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Optimization of a Laccase-Mediator Stage for TCF Bleaching of Flax Pulp

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Summary

Flax pulp obtained by anthraquinone-soda pulping, resulting in a kappa number of 11.1, a viscosity of 950 ml/g and 36.7 % ISO brightness, was bleached in a totally chlorine-free sequence using the enzyme laccase from the fungus *Pycnoporus cinnabarinus* and 1-hydroxybenzotriazole (HBT) as redox mediator (stage L), followed by a hydrogen peroxide stage (P). The laccase treatment was optimized using a three-variable sequential statistical plan over the following ranges: 1–20 U/g o.d.p. (oven-dried pulp) laccase dose, 0.5–7.5 % o.d.p. HBT dose and 1–24 h reaction time. The influence of these variables on several pulp properties after the P stage of the LP sequence was examined. The models defined from the results obtained predicted variations in ISO brightness, viscosity and kappa number of 57.6–74.8 %, 590–955 ml/g and 0–6.2, respectively. The variables most strongly influencing these pulp properties were found to be the reaction time and the enzyme dose. A compromise was adopted as regards the operating conditions in order to ensure optimum results. The study was completed by conducting a biobleaching assay in a pressurized reactor (590 kPa) to assess the effect of oxygen pressure. The high pressure level resulted in improved pulp properties by the laccase-mediator system.

Introduction

Non-wood fibers have a long history as papermaking raw materials. Although wood fibres continue to be much more widely used, some developing countries use annual plants as their major source of fibres for paper manufacturing. In developed countries, non-wood fibres are used to manufacture high-quality pulp for specialty papers. Flax (*Linum usitatissimum*) is a herbaceous annual plant highly suitable for the production of thin, strong sheets such as used in cigarette, bible and lightweight bond paper. The long flax fibres used for manufacturing paper are located in the bark of the plant and account for 20 % of the stem weight (McGovern *et al.* 1987; Moore 1996). Because of their high cost, these fibres are used mixed with core fibers. Totally chlorine-free (TCF) bleaching sequences for pulp from non-wood fibres have aroused much interest. This type of pulp is often more difficult to bleach than chemical pulps from hardwood or softwood due to reasons of anatomical and chemical composition, including the presence of core fibers.

Enzymes have high potential for improving traditional pulp and paper manufacturing processes due to their high specificity and environmental friendliness. Fungal laccases in the presence of mediators are the most promising bleaching enzymes due to their ability to degrade lignin, compared with xylanases used at the mill scale that only contribute to lignin removal in an indirect way. The laccase-mediator system (LMS) allows the development of TCF sequences for bleaching various types of pulp (Viikari 2000; Camarero *et al.* 2002). Research into the delignification chemistry of kraft pulp using LMS has been extensively examined over the past decade (Balakshin *et al.* 1998; Freudenreich *et al.* 1998; de Carvalho *et al.* 1999; Poppius-Levlin 1999; Chakar and Ragauskas 2000a; Sealey *et al.* 2000). However, LMS bleaching is not established at the mill scale yet, and its technical feasibility is to be demonstrated at pilot plant scale (Call and Mücke 1997). 1-Hydroxybenzotrizole (HBT) is one of the most efficient laccase mediators, but the possible drawbacks of this and other mediators (such as high cost and dose, limited biodegradability and potential toxicity) need to be investigated in more detail.

In previous work, a TCF sequence for bleaching flax pulp involving laccase–HBT treatment followed by hydrogen peroxide was developed (Camarero *et al.* 2001a, b, 2002). The alkaline extraction stage usually following biobleaching treatments was found to be redundant. The joint use of *Pycnoporus cinnabarinus* laccase and the mediator HBT was found to be a highly efficient choice for delignifying flax pulp. Considering a two stage sequence, the results were better than those obtained in multi-stage TCF sequences using oxygen, hy-

drogen peroxide, ozone or some other chlorine-free chemical agents. For example, in previous studies the sequence $OZ_{0.6\,\%}RP_{2\,\%}$ provides a brightness of 80.8 % ISO, a kappa number around 3 and a viscosity of 585 ml g^{-1} (García *et al.* 2001). In particular, the viscosity of the biobleached pulp (865 ml g^{-1}) was better than provided by the non-enzymatic TCF sequence. Khristova *et al.* (2002) bleached a alkaline-sulphite flax pulp by the sequence $OQP_{O;3\,\%}P_{4\,\%}$ obtaining a brightness of 82.1 % ISO and a viscosity of 387 ml g^{-1}.

