Color and COD increased with the treatment time and amount of oxygen supplied (Fig. 8 and 9). Both properties in the Ls stage were lower than those in Lair and Lo. The differences between Lair and Lo only became significant after 1.5 h of treatment.

Color and COD were found to depend on the nature of the chemical species present in the effluent, and also on their concentration. Increased treatment times and amounts of oxygen resulted in extensive degradation of lignin; part of the degraded lignin was soluble at pH 4, so the treatment increased the amount of polymer that was dissolved in the effluents. Color and COD may have increased by effect of an increased lignin concentration in the effluents; however, one should also bear in mind that the reaction medium contained some chemical species such as the enzyme laccase and the mediator HBT, their degradation products, and carbohydrates from cellulose, all of which may have affected both effluent properties.

Therefore, the color of chemical species in the effluents and the COD values during the first 3 h of treatment were directly related (Fig. 10). No similar correlation was apparent between the kappa number and color and COD; therefore, the color of the effluents is not exclusively due to lignin but, possibly, to some change in dissolved lignin or other HBT or enzyme degradation products during the treatment. HBT in the laccase-mediator system is partly converted into BT (benzotriazole) [5,

19]; therefore, it may have been responsible for the increased red color in the effluents observed at long treatment times.

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References

[1] M.B. Roncero, A.L. Torres, J.F. Colom, T. Vidal. The effect of xylanase on lignocellulosic components during the bleaching of wood pulps. Biores. Technol. 96 (2005) 21 – 30.

[2] D. Moldes, M. Díaz, T. Tzanov, T. Vidal. Comparative study of the efficiency of synthetic and natural mediators in laccase-assisted bleaching of eucalyptus kraft pulp. Biores. Technol. 99 (2008) 7959 – 7965.

[3] C. Valls, M.B. Roncero. Using both xylanase and laccase enzymes for pulp bleaching. Biores. Technol. In press.

[4] S. Camarero, O. García, T. Vidal, J. Colom, J. C. del Río, A. Gutiérrez. Flax pulp bleaching and residual lignin modification by laccase-mediator systems. Biotechnol. Pulp Pap. Ind. 21 (2002) 213 – 221.

[5] C. Sigoillot, S. Camarero, T. Vidal, E. Record, M. Esther, M. Pérez-Boada, M. J. Martínez, J. C. Sigoillot, M. Asther, J. F. Colom, A. T. Martínez. Comparison of different fungal enzymes for bleaching high-quality paper pulps. J. Biotechnol. 115 (4) (2005) 333 – 343.

[6] I. Herpöel, H. Jeller, G. Fang, M. Petit-Conil, R. Bourbonnais, J. L. Robert, M. Asther, J. C. Sigoillot. Efficient enzymatic delignification of wheat straw pulp by a sequencial xylanase-laccase mediator treatment. J. Pulp Pap. Sci. 28 (3) (2002) 67 – 71.

[7] S. Camarero, O. García, T. Vidal, J. Colom, J. C. del Río, A. Gutiérrez, J. M. Gras, R. Monje, M. J. Martínez, A. T. Martínez. Efficient bleaching of non-wood high-quality paper pulp using laccase-mediator system. Enz. Microbiol. Technol. 35 (2004) 113 – 120.

[8] O. García, S. Camarero, J. F. Colom, A. T. Martínez, M. J. Martínez, R. Monje, T. Vidal. Optimization of a laccase-mediator stage for TCF bleaching of flax pulp. Holzforschung 57 (2003) 513 – 519.

[9] M. Balakshin, C.-L. Chen, J. Gratzl, A. Kirkman, H. Jakob. Kinetic studies on oxidation of veratryl alcohol by laccase-mediator system. Part 2. The kinetics of dioxygen uptake. Holzsforchung 54 (2) (2000) 171 – 175.

[10] M. Balakshin, E. Capanema, C.-L. Chen, J. Graztl, A. Kirkman, H. Gracz. Biobleaching of pulp with dioxygen in the laccase-mediator system - reaction mechanisms for degradation of residual lignin. J. Mol. Cat. B: Enz. 13 (2001) 1 – 16.

