Chemical Engineering Journal 152 (2009) 322–329

Contents lists available at ScienceDirect

Chemical Engineering Journal

journal homepage: www.elsevier.com/locate/cej

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

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ARTICLE INFO

Article history:
Received 20 January 2009
Received in revised form 19 May 2009
Accepted 21 May 2009

Keywords:
Bleaching
Brightness
Delignification
Flax pulp
1-Hydroxybenzotriazole dose
Laccase dose
Mathematical model
Oxygen pressure
Treatment time
Optimization
Sequential statistical plan

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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doi:10.1016/j.cej.2009.05.036

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

Table 1
Kappa number, brightness and viscosity in L stage and LE and LR sequences in the preliminary study.

Time	Kappa number			Brightness (%ISO)			Viscosity (mL g^{-1})		
	L	LE	LR	L	LE	LR	L	LE	LR
Initial	10.5 ± 0.1	8.2 ± 0.3	8.9 ± 0.0	36.5	39.8	41.3	932 ± 9	858 ± 6	961 ± 3
1 h	9.0 ± 0.3	4.5 ± 0.1	7.0 ± 0.1	41.3	49.7	41.3	904 ± 22	745 ± 5	907 ± 46
4 h	7.6 ± 0.2	3.0 ± 0.1	5.2 ± 0.1	38.8	54.7	45.5	860 ± 16	736 ± 17	930 ± 16
7 h	7.8 ± 0.2	2.4 ± 0.4	5.4 ± 0.1	41.5	57.4	46.4	820 ± 30	671 ± 11	890 ± 12

oxygen pressure used or in the subsequent washings. Pulp viscosity, however, was not affected by the control treatment.

3.2. Selection of the high optimization level

Such a level was adopted from tests performed in a reactor at 0.6 MPa, using a laccase dose of 25 U g^{-1}, a mediator dose of 3%odp and a treatment time of 1, 4 or 7 h. The degree of delignification of the pulp was calculated from the following equation:

$$\text{degree of delignification } (\%) = \frac{KN_0 - KN_f}{KN_0} 100 \quad (1)$$

where KN_0 initial kappa number and KN_f final kappa number after L or LE.

As can be seen from Table 1, the kappa number changed similarly as a function of time in the three studied sequences (L, LE and LR). Thus, it exhibited a decreasing trend during the first 4 h of treatment, and a slight change between the 4th and 7th hour in LE—but none in L or LR. Subjecting the initial pulp to an E stage (without L stage) the kappa number was reduced by 1.8 units. Therefore, some lignin present in the initial pulp must have been dissolved in the alkaline extraction stage. With an L stage, the degree of delignification of the pulp after LE exceeded that obtained after L by 22% throughout the treatment (from 43 to 51%). Therefore, the L stage alters lignin in such a way that it is rendered insoluble at pH 4 in L and at neutral pH during the subsequent pulp washings, but soluble in the alkaline extraction stage.

Pulp brightness behaved differently after the L stage and the LE and LR sequences (Table 1). In previous studies [24], the brightness decrease at the beginning of the treatment was related to the formation of chromophoric groups in the pulp. The alkaline pH used in the E and R stages altered previously formed groups or resulted in pulp delignification—and in increased brightness as a result. As with the kappa number, the greatest increase in brightness was obtained within the first 4 h of treatment.

Pulp viscosity after L stage and LE sequence decreases with treatment time (Table 1). Alkaline extraction stage produces a viscosity decrease of 150 mL g^{-1} regarding L stage in the first hour of treatment. The L stage can have a twofold effect on cellulose in pulp, namely: (a) direct degradation and (b) alteration of functional groups in it and facilitation of its oxidation in a subsequent alkaline treatment. Because viscosity measurements are made in an alkaline medium, those made after the L stage actually measured the extent of degradation caused by both effects. The reductive stage (R) facilitated reduction of the functional groups in cellulose that were oxidized during L; as a result, no degradation of the previously modified cellulose occurred in the subsequent alkaline stages. These results agree with other authors [14,18].

