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Effect of process parameters in laccase-mediator system delignification of flax pulp
Part I. Pulp properties

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ABSTRACT

Flax pulp was bleached by using an enzyme treatment with laccase (L stage) in the presence of HBT as mediator in order to replace toxic chemicals (e.g. chlorine or chlorine dioxide) with environmentally friendly catalytic biotechnological products in the bleaching process, achieving a clean process technology in the pulp and paper manufacture. The operating conditions for the laccase–HBT system were optimized by using a sequential statistical plan involving four variables (laccase dose, HBT dose, treatment time and oxygen pressure), their influence on the properties of the pulp after the L stage was examined. The main objective was to minimize the reagent doses and the reaction time to make more suitable the industrial application.

A mathematical model for predicting the kappa number in terms of the process variables was developed. The kappa number decreased with increase in the value of each variable down to a minimum level of 6.9, which was obtained with a laccase dose of 13 U g^{-1}, an HBT dose of 2%odp and a treatment time of 6.5 h. The model predicted a limiting laccase dose of 13 U g^{-1} above which no additional reduction in kappa number would be obtained. By contrast, oxygen pressures of 0.2–0.6 MPa in the reactor had no effect on brightness or kappa number. Brightness was not correlated with the kappa number due to the formation of chromophores in the pulp at the beginning of the enzymatic treatment. The LP biochemical sequence allows obtaining a final brightness of 75.2%ISO.

1. Introduction

Current interest in the use of biotechnology in pulp and paper production processes has been boosted by the potential of biological treatments to meet the present environmental restrictions. Their use in bleaching processes appears to hold great promise since they could replace toxic chemicals (e.g. chlorine or chlorine dioxide) giving rise to environmentally friendly bleaching processes. Applications in pulp bleaching processes appear to hold special promise. Thus, xylanase enzymes have been industrially implemented with substantial savings in bleaching treatments, and reduced investment costs and contents in halogenated compounds in the resulting effluents [1–4]. Oxidative enzymes such as laccases, manganases peroxidases and lignin peroxidases have been extensively studied in this respect over the past 20 years and found to provide improved efficiency in the bleaching of kraft pulp and savings in reagents [5–7]. Enzyme-based biobleaching treatments are still under development; some, however, are nearly ready for industrial implementation.

Based on the specific literature published over the last decade, pulp bleaching with manganese peroxidases and laccases in the presence of a mediator has very good prospects. Laccase-mediator systems have enabled the development of efficient TCF bleaching sequences and they save reagents, bleach pulp and reduce its kappa number [5,8–12]. However, the high cost and contaminating power of mediators have led researchers to search for alternative substances or find a way to improve their recovery [13]. Therefore, an optimization of the process variables of laccase-mediator system is required for their future industrial application.

Oxidative enzymes have scarcely been used to bleach non-wood pulp [14–18]. Most studies in this context have focused on the influence of laccase-mediator systems in reactions with lignin model compounds for short times, and have failed to examine cellulose degradation via viscosity measurements [19–21].

Regarding the influence of the oxygen concentration on the efficiency of laccase-mediator systems, some authors have noted that, in pressurized reactors, raising such a pressure in the system leads to increased delignification of the pulp [6,16,20,22]. Also, using a pressurized reactor rather than atmospheric pressure has been

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found to boost delignification and reduce the viscosity of flax pulp after an LP sequence [18,23].

In a previous study [24] was examined the influence of oxygen addition on the efficiency of a laccase-mediator system applied to flax pulp at atmospheric pressure. The results show that supplying the medium with oxygen and increasing the oxygen concentration in it, influence the kinetics of the process. For this reason, it was decided to carry out the laccase-mediator treatments in an oxygen-pressurized reactor. However, other variables must to be taking into account in order to optimize the laccase-mediator system. Therefore, the aim of this study was to establish the influence of process variables on pulp properties after a laccase-mediator treatment. The process was optimized under more industrially feasible conditions, regarding mediator dose and treatment time, in an oxygen-pressurized reactor. For this purpose, a sequential statistical plan was used to study the influence of four operating variables on the efficiency of the laccase-mediator treatment (viz. the laccase and mediator doses, treatment time and oxygen pressure in the reactor) on various properties of the resulting pulp (brightness, kappa number and viscosity) measured after the enzyme treatment.