A number of experimental designs aimed at optimizing pulp bleaching, including non-wood pulp, have been reported (Abdul-Karim and Rab 1995; Iglesias *et al.* 1996; Vega *et al.* 1997; Jiménez *et al.* 1997, 1998). However, in only a few instances they have been used to optimize LMS treatments, and wood pulp bleaching was considered in all these cases (Chakar *et al.* 2000). Therefore, the optimization of LMS treatment variables with the aim of obtaining the best possible results in non-wood pulp bleaching, represents an innovative approach. In this work, a sequential statistical plan was used to examine the influence of LMS biobleaching variables on pulp properties after a hydrogen peroxide stage in a flax TCF bleaching process. The effect of the oxygen pressure used during the LMS treatment was also investigated.

Materials and Methods

Flax pulp

The unbleached alkaline flax pulp contained 15 % straw (core fibers), had a kappa number of 11.1, a viscosity of 950 ml g^{-1} and an ISO brightness of 36.7 %. It was obtained by soda anthraquinone cooking and supplied by the CELESA pulp mill (Tarragona, Spain). HBT was purchased from Aldrich (Barcelona, Spain).

Enzyme production and activity determination

Laccase was obtained from the white-rot fungus *P. cinnabarinus* IJFM A720 grown in a glucose–ammonia medium (Camarero *et al.* 2002). Production of the enzyme was induced by 150 µM $CuSO_4$. Cultures were harvested at the point of highest laccase activity. Laccase activity was determined by monitoring the oxidation of 5 mM ABTS to its cation radical (ε_{436} 29 300 mM^{-1} cm^{-1}) in 100 mM acetate buffer, pH 5. The amount of enzyme that converted 1 µmol of substrate per min was taken to be one unit of enzyme activity.

Laccase-mediator treatment (stage L)

Prior to processing, the pulp was washed with 250 mM tartrate buffer at pH 4 and room temperature for 30 min. LMS treatments were conducted on 5 g of pulp of 2 % consistency in 50 mM buffer at pH 4. The enzyme dose, mediator dose and treatment time were the three variables of the experimental design; the values used are stated below. Tween 80 (0.05 % w/v) was added as surfactant. Flasks were kept under O_2 atmosphere, at 160 rpm and 30 °C for varying time intervals.

Experimental design

Various LMS treatments were carried out following a 2^3 (two levels and three variables) sequential statistical plan, plus three replications at the central point. The three independent variables were varied over the following ranges: 1–20 U g^{-1} o.d.p. (oven-dried pulp) for the laccase dose (X_1), 0.5–7.5 % o.d.p. for the HBT dose (X_2), and 1–24 h for the reaction time (X_3). The results obtained were subjected to backward stepwise regression using the Statgraphics v. 6 software (terms with Snedecor's *F*-values less than 4 were discarded).

Biobleaching in a pressurized reactor (stage L_o)

Samples of 25 g of pulp were treated in an oxygen pressurized reactor under 590 kPa using 60 rpm stirring. The operating conditions were the same used above, except for the values of the three variables, and the use of pressurized oxygen. Two experimental points were chosen for comparison with the values predicted by the model: X_1=1, X_2 = –0.75 and X_3 = –0.5; and X_1 = 1, X_2 = –0.75 and X_3 = 0.

Hydrogen peroxide bleaching (stage P)

LMS treatments were followed by a hydrogen peroxide stage (P) involving the use of 3 % o.d.p. H_2O_2 in a solution containing 0.66 % o.d.p. NaOH, 1 % o.d.p. DTPA and 0.2 % o.d.p. $MgSO_4$ at 5 % pulp consistency at 90 °C for 2 h. Pulp brightness, kappa number and viscosity were determined in accordance to ISO 3688, ISO 302 and ISO 5351/1 standards, respectively.