The viscosity remained virtually constant after LR (Table 1). There was, however, a slight decrease (from 930 to 890 mL g^{-1}) during the first 7 h of treatment. Also, the viscosity values exceeded those obtained after the L stage. Applying a reductive stage in the bleaching sequence should thus allow part of the viscosity lost during the enzyme treatment to be recovered. Therefore, the viscosity loss in the L stage was a result of the two above-described effects. A graphical comparison of the pulp properties after each sequence

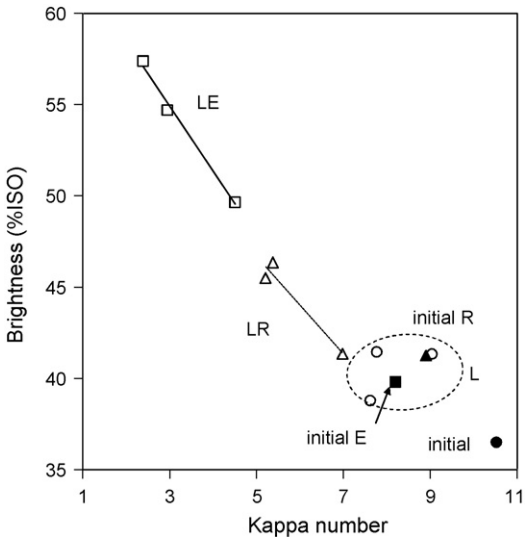

Fig. 1. Relationship between pulp properties in the preliminary study.

reveals correlation between the increase in brightness and reduction in kappa number after the LR and LE sequences (Fig. 1). No such correlation seemingly existed after the L stage. The reduction in kappa number resulted in decreased viscosity after the L stage and the LE sequence. However, the reduction in kappa number after LR was accompanied by no change in viscosity.

The values of the variables used in the statistical plan should result in a fairly large difference between the responses obtained with high and low levels of the variables. Also, the responses should fall in the region of greatest slope change from a level to the next. Because properties varied especially markedly during the first 4 h of treatment, shorter times (0.5–6.5 h) were chosen in the statistical plan. Two of the aims of optimizing the laccase treatment were to reduce the reagent doses – both the enzyme and the mediator are expensive – and avoid the toxic effects of the mediator. Both brightness and the kappa number changed markedly in the tests (to 2.4 and 57.4%ISO, respectively, after the LE sequence). This led us to also lower the high optimization levels for the enzyme and mediator doses to 20 U g^{-1} and 2%odp, respectively.

3.3. Statistical analysis

The four variables were normalized to three different values (−1, 0 and 1) for implementation of the factorial design. Table 2 shows

Table 2
Normalized values of the process variables in the L stage.

Variables	Factors	Normalized values		
		−1	0	1
Laccase dose (U g^{-1})	X_1	1	10.5	20
HBT dose (%odp)	X_2	0.1	1.05	2
Time (h)	X_3	0.5	3.5	6.5
Oxygen pressure (MPa)	X_4	0.2	0.4	0.6

Table 3
Kappa number and brightness of the pulp after L stage.

X_1	X_2	X_3	X_4	Ref.	Kappa number	Brightness (%ISO)
−1	−1	−1	−1	L 1	9.5 ± 0.1	38.6
1	−1	−1	−1	L 2	9.6 ± 0.0	37.3
−1	1	−1	−1	L 3	9.9 ± 0.2	38.6
1	1	−1	−1	L 4	8.6 ± 0.1	36.3
−1	−1	1	−1	L 5	9.5 ± 0.0	39.9
1	−1	1	−1	L 6	8.8 ± 0.2	37.7
−1	1	1	−1	L 7	8.6 ± 0.1	36.9
1	1	1	−1	L 8	7.2 ± 0.1	38.7
−1	−1	−1	1	L 9	10.1 ± 0.1	39.5
1	−1	−1	1	L 10	8.7 ± 0.1	38.7
−1	1	−1	1	L 11	9.5 ± 0.2	38.2
1	1	−1	1	L 12	9.1 ± 0.1	36.8
−1	−1	1	1	L 13	9.1 ± 0.2	39.1
1	−1	1	1	L 14	9.0 ± 0.1	35.6
−1	1	1	1	L 15	9.1 ± 0.1	36.1
1	1	1	1	L 16	7.2 ± 0.2	38.7
0	0	0	0	L 17	7.7 ± 0.3	–
0	0	0	0	L 18	8.0 ± 0.0	–
0	0	0	0	L 19	8.0 ± 0.0	–
−1	0	0	0	L 20	9.7 ± 0.1	–
0	−1	0	0	L 21	9.5 ± 0.0	–
0	0	−1	0	L 22	9.2 ± 0.0	–
0	0	0	1	L 23	8.2 ± 0.0	–
1	0	0	0	L 24	6.7 ± 0.1	–
0	1	0	0	L 25	6.4 ± 0.1	–
0	0	1	0	L 26	6.6 ± 0.0	–
0	0	0	−1	L 27	7.4 ± 0.0	–

the relationship between the process variables and their normalized values in the L stage.