[11] A. Potthast, H. Koch, K. Fischer. The laccase-mediator system - Reaction with model compounds and pulp. ISWPC - 9Th International Symposium on Wood and Pulping Chemistry - Oral Presentations (1997) F21 – F24.

[12] A. Oudia, J. Queiroz, R. Simões. The influence of operating parameters on the biodelignification of eucalyptus globulus kraft pulps in a laccase-violuric acid system. Appl. Biochem. Biotechnol. 149 (2008) 23 – 32.

[13] M. B. Roncero, A. L. Torres, J. F. Colom, T. Vidal. Effect of xylanase on ozone bleaching kinetics and properties of eucalyptus kraft pulp. J. Chem. Technol. Biotechnol. 78 (10) (2003) 1023 – 1031.

[14] M. B. Roncero, A. L. Torres, J. F. Colom, T. Vidal. TCF bleaching of wheat straw pulp using ozone and xylanase. Part B: kinetic studies. Biores. Technol. 87 (3) (2003) 315 – 323.

[15] H. P. Call, I. Mücke. History, overview and applications of mediated lignolytic systems, especially laccase-mediator systems (LignozymR-process). J. Biotechnol. 53 (1997) 163 – 202.

[16] D. Moldes, T. Vidal. Laccase-HBT bleaching of eucalyptus kraft pulp: Influence of the operating conditions. Biores. Technol. 99 (18) (2008) 8565 – 8570.

[17] D. Ibarra, J. Romero, M. J. Martínez, J. Martínez, S. Camarero. Exploring the enzymatic parameters for optimal delignification of eucalypt pulp by laccase-mediator. Enz. Microb. Technol. 39 (2006) 1319 – 1327.

[18] A. Fillat, J. Colom, T. Vidal. Exploring enzyme stability during delignification of flax pulp by laccase and natural mediators. 10th International Congress on Biotechnology in the Pulp and Paper Industry. Integrating biology with processes. (2007) 86.

[19] A. Potthast, T. Rosenau, K. Fischer. Oxidation of benzyl alcohols by the laccase-mediator system (LMS) - A comprehensive kinetic description. Holzforschung 55 (1) (2001) 47 – 56.

[20] K. Li, R. F. Helm, K.-E. Eriksson. Mechanistic studies of the oxidation of a non-phenolic lignin model compound by the laccase/1-hydroxybenzotriazole redox system. Biotechnol. App Biochem. 27 (1998) 239 – 243.

[21] D. Ibarra, S. Camarero, J. Romero, M. J. Martínez, A. T., Martínez. Integrating laccase - mediator treatment into an industrial-type sequence for totally chlorine-free bleaching of eucalypt kraft pulp. J. Chem. Technol. Biotechnol. 81 (2006) 1159 – 1165.

[22] F. S. Chakar, A. J. Ragauskas. The effects of oxidative alkaline extraction stages after laccase HBT and laccase NHAA treatments - an NMR study of residual lignins. J. Wood Chem. Technol. 20 (2) (2000) 169 – 184.

[23] J. Freudenreich, M. Amann, E. Fritz-Langhals, J. Stohrer. Understanding the lignozym-process. International Pulp Bleaching Conference (1998) 71 – 76.

[24] S. Camarero, G. C. Galletti, A. T. Martínez. Demostration of in situ oxidative degradation of lignin-side chains by two white-rot fungi using analytical pyrolysis of methylated wheat straw. Rapid. Commun. Mass. Spectrom. 11 (1997) 331 – 334.

[25] C. Crestini, L. Jurasek, D. S. Argyropoulos. On the mechanism of the laccase-mediator system in the oxidation of lignin. Chemistry - a European Journal 9 (2003) 5371 – 5378.

[26] K. Poppius-Levlin, W. Wang, T. Tamminen, B. Hortling, L. Viikari, M. L. Niku-Paavola. Effects of laccase/HBT treatment on pulp and lignin structures. J. Pulp Pap. Sci. 25 (3) (1999) 90 – 94.

Figure 1. Kappa number in the LsE, LairE and LoE treatments. In the figure, Ls (o), Lair (?), Lo (•), LsE (?), LairE (?) and LoE (■).