Results and Discussion

The flax pulp bleaching results obtained after P stage of the LP sequence (*viz*. pulp brightness, kappa number and viscosity) in each experiment, and the biobleaching conditions used are listed in Table 1. As shown in Figure 1, the three variables are mutually related, with regression coefficients of 0.93. As can be seen from Figure 1a, the kappa number decreased with increasing brightness. Brightness increase varied by 36 % at the least and 51 % at the most with respect to the initial pulp. These values exceed those obtained in a previously reported LEpo (Epo, alkaline extraction with hydrogen peroxide and oxygen) sequence for coniferous pulp (Chakar and Ragauskas 2000a). As can be seen from Figure 1b, the viscosity decreased with decreasing kappa number. The lowest extent of delignification was 46 % and the highest nearly 100 %; both figures are greater than previously reported for softwood and hardwood pulp (Nelson *et al.* 1998; Chakar and Ragauskas 2000a, b; Chandra *et al.* 2001). Finally, as can be seen from Figure 1c, the viscosity decreased with increasing brightness. The greatest viscosity decrease was 34 % and the smallest zero. This suggests that, under certain conditions, the pulp viscosity can be preserved. The greatest decrease was similar to that reported for softwood pulp (Sealey *et al.* 2000).

The experimental points obtained were accurate and exhibited trends consistent with the usual patterns for bleaching processes (Tessier and Savoie 2000; Roncero *et al.* 2000). However, these results are better than those obtained by applying an LEP sequence on other types of pulp, and hardly attainable by chemical bleaching (García *et al.* 2001; Camarero *et al.* 2002). This confirms the high efficiency of the laccase–HBT treatment for delignifying flax pulp.

Table 1. Biobleaching conditions and experimental results (LP sequence)

X_1	X_2	X_3	Enzyme dose (U g^{-1})	HBT dose (% o.d.p.)	Time (h)	Brightness (% ISO)	Kappa number	Viscosity (ml g^{-1})
−1	−1	−1	1.0	0.5	1.0	58.3	6.20	955
1	−1	−1	20.0	0.5	1.0	63.0	3.86	875
−1	1	−1	1.0	7.5	1.0	62.1	5.56	920
1	1	−1	20.0	7.5	1.0	62.1	4.83	935
−1	−1	1	1.0	0.5	24.0	64.9	4.57	855
1	−1	1	20.0	0.5	24.0	70.1	2.64	795
−1	1	1	1.0	7.5	24.0	69.4	2.49	740
1	1	1	20.0	7.5	24.0	75.8	0.10	590
0	0	0	10.5	4.0	12.5	67.6	2.18	780
0	0	0	10.5	4.0	12.5	70.1	1.95	735
0	0	0	10.5	4.0	12.5	71.1	2.06	755