The statistical analysis of pulp properties was based on the results of a planned sequence of tests. The experimental design used to this end was conducted stepwise in each sequence. Thus, the results obtained in each stage were used to decide whether the next was to be performed.

The first step in a sequential plan is establishing a factorial design. The aim is to find a linear model accurately describing the principal variables (factors) and their mutual interactions (Step 1). To this end, the variance at the centre point and the residual mean square of the factorial design are subjected to the homoscedasticity test. If the variance is homogeneous, then the model is homoscedastic, that is, the variance is constant throughout the experimental region examined and the study can be extended to the detection of potential curvature in the fitted data (Step 2). This involves estimating a linear model from the terms deemed significant in the previous factorial design and performing replications at the centre point in order to check whether any quadratic terms are significant (Step 3). If the representative quadratic term is significant, then the design is expanded with the test star in order to deconvolute quadratic terms (Step 4). Finally, the resulting model is verified (Step 5).

3.3.1. Statistical analysis of the kappa number in the L stage

3.3.1.1. Step 1: kappa number: study of the 2^4 factorial design. The 2^4 design was established from tests L 1 to L 16 (see Table 3) and the probability graph for the saturated model obtained (Eq. (2) and Fig. 2a). Those coefficients falling on the right of the significance line in the graph and departing from it were deemed significant. This allowed non-significant coefficients for the saturated model to be discarded. The model constructed from the coefficients deemed significant in the previous graphical analysis was used to determine the new coefficients for the kappa number and their significance (Eq. (3)). All terms in the model were significant ($p < 0.05$).

$$Y_{\text{KN-L}} = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{14}X_1X_4 + b_{23}X_2X_3 + b_{24}X_2X_4 + b_{34}X_3X_4 + b_{123}X_1X_2X_3 + b_{234}X_2X_3X_4 + b_{124}X_1X_2X_4 + b_{134}X_1X_3X_4 + b_{1234}X_1X_2X_3X_4 \quad (2)$$

$$Y_{\text{KN-L}} = 9.0 - 0.45X_1 - 0.33X_2 - 0.42X_3 - 0.17X_1X_2 - 0.22X_2X_3 - 0.13X_1X_2X_3 - 0.22X_1X_2X_3X_4 \quad (3)$$

3.3.1.2. Step 2: study of the variance. In order to detect potential curvature in a model, variance must be checked in order to verify if it remains constant throughout the experimental field studied. By comparing the residual mean square (RMS = 0.48) and variance for the three centre points ($S_c^2 = 0.03$), the model for the kappa number was found to be homoscedastic ($p = 0.80 > 0.05$), i.e. the variance was constant.

3.3.1.3. Step 3: is it influenced by quadratic terms?. In order to facilitate detection of potential curvature, the model provided by the previous 2^4 factorial design was expanded with the 8 coefficients previously deemed significant and that representing quadratic terms. The design matrix contained the central tests L 17 to L 19 (Table 3) and a 2^4 design with 3 central responses. Based on the statistical analysis for curvature, the coefficient for the quadratic terms was 1.0 and significant ($p = 0.00$). The central tests failed to identify the individual factor influencing the response in a quadratic manner; this required using additional tests in the statistical study.

3.3.1.4. Step 4: which factors have a quadratic effect?. In order to identify those factors exerting a quadratic effect on the response, the design was expanded with the star points (tests L 20 to L 27) in order to deconvolute quadratic terms (Table 3). The model thus obtained using the 2^4 design with 3 central tests and 8 star points was also examined.

The final model was constructed from 27 responses and fitted Eq. (4). The coefficient of determination (R^2) was 0.66 and the probability associated to F_{calc} 0.00. Table 4 shows the coefficients of the model and their significance. The combination of all quadratic coefficients was 0.96, which is similar to the previously calculated coefficient representing the quadratic terms.

$$Y_{\text{KN-L}} = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_2^1 \quad (4)$$

3.3.1.5. Step 5: verification of the final model. Fig. 2 shows the verification graphs for the model. As can be seen from Fig. 2b, the residuals distributed according to no well-defined pattern as a function of the estimated responses. Also, the kappa number responses predicted by the model, and the experimental responses, distributed around a straight line, the differences between the two ranging from −24 to 12%. In addition, the points in the normal probability graph Z:N(0,1) vs. residuals fitted a straight line (Fig. 2c). Based on these results, the model was deemed statistically accurate.

