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Application of laccase-natural mediator systems to sisal pulp: An effective approach to biobleaching or functionalizing pulp fibres?

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ABSTRACT

The effects of laccase-natural mediator systems (LMS) on sisal pulp and their potential for either biobleaching or functionalizing (via radical-coupling) its fibres were investigated. The enzyme treatment (L stage) was followed by extraction with hydrogen peroxide in order to determine whether observable effects could be enhanced by removing LMS-modified lignin. Four different plant phenols [viz. the *p*-hydroxycinnamic compounds sinapic acid (SNC), ferulic acid (FRC), coniferyl aldehyde (CLD) and sinapyl aldehyde (SLD)] were used as laccase redox mediators and their effects on pulp and effluents compared with those of the synthetic compound 1-hydroxybenzotriazole (HBT). During the L stage performed with HBT, laccase underwent a loss of 99% and 78% of the initial activity, in the absence and presence of pulp, respectively. With natural mediators inactivation was markedly reduced, being the residual activity between 65% and 100% of the initial one, in the presence of pulp. The pulp was found to protect the enzyme against inactivation: the activity was only reduced by 45% in its presence. Under the operating conditions used the natural mediators proved less efficient than HBT in facilitating pulp bleaching; rather, they tended to bind to pulp fibres. This effect could be used to functionalize fibres in order to improve intrinsic properties of pulp or introducing novel ones (e.g. antimicrobial, antioxidant, optical properties, etc.). This paper shows for the first time the application of laccase-mediator systems to sisal pulp.

1. Introduction

Wood continues to be the primary raw material for the pulp and paper industry; in fact, non-wood plants account for only 10% of the total amount of fibre used for papermaking worldwide. However, non-wood fibre plays a major role in the pulp and paper business inasmuch as it constitutes the mainstay of papermaking in many developing countries (particularly China and India) (López et al., 2004). Moreover, changes in agricultural policies, environmental concerns and wood supply issues are likely to raise the significance of non-wood fibre to the global pulp and paper industry in the near future (Ashori, 2006). In developed countries, textile-type fibre obtained from non-wood plants is used mainly to obtain specialty paper. Sisal (*Agave sisalana*) is a monocotyledon plant endemic to Central America which provides fibre with a papermaking potential. In fact, sisal, which has traditionally been used to make natural ropes, cordage and sacking, possesses some attractive properties for the production of a number of specialty paper varieties such as those used in tea bags, surgical gauze, filters or condensers (Hurter, 2001). Moreover, new opportunities exist for sisal pulp to cost-effectively replace long-fibred chemical wood pulp for the reinforcement or basis weight reduction of many paper grades (Maddern and French, 1994).

In recent years, the pulp and paper industry in Europe and North America has increasingly adopted enzyme technology, driven by the need to comply with stringent environmental legislation and improve competitiveness (Viikari, 2002). By virtue of their high specificity and environmental friendliness, enzymes possess a high potential for improving a wide range of aspects of pulp and paper production processes (Bajpai, 1999). For example, fungal laccases have been extensively studied in the presence of redox mediators in order to assess their ability to degrade lignin; this makes them useful for pulp bleaching, where the passing of increasingly strong environmental restrictions has fostered a search for effective alternatives to chlorine-containing bleaching reagents and the development of elemental chlorine-free (ECF) and totally chlorine-free (TCF) sequences (Camarero et al., 2002; Rochefort et al., 2004). The high potential of laccase-mediator systems (LMS) for bleaching some pulp types has been widely demonstrated, and *N*-hydroxy based compounds were found to be especially prominent for this purpose. Specially prominent and well-known among such mediators is 1-hydroxybenzotriazole (HBT) (Poppius-Levlin et al., 1999; Camarero et al., 2004; Fillat and Roncero, 2009; Valls and Roncero, 2009). However, the high cost of synthetic mediators and concerns about their potential

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toxicity have restricted industrial implementation of LMS and raised the need for alternative, easily available replacement substances for the pulp and paper industry such as natural phenols, which can be readily obtained from spent pulping liquors and plant materials or, directly, from fungal metabolism (Eggert et al., 1996; Johannes and Majcherczyk, 2000; Camarero et al., 2007). Some potentially cost-effective lignin-derived phenols have recently been found to perform as mediators for the laccase oxidation of recalcitrant compounds (e.g. residual lignin, industrial dyes, polycyclic aromatic hydrocarbons, lipids) (Cañas et al., 2007; Gutiérrez et al., 2007).

Although the potential involvement of laccase in the bleaching process has been thoroughly investigated, interest has recently switched increasingly to a different use of the enzyme such as the targeted modification of lignocellulose fibre surfaces with a view to improving intrinsic fibre properties or introducing novel ones (Chandra and Ragauskas, 2001; Buchert et al., 2005; Schroeder et al., 2007). This use has been facilitated by the non-specific substrate requirements of laccase and the tendency of phenolic compounds to undergo coupling reactions following enzymatic oxidation to resonance-stabilized phenoxy radicals (Chandra and Ragauskas, 2002; Milstein et al., 1994). Some authors have accomplished laccase-catalysed grafting of phenol compounds onto lignocellulose fibres; interest in this area, however, has focused mainly on wood materials and lignin-rich fibres (Chandra and Ragauskas, 2002; Suurnakki et al., 2006; Elegir et al., 2008). In this study, we investigated the effects of various laccase-natural mediator systems (LMS) on the pulp and effluents obtained in the processing of sisal pulp with low lignin content. The primary aim was to examine the tendency of mediators to either couple onto pulp or promote delignification with a view to assessing their potential for either functionalizing or biobleaching sisal fibres.