Figure 2. Variation of pulp brightness in the three treatments. In the figure, Ls (o), Lair (?), Lo (•), LsE (?), LairE (?) and LoE (■).

Figure 3. CIE L*a*b* coordinates. Relationship between a* and b* coordinates. In the figure, initial pulp (x), initial pulp E (+), L (o) and LE (?).

Figure 4. CIE L*a*b* coordinates. Variation of coordinate a* with time. In the figure, Ls (o), Lair (?), Lo (•), LsE (?), LairE (?) and LoE (■).

Figure 5. k/s curves for the Lo stage and the LoE sequence.

Figure 6. Relationship between kappa number and brightness obtained with the L stage and the LE sequence. In the figure, L (o) and LE (?).

Figure 7. Relationship between kappa number and viscosity obtained with the L stage and the LE sequence. In the figure, L (o) and LE (?).

Figure 8. COD in the effluents from the L stage during the first 3 h of treatment. In the figure, Ls (o), Lair (?) and Lo (•).

Figure 9. Color in the effluents from the L stage during the first 3 h of treatment. In the figure, Ls (o), Lair (?) and Lo (•).

Figure 10. Relationship between color and COD in the effluents from L. In the figure, Ls (o), Lair (?) and Lo (•).

FIGURES

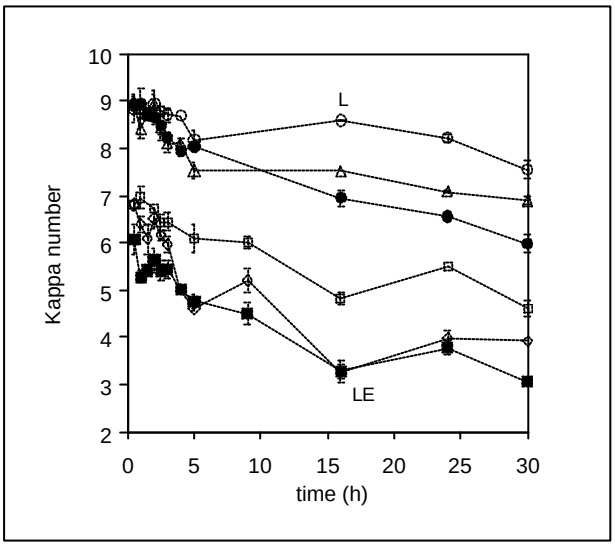

Figure 1.

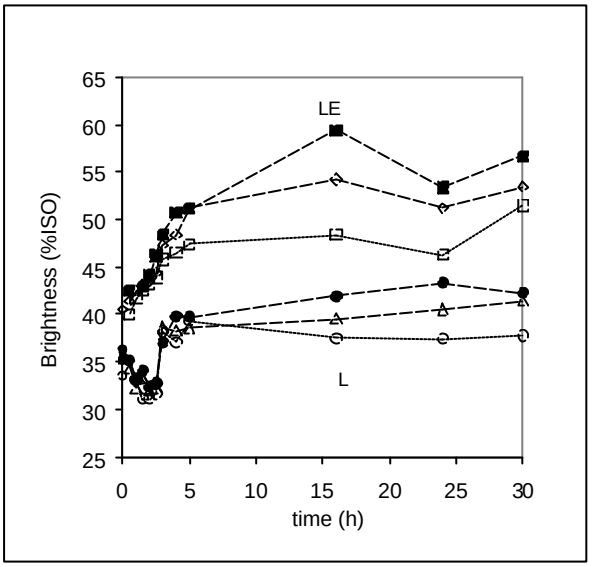
65
60
55
50
45
40
35
30
25
Brightness (%ISO)
0
5
10
15
20
25
30
time (h)
LE
L

Figure 2.