Table 4
Coefficients of the final model (Eq. (4)) as obtained from 27 responses of the kappa number in the L stage.

Coefficients	Estimated coefficients	Standard error	t value	Significance
b_0	7.9	0.23	35.1	0.00
b_1	−0.55	0.16	−3.47	0.00
b_2	−0.46	0.16	−2.89	0.01
b_3	−0.52	0.16	−3.24	0.00
b_{11}	0.96	0.28	3.46	0.00

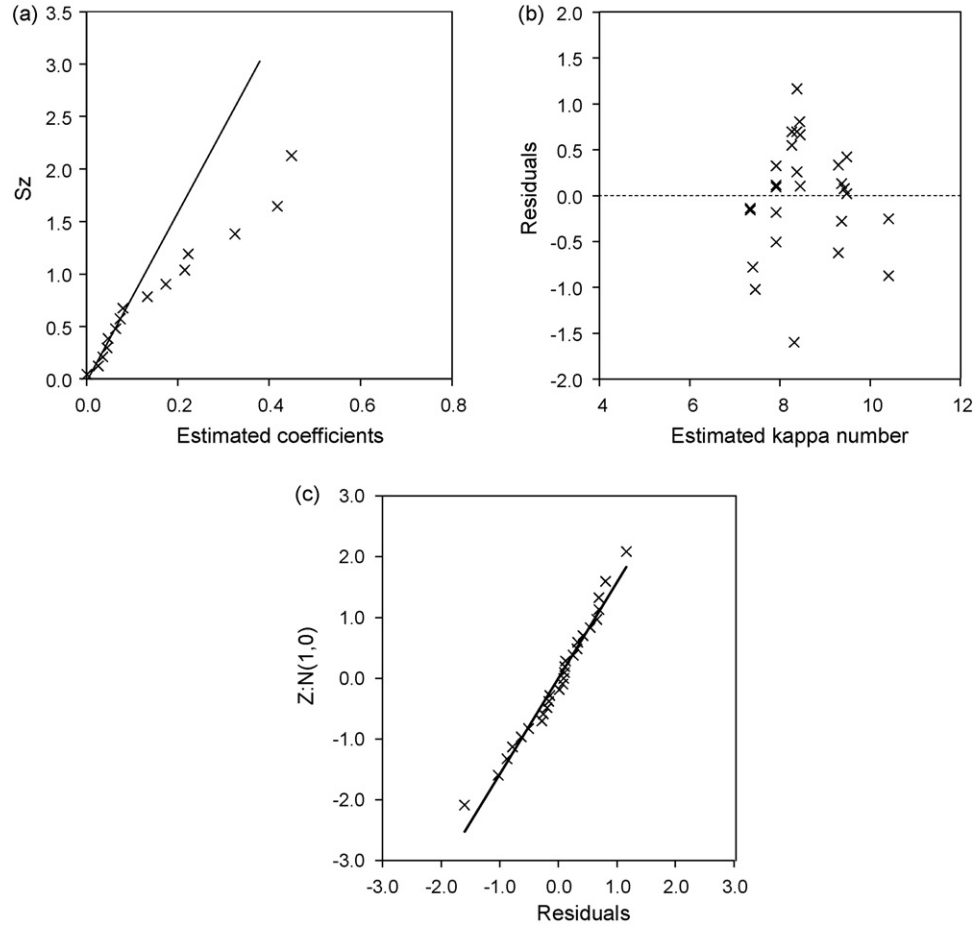

Fig. 2. Probability graphs of the model for the kappa number in the L stage. (a) Semi-normal graph of the saturated model. (b) Residuals vs. estimated kappa number. (c) Residuals vs. Z in the normal law.

3.3.2. Statistical analysis of brightness in the L stage

3.3.2.1. Step 1: brightness: study of the 2^4 factorial design. Because the 2^{4-1} factorial design allowed the establishment of no accurate model to predict brightness, a 2^4 design was tested instead. Such a design was established from tests L 1 to L 8, L 12 to L 14 and L A to L E (see Table 3) and the corresponding probability graph for the saturated model obtained (Eq. (5) and Fig. 3). The points in the semi-zeta graph grouped in no definite manner. This may have been the result of (a) heteroscedasticity (a non-constant variance), (b) the factor having little influence on the response or (c) excessive experimental noise. Available data allowed no accurate model for brightness after the L stage to be constructed from the process variables.

