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Effects of laccase-natural mediator systems on kenaf pulp

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ABSTRACT

This paper reports the first application of laccase-mediator systems (LMS) to kenaf pulp. Five natural phenolic compounds (acetosyringone, syringaldehyde, *p*-coumaric acid, vanillin and acetovanillone) were used as mediators in combination with laccase in an L stage in order to elucidate their effect on delignification. After LMS treatment, pulp samples were subjected to two alkaline treatments (an E or P stage). The results obtained were compared with those provided by the laccase-1-hydroxybenzotriazole (HBT) system. All natural mediators increased kappa number, decreased brightness and changed optical properties of the pulp after the L stage, suggesting that natural mediators tend to couple to fibers during a laccase-mediator treatment. The greatest delignification and bleaching effects after the P stage were obtained with syringaldehyde and acetosyringone, providing an effective means for delignifying kenaf, whereas those based on the other three could be used to functionalize kenaf with a view to obtaining pulp with novel properties.

1. Introduction

Kenaf *(Hibiscus cannabinus)* is a nonwood plant potentially useful for applications such as composite wood (Nourbakhsh and Ashori, 2008), insulating materials and paper production. Environmental sustainability is gradually increasing the need to reduce the amounts of wood used by the paper industry and nonwood plants could obviously play a prominent role as wood substitutes in the future (Ashori and Raverty, 2007). The use of enzyme technology by the paper industry in Europe and North America has provided a potential effective choice for complying with an increasingly restrictive environmental legislation. Laccases are multi-copper oxidase enzymes which catalyze the oxidation of various phenolic substrates by using oxygen as an electron acceptor (Leonowicz et al., 2001). Natural lignin is degraded by laccase from plants and fungi (Thurston, 1994; Mayer and Staples, 2002). However, these enzymes have a moderate oxidizing power (0.5–0.8 V) and can only attack phenolic moieties in the lignin polymer (Xu et al., 1996), thereby requiring the presence of a mediator to degrade nonphenolic lignin (Morozova et al., 2007). Mediators are low-molecular weight compounds which when oxidized by laccase generate radicals. Such radicals are ultimately responsible for the delignifying effect by virtue of their easy access to lignin linkages (Ibarra, 2006).

The synthetic mediators 2,2′-azino*bis*(3-ethylbenzthiazoline-6-sulfonate) (ABTS) and 1-hydroxybenzotriazole (HBT) possess a high oxidation power (Bourbonnais et al., 1998; Xu et al., 2000; Baiocco et al., 2003; Shleev et al., 2006). For example, the laccase-HBT system has been successfully used to oxidize lignin in flax pulp (Fillat and Roncero, 2010) and eucalyptus kraft pulp (Valls and Roncero, 2009).

The search for new and more effective mediators among natural phenolic compounds involved in natural lignin degradation has aroused increasing interest in recent years (Camarero et al., 2005, 2007). The primary advantage of using natural phenols as mediators is that they can be easily obtained from spent pulping liquors (Gutiérrez et al., 2007) or plant materials. Also, their low cost and toxicity can provide economic and environmental advantages. In this study we examined, for the first time, the effects of various laccase-natural mediator systems on kenaf pulp.

2. Methods

2.1. Pulp, enzyme and mediators

Unbleached pulp obtained by soda–anthraquinone cooking of Chinese kenaf (*Hibiscus cannabinus*) was supplied by CELESA (Tortosa, Spain) and washed with H_2SO_4 at 3% consistency at pH 2 for 30 min to remove metals. The initial pulp had a kappa number is 12.9 and 35% ISO brightness. Laccase from *Pycnoporus cinnabarinus* was supplied by INRA (Marseille, France). Activity was followed by measuring the ABTS oxidation in 0.1 M sodium acetate buffer (pH 5) at 436 nm (ϵ_{436} = 29300 $M^{-1}cm^{-1}$). One activity unit was defined as the amount of laccase that transforms

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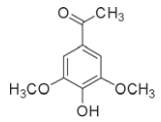
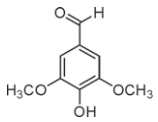
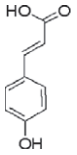
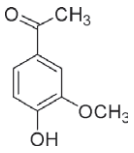
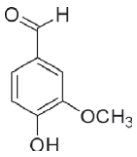
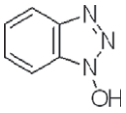
Table 1
Chemical structures of mediators.