2. Materials and methods

2.1. Raw material

Unbleached alkaline flax (*Linum usitatissimum*) pulp with 36.5%ISO brightness, 10.5 kappa number and 952 mL g^{-1} from a soda anthraquinone cooking process was provided by CELESA factory (Spain) and subjected to acid washing. The flax used as raw material contained approximately 15% core fibers. The commercial enzyme was laccase from *Trametes villosa* supplied by Novozymes® (Ref. NS-51002) with an activity of 39.4 U mL^{-1} and the mediator hydroxybenzotriazole (HBT) from Fluka (Ref. 54802).

The enzymatic activity of laccase was defined as the amount of enzyme needed to convert 1 μmol of the substrate ABTS per minute. Oxidation of ABTS was followed by an absorbance increase at 436 nm (ε_{436} = 29,300 M^{-1} cm^{-1}) in a UV–vis Shimadzu 1603 spectrophotometer. The reaction mixture contained 5 mM ABTS, 100 mM sodium acetate buffer at pH 5 and between 10 and 50 μL of enzyme.

2.2. Selection of the high optimization level

The aim of this study was to identify the high level of each variable to be used in the optimization of the laccase-mediator system for bleaching flax pulp. The chosen level should allow large enough differences in pulp properties to be observed. To this end, a pressurized reactor at 0.6 MPa was used in conjunction with high laccase and mediator doses (25 U g^{-1} and 3%odp, respectively) for 1, 4 or 7 h. This set-up was used to examine the L stage, and the LE and LR sequences.

2.3. Experimental design

Enzyme treatments were sequentially conducted according to a 2^4 factorial design with three replications at the centre point and an 8-point star. The four variables (factors) studied were examined over the following ranges: X_1 (laccase dose) from 1 to 20 U g^{-1}, X_2 (HBT dose) from 0.1 to 2%odp, X_3 (treatment time) from 0.5 to 6.5 h and X_4 (oxygen pressure in the reactor) from 0.2 to 0.6 MPa. In order to ensure experimental stability and, because the statistical study was expected to encompass all tests included in the sequential design, all 27 treatments in the L stage were performed together at the beginning of the study. The different pulp and effluent analyses were performed in accordance with the statistical results of the sequential design. The experimental results were processed by using the analytical tool “Regression” in the software Excel and the “backward stepwise regression” method.

2.4. Laccase-mediator treatment (L stage)

Treatments were performed on an amount of 40 g of initial pulp at 3%odp consistency, using a 50 mM sodium tartrate buffering solution at pH 4 at 30 °C that was supplied with Tween 80 surfactant. All tests were carried out in a pressurized reactor. The four variables used in the experimental design were the laccase and mediator doses, treatment time and oxygen pressure; the values used are stated in their respective sections. Control test was performed in the absence of enzyme and mediator, using a treatment time of 6.5 h and oxygen pressure of 0.6 MPa, which were the respective maximum values employed in the statistical plan. This test was intended to expose the effect of temperature, oxygen pressure and washing of the pulp in the absence of the laccase-mediator system.

2.5. Alkaline extraction (E stage)

The pulp samples obtained from the L stage were subjected to alkaline extraction (E stage). This treatment was intended to eliminate residual lignin not dissolved by the enzyme treatment or removed by the subsequent washing. The E stage was performed in a “Datacolor Easydye AHIBA” individual oscillating reactor. An amount of 5 g of pulp at 5%odp consistency was treated with 1.5%odp NaOH at 90 °C for 120 min in each test.

2.6. Reductive treatment (R stage)

This stage was intended to avoid degradation of carbohydrates in subsequent alkaline stages by effect of the potential formation of carbonyl groups in cellulose during the enzyme treatment. Pulp was adjusted to 5% consistency in a polyethylene bag and supplied with 2 g of solid $NaBH_4$. The reaction was allowed to develop for 30 min, the bag being shaken by hand at 5 min intervals.

2.7. Hydrogen peroxide stage (P stage)

Two pulp samples obtained from the L stage were subjected to hydrogen peroxide stage. The P stage was performed in a “Datacolor Easydye AHIBA” individual oscillating reactor. An amount of 5 g of pulp at 5%odp consistency was treated with 3%odp de H_2O_2, 1.5%odp de NaOH, 1%odp de DTPA, 0.2%odp de $MgSO_4$ at 90 °C for 120 min in each test.

2.8. Pulp properties

The pulp samples obtained from the L and P stage were analyzed for brightness, kappa number and viscosity according to the applicable ISO standards, ISO 2470, ISO 302 and ISO 5351-1, respectively.

3. Results and discussion

3.1. Control test

A comparison of the properties of the initial pulp and control pulp (kappa number of 8.7 and viscosity of 947 mL g^{-1}) reveals that the latter reduced the kappa number. Therefore, some lignin in the pulp may have been dissolved by effect of the temperature and