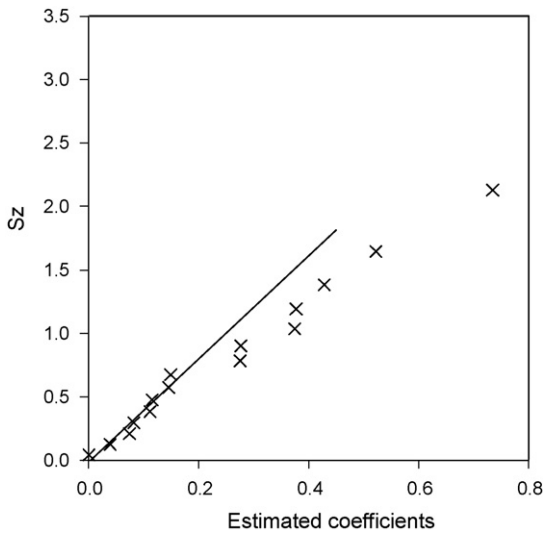

Fig. 3. Semi-normal graph of the saturated model for brightness in the L stage.

$$Y_{\text{B-L}} = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{14}X_1X_4 + b_{23}X_2X_3 + b_{24}X_2X_4 + b_{34}X_3X_4 + b_{123}X_1X_2X_3 + b_{234}X_2X_3X_4 + b_{124}X_1X_2X_4 + b_{134}X_1X_3X_4 + b_{1234}X_1X_2X_3X_4 \quad (5)$$

3.4. Pulp property models

3.4.1. Model for the kappa number in the L sequence

The model relating the kappa number of the pulp after the L stage to the process variables fitted Eq. (6) and predicted kappa numbers from 10.4 to 6.9. The variables influencing the kappa number were X_1 (laccase dose), X_2 (mediator dose) and X_3 (treatment time). The oxygen pressure in the reactor, X_4, had no effect on this property. The most influential variable in the studied experimental region was X_2 (the HBT dose), with a coefficient of −0.55. All binary interactions between the variables laccase dose (X_1), mediator dose (X_2) and treatment time (X_3) do not influence the kappa number. The factor laccase dose had a quadratic influence on the response (X_1^2). An increase in the individual factors resulted in a decrease in kappa number whereas the quadratic terms tended to increase it.

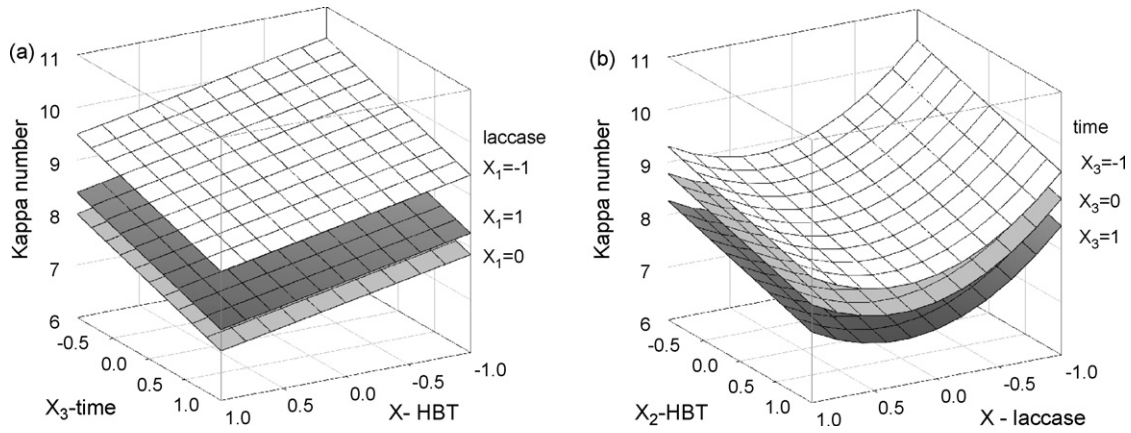

Fig. 4. Variation of the kappa number in the L stage as a function of the factors of the statistical plan.

$$Y_{\text{KN-L}} = 7.9 - 0.55X_1 - 0.46X_2 - 0.52X_3 + 0.96X_2^1 \quad (6)$$

where X_1 = (L − 10.5)/9.5, L = laccase dose (U g^{-1}); X_2 = (M − 1.05)/0.95, M = HBT dose (%odp); X_3 = (t − 3.5)/3, t = reaction time (h).

