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Kraft pulp biobleaching using an extracellular enzymatic fluid produced by *Pycnoporus sanguineus*

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ABSTRACT

The aim of this work was to obtain a LMS pre-treatment applicable to industrial TCF bleaching. Kraft pulp from *Eucalyptus globulus* was treated at 40 °C/pH 3 and 60 °C/pH 5 for 1 h using an extracellular fluid enriched in laccase produced by *Pycnoporus sanguineus* and acetosyringone as mediator (HBT was used as a control mediator) (L). Alkaline extraction (E) and hydrogen peroxide (P) stages were then assayed. The LEP alternative was an efficient sequence to bleach kraft pulp since the enzymatic pre-treatment boosted the subsequent chemical bleaching. The best L pre-treatment was obtained with laccase–acetosyringone at 40 °C/pH 3. It reduces kappa number and hexenuronic acids, increases pulp viscosity, lowers hydrogen peroxide consumption down to an 87.4% (94.0% without L) and enhances brightness up to a 59% ISO (51% ISO without L).

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1. Introduction

The conventional kraft pulp bleaching process uses chlorine compounds to selectively degrade lignin but causing environmental impact. The chlorine in these chemicals links to the residual lignin generating chloro-lignins and molecules, such as dioxins, which are highly toxic even at low concentrations. The use of oxygen, ozone or hydrogen peroxide in bleaching, instead of chlorinated chemicals, can reduce toxic contaminants in the effluents. However, due to their poorer selectivity towards lignin, these agents are not as efficient as chlorine derivatives and they produce bleached pulps of lower quality.

Recently, biotechnology applications in this field have attracted considerable interest. Its first application in biobleaching was proposed by Viikari et al. (1986), who used xylanases as bleaching booster. Since then, many authors have used enzymes, such as xylanases and laccases, to treat pulps before applying the standard bleaching sequences (Bajpai et al., 2006; Kapoor et al., 2007; Fillat et al., 2007; Singh et al., 2008; Valls and Roncero, 2009). These experiences showed that the pre-treated pulps required smaller number of chemical agents in further bleaching than the non-pre-treated pulps, maintaining the same pulp quality and reducing the pollutant load in the process effluents.

Among these enzymes, laccases are highly interesting because they can oxidize a wide variety of lignin derivatives and phenolic compounds requiring only molecular oxygen and the presence of some low molecular weight compounds called mediators. These substances act as electron transporters, inhibiting further re-polymerization of the oxidized radicals. The so called "laccase–mediator system" (LMS) was first described by Bourbonnais and Paice (1990), who studied the selective delignification of softwood and hardwood kraft pulps using 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonate (ABTS) as mediator. Other substances proposed as mediators were 1-hydroxybenzotriazole (Call, 1994) or 3-hydroxyanthranilic acid (3-HAA), a natural compound produced by the white rot fungus *Pycnoporus cinnabarinus* (Eggert et al., 1996). Recently, new studies about the application of the LMS to the bleaching process have been reported (Moldes and Vidal, 2008; Moldes et al., 2008; Oudia et al., 2008; Da Re et al., 2008).

However, the industrial application of the LMS into the bleaching sequences has some restrictions. One of them is the toxicity of the mediators, such as ABTS or HBT (1-hydroxy-benzotriazol), widely used in biobleaching research. The other drawback concerns the conditions of the enzymatic pre-treatment, which are dependent on laccase stability and very different from the temperature and pH employed in a conventional TCF bleaching sequence. The search for natural mediators can solve the first problem. Acetosyringone, the natural compound used in this study, has shown its effectiveness as mediator in a previous work (Camarero et al., 2007). The search for fungi which endure high temperatures had

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as its aim to obtain laccases which also endure high temperatures for some industrial applications such as biobleaching. Laccases produced by *Pycnoporus sanguineus* are able to endure temperatures around 60–75 °C for more than 1 h (Litthauer et al., 2007). These temperatures are closer to those used in bleaching at industrial scale, which would avoid the need for an excessive number of intermediate cooling and heating steps.

On the other hand, commercial laccases have been used in most studies on the application of LMS to pulp bleaching. A non-purified extracellular fluid with high laccase activity have been used in order to retain possible natural mediators generated by fungal metabolism and also to increase the LMS effectiveness at high temperature by introducing laccases which endure high temperatures for more than 1 h.

The aim of this work was to obtain a feasible laccase pre-bleaching treatment applicable to an industrial TCF process. So, the laccase treatment was tested at the highest temperature and pH (depending on laccase stability); and the effectiveness of a non-toxic natural compound (acetosyringone) was evaluated looking for possible benefits in the implementation of the LMS into a conventional TCF bleaching sequence. Acetosyringone's efficiency as mediator was compared with HBT's (a chemical with demonstrated capacity as mediator). Experiments were performed on an industrial *Eucalyptus globulus* pulp supplied by a Spanish mill and, after the laccase pre-treatment of the pulp, an alkaline extraction was applied to remove most of the enzymatically oxidized lignin. Finally, a hydrogen peroxide stage was carried out to verify if the laccase pre-treatment boosts the global bleaching sequence or if the effects of the enzymatic stage are masked by the chemical bleaching stage.

