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A new biobleaching sequence for kenaf pulp: Influence of the chemical nature of the mediator and thermogravimetric analysis of the pulp

Glòria Andreu [a], Agustín G. Barneto [b], Teresa Vidal [c,*]

[a] Chemical Engineering Department, ETSEIAT, Universitat Politècnica de Catalunya, Colom 11, E-08222 Terrassa, Spain
[b] Chemical Engineering Department, Physical Chemistry and Organic Chemistry, University of Huelva (ceiA3), Spain
[c] Textile and Paper Engineering Department, ETSEIAT, Universitat Politècnica de Catalunya, Colom 11, E-08222 Terrassa, Spain

HIGHLIGHTS

- We report a novel laccase-mediator treatment for kenaf pulp biobleaching.
- Relevance of phenoxy radicals formed in the enzymatic stage.
- Kenaf increases oxidative efficiency of laccase.
- Thermogravimetric analysis realizes cellulose surface changes.

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ABSTRACT

This paper evaluates five phenolic compounds as mediators for kenaf pulp biobleaching by laccase. The results have been compared with the treatment using a non-phenolic mediator, 1-hydroxybenzotriole and laccase alone. The influence of the nature of the chemical mediators used on various pulp properties is discussed. In addition to oxidizing lignin, the phenolic radicals formed in the process take part in condensation and grafting reactions in enzymatic stage. After biobleaching sequence (LP), syringaldehyde was shown to be the best phenolic mediator, allowing a delignification of 43% and 72% ISO brightness. These results were similar to the use of laccase alone due to the role as mediators of syringyl units resulting from oxidative lignin degradation. As a novelty, the study was supplemented with thermogravimetric analysis, with emphasis on the crystallinity degree of the cellulose surface and the aim of elucidating the action mechanisms of laccase-mediator systems on fiber.

1. Introduction

Kenaf (*Hibiscus cannabinus*) is an annual dicotyledonous plant which grows in temperate and tropical areas. Kenaf adapts easily to various types of soil and requires only minimal chemical treatment to grow effectively (Elsaid et al., 2011). Kenaf plants can adsorb approximately 1.5 times their weight in carbon dioxide, which is an increased level of adsorption relative to other plants (Mohanty et al., 2005). This fact, its rapid growth and its high yield makes kenaf one of the most promising nonwood plants. In the developed world, fiber from nonwood plants has a growing market for producing paper with high added value (Moore, 1996). The contents in long (bast) and short (core) fibers of kenaf are in fact suitable for manufacturing paper and various other products (Ahmed et al., 1998); however, kenaf bast fibers are especially suitable for producing high-quality paper.

The use of laccases in combination with various natural phenolic compounds is receiving increasing attention for various purposes including pulp delignification, wood fiber modification, dye or stain bleaching, contaminated water purification and soil remediation (Widsten and Kandelbauer, 2007). Laccases hold much promise for the paper and pulp industry in its search for ways to avoid the environmental impact of the chlorine-based oxidants currently in use in delignification and bleaching processes (Cañas and Camarero, 2010).

Laccases are multi-copper oxidases catalyzing the oxidation of phenolic substrates with the concomitant reduction of oxygen (Leonowicz et al., 2001). However, these enzymes have a moderate oxidizing power and can only attack phenolic moieties in lignin polymers (Xu et al., 1996), so they require the assistance of a natural or synthetic mediator to efficiently degrade nonphenolic lignin (Morozova et al., 2007). Mediators are low-molecular weight compounds that form radicals upon oxidation by laccase; such radicals are indeed capable of oxidizing lignin linkages. Laccase-mediator systems (LMS) have been successfully used to oxidize lignin in sisal

* Corresponding author. Tel.: +34 937 398 180; fax: +34 937 398 101.
E-mail address: tvidal@etp.upc.edu (T. Vidal).

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(Aracri et al., 2009), flax (Fillat et al., 2010) and kenaf (Andreu and Vidal, 2011). However, the effect of an LMS depends on the balance between oxidative degradation and other reactions taking place during the enzymatic stage (Moldes et al., 2008; Barneto et al., 2012).

Thermogravimetric analysis is a powerful tool for detecting chemical changes in microfibril surfaces, as well as for quantifying crystalline and amorphous cellulose in fiber. Clean and crystalline cellulose are thermally degraded at high temperatures spanning a narrow range because ordered cellulose chains yield crystallites that are protected from external attack by a surface hydrogen bond network (Barneto et al., 2011). For example, reaching the highest possible mass loss rate for crystalline cellulose requires heating at about 360 °C in a nitrogen environment and at only 20 °C less in the air because oxygen reacts much more easily with volatiles evolved during heating than with cellulose (Mamleev et al., 2007). Amorphous and paracrystalline cellulose respond differently to heating (Ioelovich et al., 2010); thus, they are degraded at lower temperatures over a broad range. Amorphous cellulose is located between cellulose crystallites in cellulose microfibrils; by contrast, paracrystalline cellulose is unordered crystalline cellulose located on crystallite surfaces, onto which it is chemically or enzymatically adsorbed.