Fig. 4 shows the surface plots for the model corresponding to the equation obtained with one of the variables at its low, medium or high level, and Fig. 5 the contour lines obtained with one of the factors at its high level, and also the combinations of reagent doses and time which afford a given brightness level.

The kappa number decreased to a minimum value with increasing value of each factor beyond which it became slightly greater (Fig. 4b). The smallest kappa number provided by the statistical plan was 6.9 and corresponded to X_1 = 0.29, X_2 = 1 and X_3 = 1 (viz. a laccase dose of 13 U g^{-1} an HBT dose of 2%odp and a treatment time of 6.5 h). Therefore, the smallest possible kappa number was obtained at the highest HBT dose and longest time used in the plan. Increasing the HBT dose and treatment time over the studied ranges decreased the kappa number irrespective of the particular laccase dose used (Fig. 4a). The model predicted a limiting laccase dose above which a further increase would result in no additional reduction in kappa number. This result suggests that some lignin was inaccessible to the reagents and remained in the pulp, thereby preventing the L treatment form further reducing the kappa number [25].

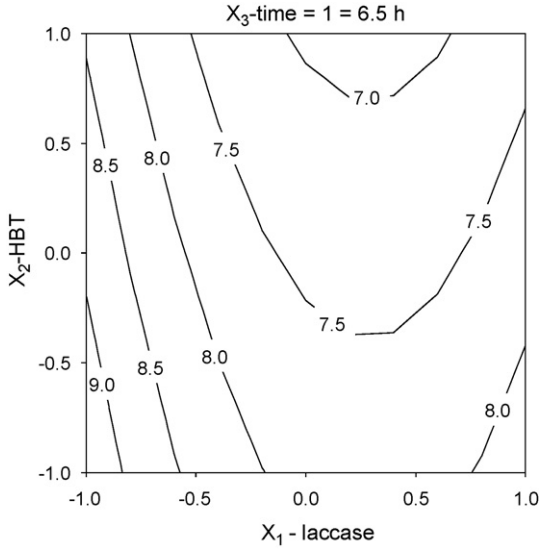

Fig. 5. Contour lines for kappa number in the L stage as obtained at a treatment time X_3 = 1 (6.5 h).

When laccase and HBT doses are low (X_1 = −1, X_2 = −1) there is practically no delignification (Fig. 4a), kappa number is 10.4 at 0.5 h and decrease to 9.4 at 6.5 h. By other hand, when both doses are high the kappa number is reduced in the first 30 min to 8.4 h and to 7.4 at 6.5 h. Independently of the doses, increasing the treatment time from 30 min to 6.5 h reduced the kappa number by 1 unit (the curves obtained at the different times were parallel). As can be seen from Fig. 4b, with a low laccase dose (X_1 = −1) and a high HBT dose (X_2 = 1), the kappa number remained unchanged at 9.5 over the first 30 min of treatment. Increasing the time from 0.5 to 6.5 h the kappa number is reduced to 8.5.

As can be seen in Fig. 4, increasing laccase dose from a low value resulted in substantial reductions in kappa number. As the dose was increased and the kappa number approached its limit, obtaining an equivalent reduction required using a substantially higher enzyme dose. Increasing HBT dose from 0.1 to 2%odp, similarly to time, reduced the kappa number by 1 unit, the curves obtained were parallel (Fig. 4).

3.4.2. Determination of the reagent limiting dose

Equation Eq. (7), which was obtained by zeroing the derivative of the model equation with respect to factor X_1, allowed the limiting laccase dose, $X_{1-\text{lim}}$, above which a further increase would provide no additional decrease in kappa number to be calculated. Based on Eq. (7), the limiting dose, $X_{1-\text{lim}}$ = 0.29 (13 U g^{-1}), provided the smallest possible kappa number: 6.9. However, a lower laccase dose resulted in similar reductions.

$$X_{1-\text{lim}} = \frac{-b_1}{2\ b_{11}} = 0.29 \quad (7)$$

3.4.3. Determination of the reagents optimal doses

Obtaining a kappa number less than 7.0 required using a time level X_3 = 1 and a laccase dose X_1 < 1 (Fig. 5). With X_3 = 1 and the lowest possible HBT dose, the laccase dose (X_1) should exceed 0.3; also, the HBT dose (X_2) should exceed 0.6. Accepting a 0.5 unit greater kappa number (i.e. 7.5 instead of 7) would provide a 30% saving in HBT, since X_2 should be −0.3 (0.8%odp) and laccase dose X_1 = 0 (10 U g^{-1}).