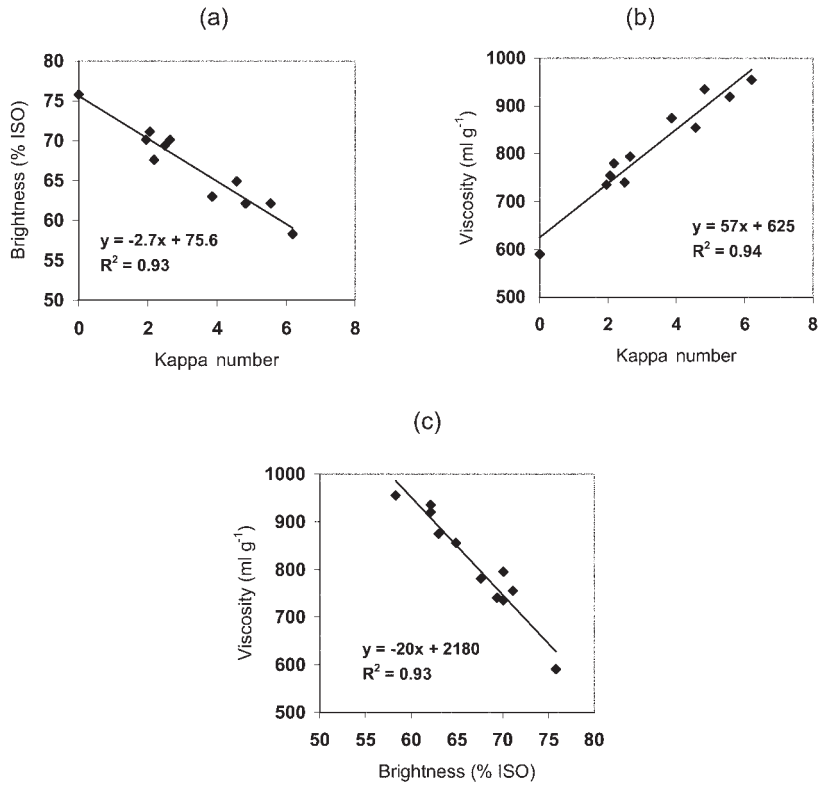

Fig. 1. Properties of experimental points of LP sequence: (a) brightness vs. kappa number, (b) viscosity vs. kappa number and (c) viscosity vs. brightness.

Modeling

Experimental data were fitted to a second-order polynomial equation. Responses were pulp brightness (Y_B), kappa number (Y_{KN}) and viscosity (Y_V). The independent variables [*viz.* X_1 (1–20 U g^{-1}), X_2 (0.5–7.5 % o.d.p.) and X_3 (1–24 h)] were coded as −1 or +1, both for direct comparison of coefficients and for easier understanding the effect of variables on the responses.

A preliminary experimental analysis revealed the quadratic term to be significant. Two additional experiments were therefore required to identify those variables possessing a significant term and discriminate them. The experimental results are shown in Table 2. A second analysis of the modelling equations provided the following responses:

$$Y_B(\%ISO) = 70.00 + 2.18X_1 + 1.53X_2 + 4.33X_3 - 4.28X_3^2 \quad (1)$$

with R^2=0.90 for brightness,

$$Y_{KN} = 2.06 - 0.94X_1 - 0.54X_2 - 1.34X_3 - 0.63X_2X_3 + 1.69X_2^2 \quad (2)$$

with R^2=0.97 for the kappa number and

$$Y_V(ml/g) = 756.6 - 38.2X_1 - 33.5X_2 - 88.1X_3 - 43.1X_2X_3 + 72.6X_1^2 \quad (3)$$

with R^2=0.92 for viscosity.

In the above equations, $X_1 = (L - 10.5)/9.5$ (L denoting the laccase dose, in U g^{-1}), $X_2 = (M - 4)/3.5$ (M denoting the HBT dose, as a percentage) and $X_3 = (t - 12.5)/11.5$ (t being the reaction time, in hours). As can be seen from the R^2 values obtained, the fit was quite good (particularly for the kappa number).

Brightness

As shown by Eq. (1), brightness is influenced by the three variables with a positive sign, and by X_3^2 with a negative sign, time (X_3) being the most influential variable. As can be seen from Figure 2a, obtained at fixed laccase doses, there is a quadratic dependence on time and a linear one on the HBT dose. With $X_1 = 1$, the variation of the ISO brightness with time (65.1 to 73.7 %) is more marked than with the HBT dose (from 70.7 to 73.7 %); a maximum occurs at $X_3 = 0.5$ (74.8 % ISO) beyond which a brightness reversion occurs.

Kappa number

As can be seen from Eq. (2), the kappa number is influenced by the three variables, as well as by the interaction between the HBT dose and time (X_2X_3); it decreases with the increase in any of them. The mediator dose also

Table 2. Biobleaching conditions and experimental results (two more experiments) (LP sequence)

X_1	X_2	X_3	Enzyme dose (U g^{-1})	HBT dose (% o.d.p.)	Time (h)	Brightness (% ISO)	Kappa number	Viscosity (ml g^{-1})
1	–1.00	0	20	0.500	12.5	70.4	3.31	810
1	–0.75	0	20	1.375	12.5	72.5	2.50	800

