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Paper strength improvement by oxidative modification of sisal cellulose fibers with laccase–TEMPO system: Influence of the process variables

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ABSTRACT

The oxidation of sisal pulp fibers by the laccase–TEMPO system was investigated and the influence of process variables including the laccase and TEMPO doses, and reaction time, on various properties of the oxidized fibers and of handsheets made from them was for the first time assessed using a three-variable statistical plan. The laccase–TEMPO system was found to oxidatively modify cellulose fibers, largely by introducing aldehyde groups and, to a much lesser extent, by introducing carboxyl groups. Based on the mathematical models used, increasing the TEMPO dose and reaction time increases the aldehyde content of the fibers, thereby also increasing their wet strength by effect of inter-fiber covalent bonding *via* hemiacetal linkages. Although no accurate model for the carboxyl content could be established, this property was found to peak under the specific conditions yielding the highest response in the dry tensile index model. The fact that the oxidative treatment diminished pulp viscosity is indicative of partial depolymerization of cellulose. This was especially marked under the conditions providing the highest contents in aldehyde and carboxyl groups, and the greatest improvements in the dry and wet tensile indices.

1. Introduction

Laccase (EC.1.10.3.2.) is a family of blue multi-copper oxidases produced by microorganisms and plants which catalyze the one-electron oxidation of phenols and aromatic or aliphatic amines to reactive radicals with the concomitant reduction of oxygen to water. The broad substrate range for this enzyme makes it attractive for a number of biotechnological applications (Kunamneni et al., 2008; Riva, 2006). Moreover, the range can be expanded using the enzymes in combination with a chemical mediator enabling the oxidative transformation of compounds with a redox potential higher than that of the enzyme (Bourbonnais & Paice, 1990; Galli & Gentili, 2004). In the last few decades, laccase has played an increasingly important role in pulp and paper research by virtue of its wide applicability along the entire production chain of paper products (Bajpai, 1999; Widsten & Kandelbauer, 2008). Special emphasis has been placed on the potential of laccase and laccase-mediator systems (LMS) for use in biobleaching and mill water treatments (Font, Caminal, Gabarrell, Romero, & Vicent, 2003; Valls & Roncero, 2009). One attractive, fast-growing field of research at present is the enzymatic modification of fibers with a view to improving the chemical or physical properties of fiber products or developing novel alternatives (Chandra & Ragauskas, 2001; Viikari, 2002). One well-known procedure for functionalizing polysaccharides is the catalytic oxidation of primary hydroxyl groups into aldehyde and/or carboxyl groups using the stable nitroxyl radical 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) in aqueous media at room temperature (de Nooy, Besemer, & van Bekkum, 1995b). In virtually all studies concerning carbohydrate oxidation, the established NaClO/NaBr system has been used as a primary oxidant (Bragd, Besemer, & Bekkum, 2001; Chang & Robyt, 1996; de Nooy, Besemer, & van Bekkum, 1995a; Isogai & Kato, 1998). In this process, TEMPO and its oxoammonium cation (the actual oxidant) are reduced to an *N*-hydroxyderivative that is continuously reoxidized by sodium hypochlorite, thus affording the use of TEMPO in catalytic amounts. Environmental concerns have shifted research interest toward halide-free oxidative systems. One promising approach for this purpose is the use of oxidative enzymes such as laccases in combination with oxygen as primary oxidants (Arends, Li, Ausan, & Sheldon, 2006; Viikari, Kruus, & Buchert, 1999). Similarly to the NaClO/NaBr system, oxoammonium ion is regenerated *in situ*, so only oxygen is consumed during the reaction. In addition to the environmental benefits associated with the use of an enzyme, this method has the

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Abbreviations: BV, borohydride viscosity; Cns, pulp consistency; CS, cellulose chain scission number; DP, degree of polymerization; DTI, dry tensile index; LT, laccase–TEMPO; Odp, oven dried pulp; Rpm, revolutions per minute; TEMPO, 2,2,6,6-tetramethylpiperidine-1-oxyl free radical; TR, pulp treated prior to refining; WTI, wet tensile index.

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advantage that it operates at near-neutral pH, which reduces the occurrence of β-elimination reactions causing cleavage of polysaccharide chains—a major drawback of the traditional method using pH 10–11 (Isogai & Kato, 1998).

Although the laccase–TEMPO system has been shown to catalyze the oxidation—and consequent functionalization—of various types of polysaccharides (Jetten, Van den Dool, Van Hartingsveldt, & Besemer, 2000; Marzorati, Danieli, Haltrich, & Riva, 2005; Viikari, Niku-Paavola, et al., 1999; Viikari, Kruus, et al., 1999), the influence of the experimental conditions and the characteristics of the resulting products have scarcely been examined. Much research aimed at developing specific applications for cellulose pulp has focused on oxidation reactions mediated by the TEMPO–NaBr–NaClO system on the grounds of its efficiency in functionalizing fiber surfaces with large amounts of aldehyde and/or carboxyl groups. This oxidative modification has been exploited to improve or modulate several physical properties of various types of pulp fibers including inter-fiber bonding capacity—and hence strength-related properties in the resulting paper (Dang, Zhang, & Ragauskas, 2007; Duarte et al., 2006; Lianshan, Kweinam, Deneault, & Brouillette, 2008). Paper from TEMPO-oxidized fibers was recently shown to have an increased wet strength; this was ascribed to the ability of aldehyde groups to form inter-fiber covalent bonds through hemiacetal linkages with hydroxyl groups in adjacent fiber surfaces (Saito & Isogai, 2005, 2006). The potential of laccase for replacing halide-based co-oxidizer systems in the TEMPO-mediated oxidation of cellulose fibers has scarcely been explored to date, however (Viikari, Kruus, et al., 1999). In recent work (Aracri, Vidal, & Ragauskas, 2011), we studied the oxidation of sisal pulp fibers by the laccase–TEMPO system and found it to slightly increase the carboxyl group content of the fibers and to considerably improve the wet strength of the resulting paper, the latter suggesting the formation of a substantial amount of aldehyde groups providing inter-fiber bonding through hemiacetal linkages. In this work, we further investigated the process and examined the influence of the operating conditions (*viz.* the laccase and mediator doses, and treatment time) on the distribution of carboxyl and aldehyde groups in fibers, pulp borohydride viscosity, and dry and wet strength properties of the handsheets for the first time using a three-variable sequential statistical plan.