3.4.4. Brightness in the L sequence

Brightness ranged from 35.6 to 39.9%ISO (Table 3). As noted earlier, no accurate mathematical model for this pulp property could be established. Pulp brightness was correlated with none of the process variables. In fact, it was uncorrelated with the kappa number (Fig. 6) – as is usually the case with conventional bleaching processes – and hence with the reduction in lignin content of the pulp. As revealed by the pulp color tests in previous studies [24],

Table 5
Viscosity after L stage.

Ref.	Laccase dose (U g^{-1})	HBT dose (%odp)	Time (h)	Oxygen pressure (MPa)	Viscosity (mL g^{-1})
Low	0.5	0.1	0.5	0.2	930 ± 38
				0.6	939 ± 11
Medium	10	1.1	3.5	0.4	876 ± 16
				0.4	857 ± 02
High	20	2	6.5	0.6	843 ± 08
				0.2	835 ± 15
Initial	–	–	–	–	952 ± 10
Control	–	–	6.5	0.6	947 ± 20

the brightness difference between enzyme-treated and initial pulp was a result of the formation of chromophores during the first few hours of treatment.

3.4.5. Viscosity in the L sequence

Table 5 gives the viscosity values obtained after the L stage by using the variables at their low, medium and high levels. The response ranged from 835 to 939 mL g^{-1}. Pulp viscosity decreased with increasing reagent doses and treatment time. However, the results allowed no individual factor to be identified as that resulting in the greatest viscosity loss. Such a loss was a consequence of direct degradation of cellulose, alteration of its functional groups or both. The greatest viscosity change was 104 mL g^{-1}, which was deemed inadequate to develop an accurate mathematical model to predict the viscosity as a function of the variables of the L stage. A graphical comparison of the pulp properties studied reveals that the viscosity and kappa number followed a clear-cut trend that fitted a straight line with a coefficient of determination R^2 better than 0.94 (Fig. 7); there was thus a linear relationship between both properties. Therefore, the viscosity may be influenced similarly to the kappa number by the process variables.

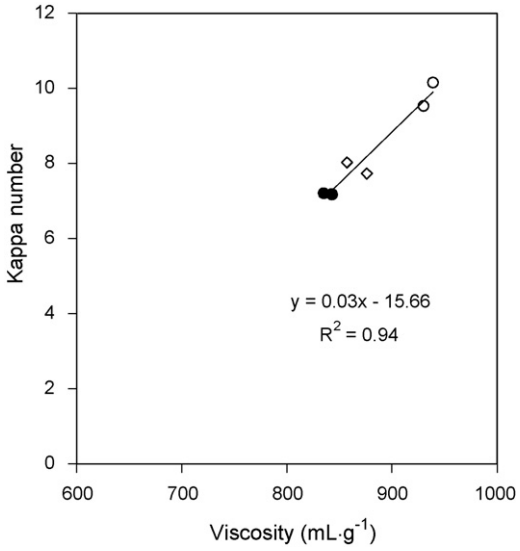

Fig. 7. Viscosity vs. kappa number in L stage. In the figure, $X_1 = X_2 = -1$ (○), $X_1 = X_2 = 0$ (□) and $X_1 = X_2 = 1$ (●).

3.4.6. LP sequence

Kappa number and brightness after the LP sequence in those enzymatic treatments when process variables in L stage are in a low level are (5.6 ± 0.1 and 60.7%ISO) and in a high level (2.4 ± 0.1 and 75.2%ISO). A P stage produces an increase in pulp delignification and brightness regarding enzymatic treatment. Pulp properties after P stage depends on L stage variables. Differences of 3.2 and 9.5%ISO on kappa number and brightness, respectively, can be observed between both treatments. The LP biochemical sequence allows obtaining a high final brightness on flax pulp.