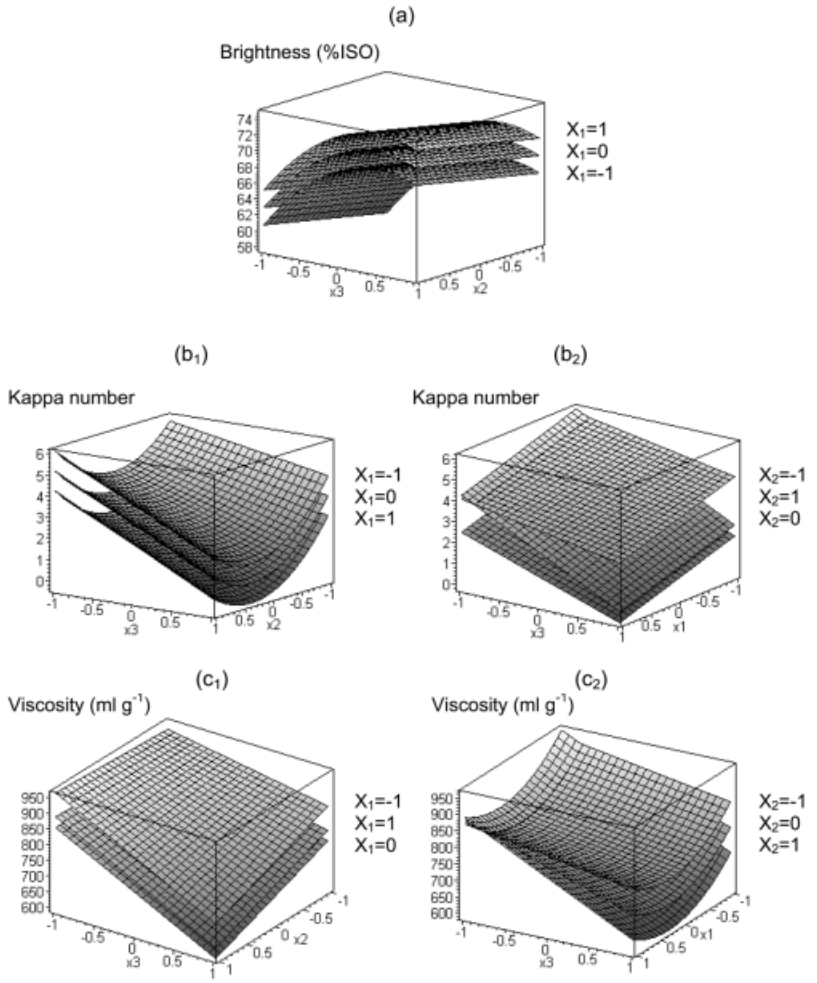

Fig. 2. Variation of estimated properties after LP sequence at three constant laccase dosages (X_1): (a) brightness, (b_1) kappa number, (c_1) viscosity; and three constant HBT dosages (X_2): (b_2) kappa number, (c_2) viscosity.

influences this pulp property *via* a quadratic, positive term (X_2^2). The effect of X_3 is the highest one as in brightness equation. At a fixed laccase dose (Fig. $2b_1$), there is a linear variation with time (X_3) and a quadratic variation with the HBT dose (X_2). With $X_1 = -1$, the variation with time (from 6.14 to 2.19) is greater than with the HBT dose (from 4.53 to 2.19); a minimum occurs at $X_2 = 0.34$ where the kappa number is 1.45. Therefore, maximizing delignification entails using the highest HBT dose corresponding to this value (*viz.* 2.64 % HBT o.d.p.), whatever the laccase dose. Higher HBT doses inhibit the action of laccase (Amann 1997; Li *et al.* 1999). As can be seen from Figure $2b_2$, obtained at a fixed HBT dose, there is a linear variation with time and laccase dose. As the HBT dose is increased, the effect of time changes. Thus, with $X_2 = -1$, the variation of the kappa number with time (from 4.08 to 2.65) is similar to that with the laccase dose (from 4.53 to 2.65).