2. Methods

2.1. Laccase assays and mediators

Laccase (EC 1.10.3.2) from *Trametes villosa*, 588 U/ml, was supplied by Novozymes (Denmark). One activity unit was defined as the amount of laccase transforming 1 μmol/min ABTS to its cation radical (ε_{436} nm = 29,300 M^{-1} cm^{-1}) in 0.1 M sodium acetate buffer (pH 5) at 25 °C. Sinapic acid (SNC), ferulic acid (FRC), coniferyl aldehyde (CLD), sinapyl aldehyde (SLD) and 1-hydroxybenzotriazole (HBT), all purchased from Sigma–Aldrich, were assayed as laccase-mediators.

Oxidation of 50 μM HBT and *p*-hydroxycinnamic compounds by *T. villosa* laccase was assayed by using 300 mU/mL of enzyme in 50 mM sodium tartrate buffer (pH 4) at 25 °C. UV–vis spectra for the reaction mixture were recorded at different times (0, 3, 5, 10, 15, 20 min) during the first 20 min of enzymatic oxidation, using a Thermo Scientific Evolution 600 spectrophotometer.

2.2. Pulp and laccase-mediator treatments

Sisal (*A. sisalana*) alkaline pulps from a soda–anthraquinone cooking process were supplied by CELESA (Spain). Prior to initial characterization, the samples were washed with H_2SO_4 at 2% consistency at pH 4 for 30 min, which was followed by filtration and extensive washing with de-ionized water. This step ensured removal of contaminants and metals, and brought the pulp to the pH required for the enzyme treatment. The initial pulp samples had 47.3% ISO brightness, a kappa number of 7.75 and a viscosity of 784 mL/g.

Laccase-mediator treatments (L stage) were performed by using an amount of 40 g of sisal pulp at 5% consistency in 50 mM sodium tartrate buffer at pH 4, 20 U/g of *T. villosa* laccase and a proportion of 1.5% (w/w) HBT or natural mediator (all relative to pulp dry weight). Tween 80 (0.05% w/v) was added as surfactant. The treatments were carried out in a reactor under pressurized O_2 (6 bar) at 30 rpm at 50 °C for 4 h. Pulp samples treated under identical conditions in the absence of enzyme and mediator or only the latter were used as controls. Once treated, each pulp was filtered, its residual liquor collected and the pulp extensively washed with de-ionized water (Valls and Roncero, 2009).

2.3. Residual laccase activity

The enzyme activity, using ABTS as substrate, was monitored for 4 h under the conditions of an L stage performed at a small scale in an O_2 atmosphere (continuous bubbling) in the absence or presence of mediator, and with or without pulp. Residual activity values were expressed as percentages of initial activity, which was measured at the outset (time 0) of the incubation period.

2.4. Bleaching treatment

The laccase-mediator treatment was followed by an alkaline peroxide bleaching treatment (P stage) that was performed in an Ahiba Spectradye dyeing apparatus from Datacolor equipped with closed vessels 150 ml in volume that were loaded with 5 g oven-dried pulp (odp) at 5% consistency, 3% odp H_2O_2, 1.5% odp NaOH, 1% odp DTPA (diethylenetriaminepentaacetic acid) and 0.2% odp $MgSO_4$ at 90 °C for 2 h. Then, each treated pulp sample was filtered and extensively washed with de-ionized water.

2.5. Analysis of pulp properties

Pulp brightness, kappa number (an estimation of lignin content) and viscosity (determined as the intrinsic viscosity of a sample of cellulose dissolved in a dilute solution of cupri-ethylenediamine) were determined in accordance with ISO 3688, ISO 302 and ISO 5351/1, respectively, at the different stages of the process.

2.6. Determination of anionic charge

The surface anionic charge of sisal fibres was determined by polyelectrolyte adsorption as described elsewhere (Wagberg et al., 1989). The cationic polyelectrolyte used for adsorption was methylglycochitosan (MGCh) from Wako Pure Chemical Industries, Ltd. (Japan). The polymer molar mass and charge density were 1.5×10^5 and 4.04 meq/g, respectively. An amount of 0.25 g of pulp sample was diluted with 50 ml of 0.5×10^{-3} N MGCh and stirred for 30 min to facilitate the adsorption equilibrium. The resulting suspension was centrifuged and part of the clear supernatant (10 ml) collected for titration with the anionic polyelectrolyte (Cadena et al., 2009). The point of equivalence was determined with the cationic dye indicator Toluidine Blue (Wako) or a Mütek PCD 03 particle charge detector (Germany). With the dye indicator, the supernatant was titrated with potassium polyvinyl sulphate (PVSK) (Wako); with the PCD, titration was done with sodium polyethensulphonate (PES-Na) (Oy G. W. Berg & CO Ab/BTG Mütek GmbH, Germany). Blank samples were titrated exactly in the same way as those brought into contact with pulp. The amount of fibre in each sample upon titration was determined gravimetrically following filtering on pre-weighed filter paper and drying in an oven at 105 °C overnight.

2.7. Characterization of effluents

The effluents from the L and P stages were analysed for chemical oxygen demand (COD) and colour following ASTM D1252-00 and ASTM D1209-00, respectively. Coefficient of variation was