2. Methods

2.1. Chemicals

2,2′-Azino-bis-3-ethylbenzthiazoline-6-sulphonate (ABTS) was purchased from Roche (Madrid, Spain). All other chemicals: acetosyringone (ASG), 1-hydroxy-benzotriazol (HBT), NaOH and H_2O_2 were reagent-grade and obtained from Merck (Barcelona, Spain) or Sigma–Aldrich (Madrid, Spain).

2.2. Enzymatic pre-treatment

Assays were performed using 50 g of an industrial *E. globulus* kraft pulp (14.2 kappa number; 33% ISO for brightness; 842 ml/g for viscosity and 40.7 μmol/g of HexA) which was introduced into polyethylene bags where it was intensively mixed with the enzymatic fluid and chemicals before being submerged in a thermostatic bath. Consistency, reaction time, laccase dose and mediator concentration stayed unchanged during laccase pre-treatment and fixed at 10%, 1 h, 2.4 U/g and 0.05 mmol/g, respectively, while the type of mediator and the reaction conditions varied. Acetosyringone and HBT (used as reference) were the mediators used. Furthermore, for each mediator, two sets of temperature and pH were chosen: 40 °C/pH 3 (where laccase activity showed the best activity and stability, results not shown) and 60 °C/pH 5 (more favourable conditions for a hypothetical application to the industry).

Controls were included in the experimentation. In one of them (called conventional E/P bleaching), the laccase pre-treatment was omitted and it would correspond to a classical peroxide bleaching sequence. In two of them (called No-LM/E/P bleaching), the laccase and the mediator were also omitted but the pulp was treated with an aqueous solution (buffer) with the same pH, time and temperature as were used in both laccase–mediator treatments. These controls were included to distinguish between the contributions of the laccase (LMS) and that of the buffer extraction to the biobleaching process. Although it is known that oxygen boosts the effects of LMS in pulp bleaching, it was not applied to the enzymatic stage because the objective of this work is to compare the effectiveness of different mediators (acetosyringone and HBT) during this enzymatic process.

After every enzymatic treatment, pulps were washed and the laccase activity was measured in each effluent using ABTS as substrate according to Mansur et al. (2003) to find out if inactivation of the enzyme was occurred during the enzymatic pre-treatment.

2.3. Alkaline extraction and hydrogen peroxide treatments

After the enzymatic pre-treatment, an alkaline extraction was carried out using 1.5% NaOH, 5% of consistency and 90 °C during 120 min. After this treatment, pulps were washed and a hydrogen peroxide bleaching stage was applied in the following conditions: 1% H_2O_2, 1.5% NaOH, 1% DTPA, 0.2% $MgSO_4$, and 5% of consistency and 90 °C during 90 min. Residual hydrogen peroxide was analyzed in the bleaching effluent by standard titration.

2.4. Pulp characterization

Treated pulps were characterized in terms of their kappa number, brightness and viscosity according to the standards UNE 57034, UNE 57061 and UNE 57-039-92, respectively. Also, hexenuronic acids (HexA) content were analyzed following the method of Gellerstedt and Li (1996) and its contribution to the pulp kappa number was calculated using the molar oxidation equivalent of 8.6. A quantity of 11.6 μmol of HexA in 1 g of pulp corresponds approximately to 1 kappa number unit (Sevastyanova, 2005).

3. Results and discussion

The application of the LMS to the biobleaching of an *E. globulus* kraft pulp at all conditions assayed is shown in Figs. 1–3, which represent the evolution of kappa number, pulp viscosity and brightness, respectively, after the laccase–mediator stage (L), alkaline extraction (E) and hydrogen peroxide bleaching (P).

It is well known that, after cooking, eucalyptus kraft pulps contain significant amounts of hexenuronic acids in addition to the residual lignin. These acids behave in a similar way to lignin in the kappa number test, increasing its value (Sevastyanova, 2005). They also consume chemicals in pulp bleaching and must be removed to avoid brightness reversion (Forsström et al., 2007). There are other no-lignin substances which also increase the kappa number, but its significance is minor. For this reason, the kappa number is shown in Fig. 1 only as the addition of hexenuronic acids and lignin contents.

The conventional bleaching sequence reduces the total kappa number in 4.7 units, mainly in the P stage. The application of the LMS clearly improves these results; and the kappa number is reduced by 7 units when the LMS operates at 40 °C (pH 3) with acetosyringone as mediator. At 60 °C (pH 5), the effectiveness of the *P. sanguineus* laccase is not outstanding and, when HBT is the mediator, the kappa number reduction is only slightly better (5.3) than that obtained without enzymatic pre-treatment (4.7).

The pre-treatment of the pulp with the same buffer solution and at the same temperature as those used with the LMS was made to determine if the kappa number reduction is caused by the direct action of the laccase on the lignin or, on the contrary, results from lignin removal by the buffer solution. The pre-treatment without laccase and mediator means a slight decrease, although significant, in the kappa number (about 0.8). However, when the whole sequences (No-LM/E/P and conventional E/P) are compared, the ef-

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