Viscosity

As shown by Eq. (3), pulp viscosity is influenced by the three variables plus the interaction of the HBT dose with time (X_2X_3) and the quadratic term of the enzyme dose (X_1^2). All terms except the last one have a negative sign. Once again, X_3 is the most influential variable. As can be seen from Figure $2c_1$, obtained at fixed laccase doses, there is a linear variation with time and the HBT dose. With $X_1 = -1$, the variation with time (from 965 to 703 ml g^{-1}) is greater than with the HBT dose (from 856 to 703 ml g^{-1}). At a fixed HBT dose (Fig. $2c_2$), the viscosity varies linearly with time and quadraticaly with the laccase dose. The higher the HBT dose is, the more marked is the adverse effect of time, and the pulp is more strongly degraded as a result of the interaction between time and mediator dose (X_2X_3). When $X_2 = 1$, the viscosity varies more markedly with time (from 889 to 626 ml g^{-1}) than with the laccase dose (from 703 to 626 ml g^{-1}); a minimum (587 ml g^{-1}) occurs at $X_1 = 0.26$. When $X_2 = -1$, the variation with time (from 870 to 780 ml g^{-1}) is virtually the same as that with the laccase dose (from 856 to 780 ml g^{-1}), with a minimum (740 ml g^{-1}) at $X_1 = 0.26$. It is therefore interesting to use low HBT doses in order to avoid excessive degradation of the pulp.

All three variables influence the properties of flax pulp. On the whole, time exerts the strongest effect, followed by the enzyme dose. The interaction between time and mediator dose affects the viscosity and kappa number of the pulp. Figures 3a, b and c were obtained by plotting the experimental values for the properties against the values predicted by the models. The figures also show the widest variation range for each property. The prediction errors for the three pulp properties are small: ± 5 % for brightness, ± 6 % for viscosity and ± 15 % for the kappa number.

Identification of optimum point and model validation

The optimum points for each equation were identified using the Solver application of Excel. As can be seen

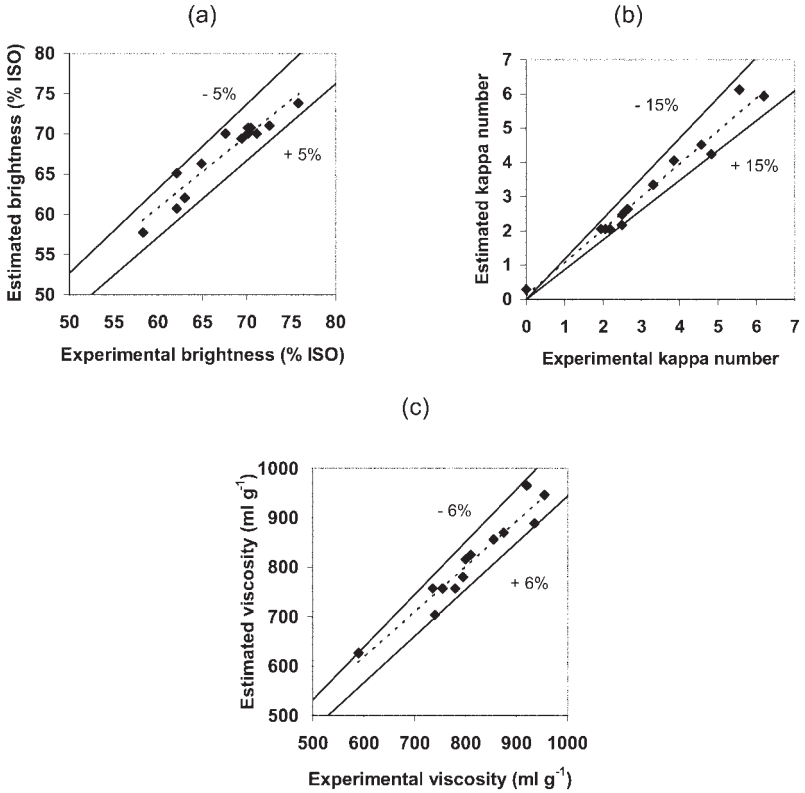

Fig. 3. Experimental vs. estimated values (from models) and errors (LP sequence): (a) brightness, (b) kappa number and (c) viscosity.