2. Experimental

2.1. Chemicals, enzyme and pulp

All chemicals were purchased from Aldrich and used as received. Laccase from *Trametes villosa* was supplied by Novozymes (Bagsvaerd, Denmark). One activity unit was defined as the amount of laccase transforming 1 μmol/min ABTS to its cation radical ($\varepsilon_{436\,\mathrm{nm}} = 29{,}300\,\mathrm{M^{-1}\,cm^{-1}}$) in 0.1 M sodium acetate buffer at pH 5 at 25 °C. Sisal (*Agave sisalana*) pulp (1% lignin content) from a soda–anthraquinone cooking process was supplied by CELESA (Tortosa, Spain). Following conditioning at 2% consistency at pH 4 (H_2SO_4) under stirring for 30 min, the pulp was filtered and extensively washed with de-ionized water. This step was needed to remove contaminants and metals, and also to bring the pulp to a suitable pH for the enzyme treatment. The main properties of the initial pulp were as follows: 108 ± 5 μmol/g carboxyl groups content, 1 ± 0 aldehyde groups content μmol/g and 736 ± 0 ml/g borohydride viscosity.

2.2. Pulp treatments

A series of preliminary tests was performed using an amount of 30 g of pulp in a 5 L reactor stirred at 60 rpm, using 50 mM acetate buffer at pH 5 in the presence of 60 U/g odp laccase and 8% odp TEMPO, and variable conditions of time, temperature, applied oxygen pressure and consistency (Table 1). Pulp samples treated in the absence of TEMPO, or both laccase and TEMPO, at room temperature and 1% consistency under an oxygen pressure of 0.6 MPa for 18 h were used as controls.

Table 1
Operating conditions used in the laccase–TEMPO (LT) treatments of the preliminary study.

Sample ID	Time (h)	Temperature	Applied O_2 pressure (MPa)	Pulp consistency (% odp)
LT – 18 h	18	Room	0.6	1
LT – 30 h	30	Room	0.6	1
LT – 50 °C – 30 h	30	50 °C	0.6	1
LT – no P_{O_2} – 30 h	30	Room	–	1
LT – 4 h	4	Room	0.6	1
LT – cns 5% – 30 h	30	Room	0.6	5

Subsequently, laccase–TEMPO treatments were performed according to the established experimental design in plastic containers, using a jar testing apparatus at a stirring speed of 60 rpm, 15 g of pulp at 1% consistency in 50 mM acetate buffer at pH 5 at room temperature under oxygen bubbling. The operating variables (factors) studied were the laccase dose, mediator dose and reaction time. After treatment, each pulp was filtered and washed with de-ionized water until a colorless, neutral filtrate was obtained.

2.3. Experimental design

Laccase–TEMPO treatments were conducted in accordance with a 2^3 experimental design involving three variables at two levels each and three replicates at the central point, which required a total of 11 tests. The ranges examined for the independent variables were 20–100 U/g odp (laccase dose, x_1), 2–8% odp (TEMPO dose, x_2) and 8–20 h (reaction time, x_3), and the results were coded as −1 or +1, both for direct comparison of coefficients and for easier understanding of the effect of the variables on the responses. The independent variables were zeroed at the central point. The results of the three replicates at the central point, and their variance, were used in combination with the variance of the saturated model to calculate Snedecor's *F*-value in order to determine whether the variance was homogeneous or heterogeneous. The variance was homogeneous in all cases, so a linear model was constructed, its significant terms identified and its potential curvature detected. Linear multiple regression was applied by means of an Excel spreadsheet in order to implement the stepwise backward regression method and discard all terms with a probability $p < 0.05$.

2.4. Analysis of pulp properties

Pulp viscosity was determined in accordance with ISO 5351/1. Borohydride viscosity was measured after treatment with 2% $NaBH_4$, at 5% consistency at room temperature for 30 min (Roncero, Queral, Colom, & Vidal, 2003). The bulk acid group content was determined by conductimetric titration as described elsewhere (Aracri et al., 2011). TEMPO-oxidized pulp samples were further oxidized with $NaClO_2$ for selective conversion of aldehyde groups into carboxyl groups at room temperature for 48 h. The carboxyl content was determined with the above-described conductimetric titration method. The carboxyl groups formed by effect of $NaClO_2$ oxidation were assumed to derive from aldehyde groups originally present in the pulp (Saito & Isogai, 2005).

2.5. Paper testing

Prior to oxidative treatment, each pulp was disintegrated for 30,000 revolutions and then refined for 4500 revolutions according