Acetosysiringone **(AS)** 	Syringaldehyde **(SA)** 	p-coumaric acid **(PC)** 
Acetovanillone **(AV)** 	Vainillin **(V)** 	1-hidroxibenzotriazole **(HBT)** 

Abbreviations: AS (acetosyringone); SA (syringaldehyde); PC (p-coumaric acid); V (Vainillin); AV (acetovanillone) and HBT (1-hydroxybenzotriazole).

μmol/min of ABTS at 25 °C. All measurements were carried out using a Shimadzu UV–vis 1603.

The mediators studied and their chemical structures are shown in Table 1.

2.2. Enzyme treatments (L stage)

The different treatments applied to kenaf pulp, designated as Treatment n^o time Laccase$_{\text{Mediator}-\%(w/w)\text{mediator}}$, were conducted according to the following conditions:

2.2.1. Treatment 1_2L_{M3}

2 g of pulp at 1% consistency was treated with 20 U/g of *P. cinnabarinus* laccase and 3% (w/w) mediator in 50 mM tartrate buffer (pH 4.0) at 30 °C under oxygen saturation conditions in an open reactor for 2 h (all relative to pulp dry weight).

2.2.2. Treatment 2_2L_{M3}

10 g of pulp at 5% consistency was treated with 20 U/g of *P.cinnabarinus* laccase and 3% (w/w) mediator in 50 mM tartrate buffer (pH 4.0), 50 rpm at 30 °C under oxygen saturation conditions in a closed reactor for 2 h (all relative to pulp dry weight).

2.2.3. Treatment 2_4L_{M3}

10 g of pulp at 5% consistency was treated with 20 U/g of *P.cinnabarinus* laccase and 3% (w/w) mediator in 50 mM tartrate buffer (pH 4.0), 50 rpm at 30 °C under oxygen saturation conditions in a closed reactor for 4 h (all relative to pulp dry weight).

After L stage, the pulp was filtered and washed with de-ionized water.

2.3. E and P stage

The enzyme treatment was followed by two parallel treatments, namely: *Stage E*, which involved extraction with 1.5% (w/w) NaOH at 5% consistency at 90 °C in an Ahiba Easydye apparatus from Datacolor International for 2 h; and *Stage P*, which consisted in an alkaline peroxide bleaching treatment performed under the same conditions as E, but using 3% H_2O_2, 1% DTPA and 0.2% $MgSO_4$ (all relative to pulp dry weight).After each stage, the pulp was filtered and washed with de-ionized water.

2.4. Analysis of pulp properties

Brightness and kappa number of the pulp after the L stage, and the LE and LP sequences were assessed according to ISO 3688 and ISO 302, respectively (ISO, 1998).

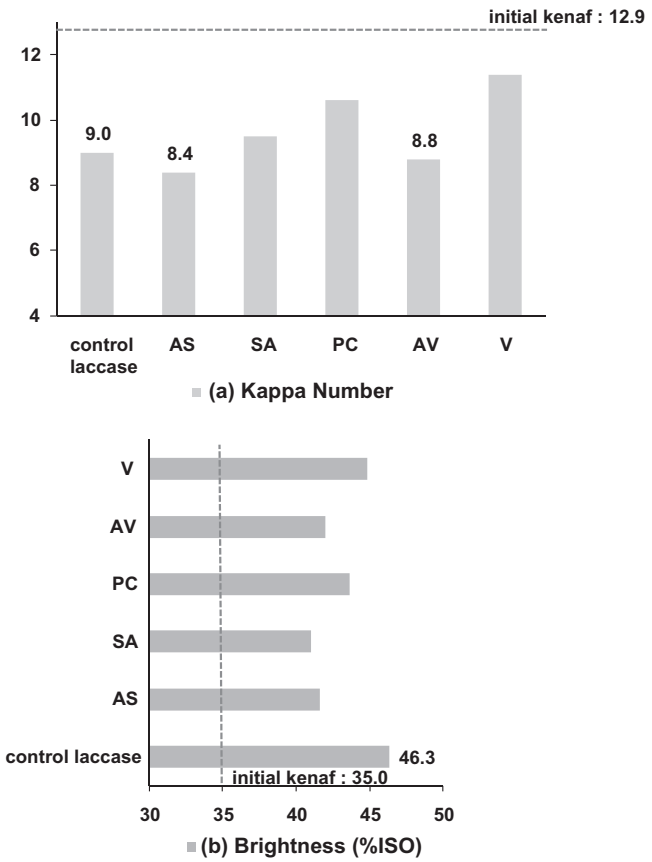


Fig. 1. *$1_2L_{M3}E$ treatment:* Changes in kappa number (a) and brightness (b) of kenaf pulp treated with laccase in the presence of mediators. The control laccase was treated in the absence of mediator. The dashed line corresponds to the initial kenaf pulp.