The aim of the present work is to evaluate the efficiency of five phenolic mediators on a kenaf bleaching sequence. These treatments will be compared with laccase alone and a non phenolic mediator, 1-hydroxybenzotriole (HBT). The results will be evaluated in terms of delignification, brightness and viscosity. Their effect on optical properties of the treated pulp will be emphasized. Finally, thermogravimetric analysis will be employed by first time to kenaf treated pulp.

2. Methods

2.1. Raw materials, laccase and natural phenols

Kenaf (*Hibiscus cinnabinus*) alkaline pulp samples were obtained by soda–anthraquinone cooking in the CELESA pulp mill (Tortosa, Spain). Prior to the enzyme treatments, the pulp was washed with acidified water (pH 2) at 3% pulp consistency for 30 min, followed by filtration and extensive washing with deionized water. This procedure was needed to remove contaminants and metals, as well as to adjust the pulp to the pH required for the enzyme treatments. The properties of the washed pulp were as follows: kappa number 12.9 ± 0.1, ISO brightness 35.0%, viscosity 925 ± 23 mL g^{-1}, glucan content 83.5% ± 0.2, xylan content 14.3% ± 0.06 and klason lignin content 2.1% ± 0.1. The pulp was treated with laccase and either HBT or one of the natural mediators shown in Table 1. Laccase (EC 1.10.3.2) from *Trametes villosa* (*Tv*L) was supplied by Novozymes (Bagsvaerd, Denmark). Its activity was assessed by monitoring the oxidation of ABTS in 0.1 M sodium acetate buffer (pH 5) at 436 nm (ε_{436} = 29 300 M^{-1} cm^{-1}). One activity unit was defined as the amount of laccase converting 1 μmol min^{-1} ABTS at 25 °C. All absorbance measurements were made with a Shimadzu UV–Vis 1603 Spectrophotometer. The natural laccase mediators (syringaldehyde, acetosyringone, *p*-coumaric acid, vanillin and acetovanillone), and 1-hydroxybenzotriole (HBT), were all purchased from Sigma–Aldrich.

2.2. Bleaching sequence (LP)

Each laccase-mediator treatment (L stage) was performed by using an amount of 40 g of pulp in 50 mM sodium tartrate buffer at pH 4, 20 U/g *Tv*L and a proportion of 1.5% HBT or natural mediator (all relative to pulp dry weight). The treatments were carried out in a reactor under O_2 pressure (0.6 MPa) at 30 rpm at 50 °C for 4 h. Pulp consistency was 5% in all treatments. Pulp samples treated under identical conditions except for the absence of a mediator were used as controls. After the enzyme treatment, samples were filtered and extensively washed with deionized water.

The L stage 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 of 150 mL volume; the vessels were loaded with 5 g odp (oven-dried pulp) 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 sample was filtered and extensively washed with deionized water (García et al., 2003).

2.3. Soxhlet extraction (L_{Sox})

After enzymatic treatment, pulps were extracted with acetone in a Soxhlet apparatus for 2 h and 15 min in order to remove residual phenolic compounds adsorbed in the pulp (Aracri et al., 2010). The pulp samples after this extraction are named L_{Sox} in the text.

2.4. Analysis of pulp properties

The treated pulp samples were characterized in terms of kappa number, brightness and viscosity according to ISO 302:2004, 2470–1:2009 and 5351:2011, respectively. Analyses were performed in duplicate for kappa number (errors associated to measurement were lower than 0.5 standard deviation) and viscosity, and in quadruplicate for brightness (standard deviation = 0.1).

The optical properties of each pulp were determined by using a Technidyne Colour Touch reflectometer in accordance with ISO 11475. Reflectance spectra and the *k*/*s* ratio for paper sheets made from the pulp were obtained by following the Kulbelka–Munk theory (Andreu and Vidal, 2011). The area decrease rate (ADR) is a measure of the area decrease in the *k*/*s* plot with respect to the initial pulp: $ADR = \frac{(A_0 - A_1)}{A_0} \times 100$, where A_0 is the initial area and A_1 the final area (Andreu and Vidal, 2011). The area difference represents the amount of chromophoric groups removed (positive values) or introduced (negative values) by the treatment. The initial kenaf pulp was used as reference.

Sample color was described in terms of the CIE $L^*a^*b^*$ color space. L^*, a^* and b^* are the coordinates of the space, which is based on the assumption that color is perceived as L^* (lightness, which ranges from 100 for perfect white to 0 for absolute black), a^* (which ranges from greenness to redness), and b^*(which ranges from blueness to yellowness, from negative to positive values) (Hunt, 1998).

Table 1
Natural mediators used in the enzymatic stage: name mediator (abbreviation).

Substituents in ortho-phenol position	Mediators		
Two methoxy groups	Syringyl derivatives:	Acetosyringone (AS)	Syringaldehyde (SA)
One methoxy group	Coniferyl derivatives:	Acetovanillone (AV)	Vainillin (V)
Without substituents	Guaiacyl derivatives:	*p*-coumaric acid (PC)	