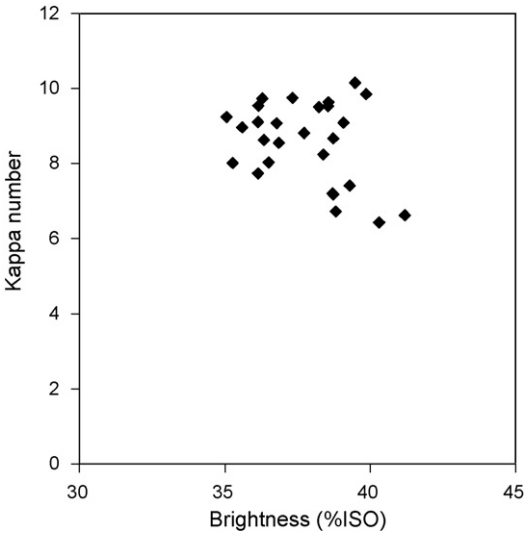

Fig. 6. Brightness vs. kappa number in L stage.

4. Conclusions

Flax pulp was bleached by using laccase–HBT system, where their operating conditions were optimized. The pre-experiences (high HBT and laccase doses and pressure, and variable treatment times) revealed that the pulp properties obtained after the L stage, and the LE and LR sequences, were essentially identical with treatment times from 4 to 7 h. This, together with the need to minimize the laccase and HBT doses, led us to reduce the reagent doses and reaction time to be used in the optimization process.

The ranges of the operational variables were: 1–20 U g^{-1} for the laccase dose, 0.1–2%odp for the HBT dose, 0.5–6.5 h for the treatment time and 0.2–0.6 MPa for the oxygen pressure inside the reactor. A comparison of the properties of the control pulp with those of the pulp obtained after the L stage revealed that the treatments involving a low laccase and HBT doses resulted in properties similar to those provided by the control treatment. Therefore, the laccase-mediator system is ineffective with a laccase dose of 1 U g^{-1} and an HBT dose of 0.1%odp.

The experimental responses of the statistical study revealed that changing the oxygen pressure in the reactor from 0.2 to 0.6 MPa had no effect on delignification; therefore, a pressure of 0.2 MPa was enough for the intended purpose. The kappa number decreased with increasing value of each process variable up to a limiting level of 6.9, which was obtained with a laccase dose of 13 U g^{-1}, an HBT dose of 2%odp and a time of 6.5 h. The model predicted a limiting

laccase dose of 13 U g^{-1} above which further increasing it would result in no additional reduction in kappa number. On the other hand, the model predicted no limiting HBT dose or treatment time; therefore, increasing the HBT dose and treatment time above 2%odp and 6.5 h, respectively, might in theory reduce the kappa number below 6.9. The kappa number obtained in the tests involving somewhat higher values of the process variables (viz. a laccase dose of 25 U g^{-1}, an HBT dose of 3%odp, a treatment time of 7 h and an oxygen pressure of 0.6 MPa) was 7.8, which is higher than the smallest level provided by the statistical plan. Therefore, further increasing the HBT dose and/or treatment time would probably not reduce the kappa number in a substantial manner.

No accurate mathematical model relating the brightness of pulp to the process variables could be obtained. Unlike conventional bleaching processes, brightness was not correlated with the kappa number, nor with the reduction of the amount of lignin in the pulp as a result. This behavior, and the increased or decreased brightness of enzyme-treated pulp relative to the initial pulp, was a result of the formation of chromophores in the pulp during the first few hours of treatment.

Pulp viscosity decreased with increasing reagent doses and treatment time. This viscosity loss was a result of direct degradation of cellulose, alteration of its functional groups or both. The greatest viscosity change was 104 mL g^{-1}, which was deemed inadequate to establish an accurate mathematical model for viscosity in terms of the process variables of the L stage.

Pulp properties after P stage depends on L stage variables. Differences of 3.2 and 9.5%ISO on kappa number and brightness, respectively, can be observed between both treatments. The LP biochemical sequence allows obtaining a final brightness of 75.2%ISO.

Acknowledgements

This work was supported by a Spanish MEC ENZPULP Project (CTQ2005-08925-C02-01) and a BIORENEW Integrated European Project (NMP2-CT-2006-026456). Dr. U. Fillat is grateful to Department of University, Research and Society of the Information of the Generalitat de Catalunya and the Social European Fund for the research fellowship (2005 FI grant). The authors are gratefully to CELESA (Spain) for providing starting pulp and Novozymes (Denmark) for supply the laccase.

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