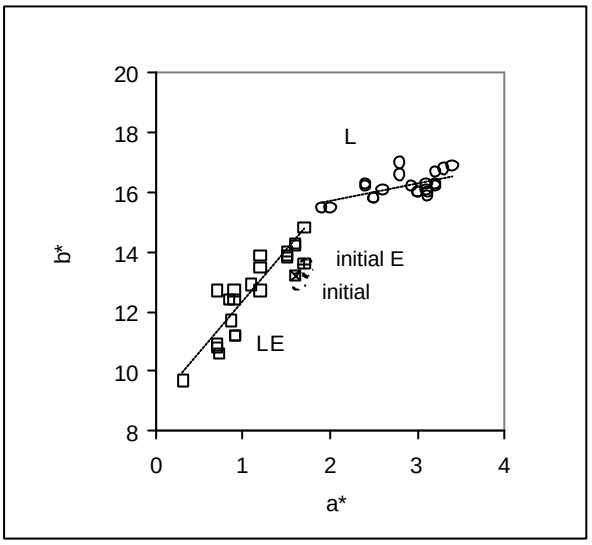

Figure 3.

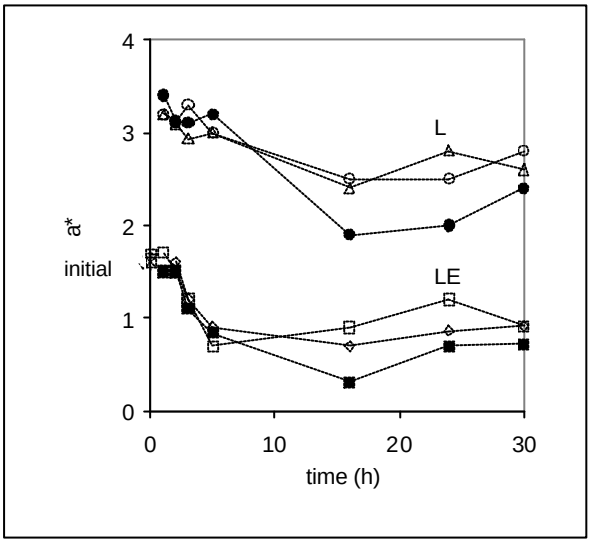

Figure 4.

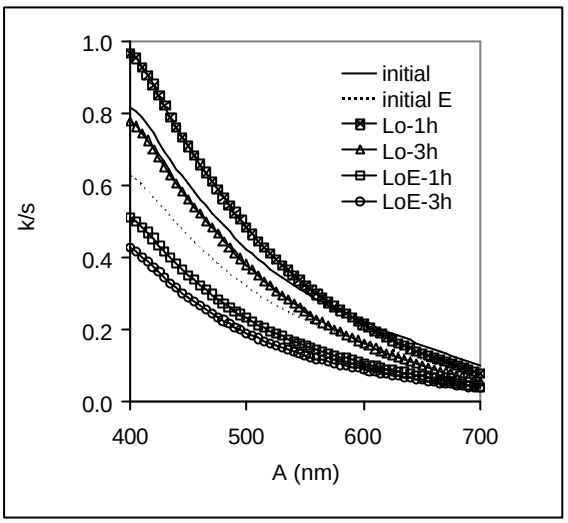
1.0
0.8
0.6
0.4
0.2
0.0
k/s
400
500
600
700
A (nm)
initial
initial E
Lo-1h
Lo-3h
LoE-1h
LoE-3h

Figure 5.

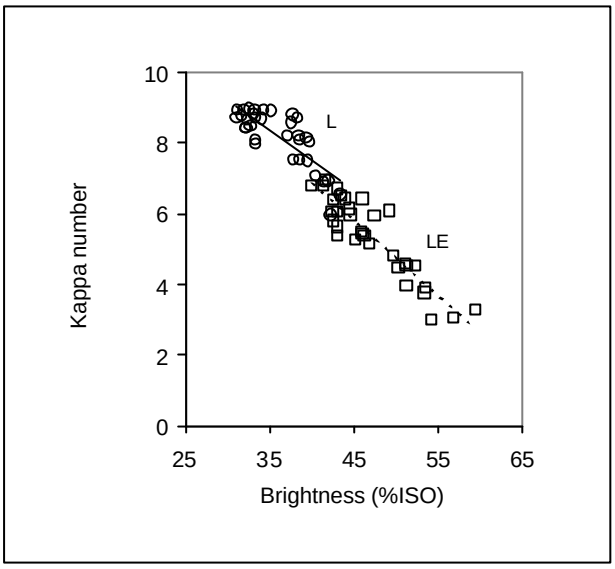

Figure 6.