Table 3. Optimum values predicted by each modeling equation (LP sequence)

X_1	X_2	X_3	Brightness (% ISO)	Kappa number	Viscosity (ml g^{-1})	Observations
1	1.00	0.505	74.82	1.28	691	Brightness optimum
1	0.34	1.00	72.76	0.00	677	Kappa number optimum
–1	1.00	–1.00	60.72	6.14	965	Viscosity optimum

Table 4. Laboratory experiments in pressurized bioreactor (LoP) and estimated results from the models (LP)

Point	X_1	X_2	X_3	Enzyme dose (U g^{-1})	HBT dose (% o.d.p.)	Time (h)	Brightness (% ISO)	Kappa number	Viscosity (ml g^{-1})
Estimated LP	1	–0.75	–0.5	20	1.375	6.75	67.8	2.92	844
Experimental LoP	1	–0.75	–0.5	20	1.375	6.75	71.9	1.90	815
Estimated LP	1	–0.75	0	20	1.375	12.50	71.0	2.49	816
Experimental LoP	1	–0.75	0	20	1.375	12.50	73.3	1.46	765

from Table 3, the optimum points for brightness and kappa number were very similar, which is consistent with the linear relation they exhibit in Figure 1a. However, the optimum viscosity was rather distant from them. Therefore, a compromise had to be made in choosing the optimum point. Figure 2a shows the variation of brightness as a function of the HBT dose and time at three different enzyme doses ($X_1 = 1, 0$ and -1). In order to set the optimum time, we chose the highest enzyme dose (X_1=1), which was that leading to the highest brightness and lowest kappa number (Figs $2b_1$ and $2b_2$). This laccase dose should be used in conjunction with as short a time and as low an HBT dose as possible. In order to obtain an ISO brightness value of *ca.* 70 % and a high viscosity (around 800 ml g^{-1}), one must use a short time $X_3 = -0.5$ (6.75 h) and an HBT dose not greater than $X_2 = -0.75$ (1.375 % o.d.p.). Consequently, the most suitable optimum point is that at $X_1 = 1$, $X_2 = -0.75$ and $X_3 = -0.5$, which corresponds to a laccase dose of 20 U g^{-1}, an HBT dose of 1.375 % o.d.p. and a reaction time of 6.75 h. Under these conditions, the models predict an ISO brightness of 67.8 %, a kappa number of 2.92 and a viscosity of 844 ml g^{-1} (Table 4).

A new experiment was conducted to validate the proposed models, and the optimum point defined above. Estimated values compared with the experimental values were: for brightness 67.8 % ISO vs. 68.2 % ISO, for kappa number 2.92 vs. 3.44 and for viscosity 844 ml g^{-1} vs. 865 ml g^{-1}. As shown, the experimental values fitted the predictions of the models quite well, thus validating the proposed models.

Biobleaching in a pressurized reactor: The effect of pressure

The statistical study was followed by a test in a stirred reactor under oxygen pressure. Two different treatments were performed, one at the previously chosen optimum point, and the other at $X_1 = 1$, $X_2 = -0.75$ and $X_3 = 0$. As can be seen from Table 4, the results were better than those predicted by the model at both points. Thus, the ISO brightness was 2–4 % higher, delignification was increased by 35–41 % and viscosity was reduced by 50 ml g^{-1} at the most (only slightly lower than estimated at atmospheric pressure). Using high pressure during biobleaching with laccases has a favorable effect as it increases the solubility of oxygen in water. These results are consistent with those obtained in previous studies (Balakshin *et al.* 1999).

Conclusions

The pulp properties brightness, kappa number and viscosity after laccase-mediator treatment followed by a hydrogen peroxide stage were modeled in terms of the variables involved in the biobleaching process. The equations thus derived were used to select an optimum point that was in turn employed to define the most suitable values for the process variables, namely a laccase dose of 20 U g^{-1}, an HBT dose of 1.375 % o.d.p. and a reaction time of 6.75 h. Under these conditions, an ISO brightness of 68.2 %, a kappa number of 3.4 and a viscosity of 865 ml g^{-1} were obtained. A short reaction time and a low HBT dose are desirable for a potential industrial application of the process. In fact, the HBT dose should not exceed 2.64 %; otherwise, delignification of the pulp is inhibited and its degradation favored. The use of a TCF sequence consisting of only two stages (*viz.* the laccase treatment and the subsequent bleaching with hydrogen peroxide) affords very good results in the biobleaching of flax pulp. The results are even better if high pressure is used. The good results obtained testify to the high potential of LMS for the TCF bleaching of flax pulp.

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