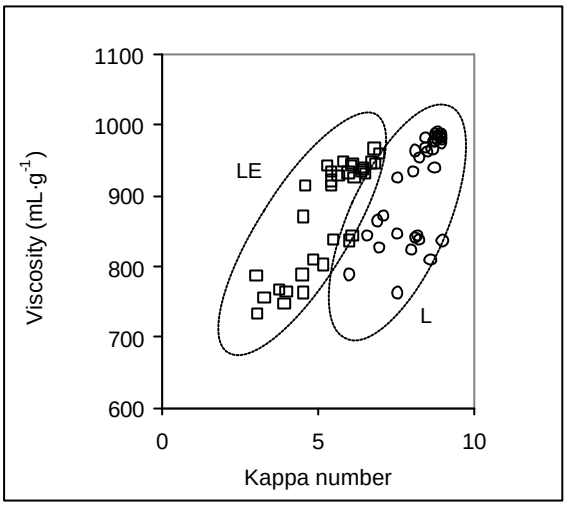
1100
1000
900
800
700
600
0
5
10
Viscosity (mL·g-1)
Kappa number
LE
L

Figure 7.

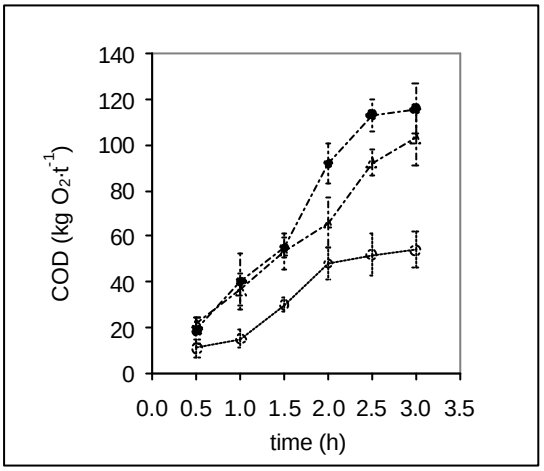

Figure 8.

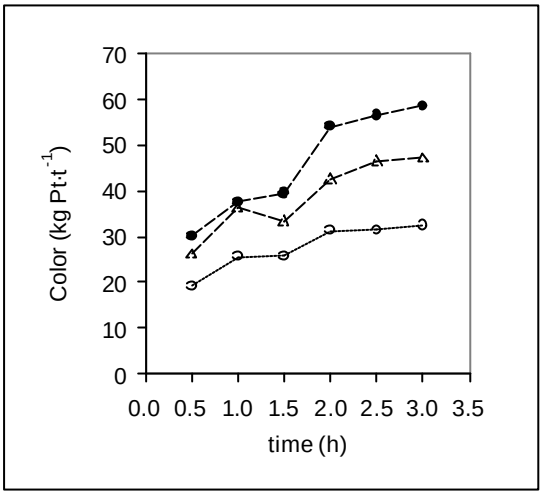

Figure 9.

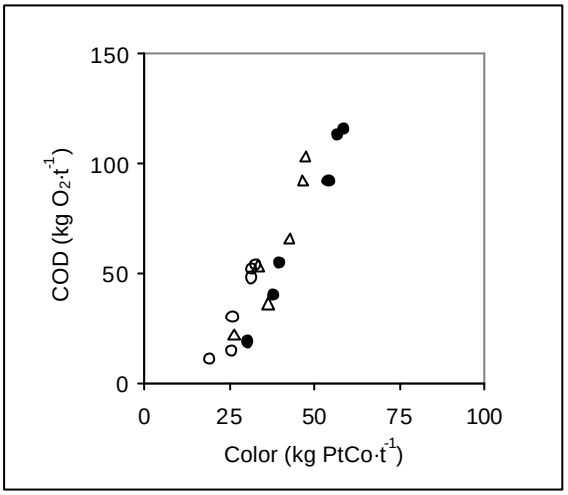

Figure 10.