Optimization of Laccase-Aided Chlorine Dioxide Bleaching of Bagasse Pulp

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The laccase-mediator system in laccase-aided chlorine dioxide bleaching of bagasse pulp was optimized using response surface methodology (RSM). The effects and interactions of the laccase enzyme dosage, the dosage of the mediator 1-hydroxybenzotriazole (HBT), and the reaction time on the adsorbed organic halogen (AOX) content of the wastewater as well as the brightness and kappa number of the pulp were examined. The optimal reaction conditions to achieve a balance of lower AOX content, higher brightness, and lower kappa number were as follows: laccase enzyme dosage of 20.3 U/g, HBT dosage of 1.51%, and reaction time of 154.5 min. Under these conditions, an AOX content of 20.67 mg/L, brightness of 58.94% ISO, and kappa number of 2.71 were observed. These results will offer a favorable option for pulp and paper mills as well as the natural environment and therefore provide a theoretical foundation for the industrial application of laccase in bleaching processes.

Keywords: Response surface methodology; Laccase; AOX; ECF; Bagasse soda pulp

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INTRODUCTION

Chlorine dioxide is the most important bleaching chemical in the elemental chlorine-free (ECF) bleaching of pulp. It has shown a series of fundamental benefits compared with traditional bleaching chemicals (Bajpai *et al.* 2006; Nie *et al.* 2014a). ECF bleaching can restrain the formation of adsorbed organic halogen (AOX) and other chlorine compounds (Li *et al.* 2014). AOX is regarded as one of the most generally significant criteria to assess the composition of a bleaching effluent (Nie *et al.* 2014b). Many governments have set emission standards for these compounds, especially AOX. The Chinese government, for example, has imposed a limit for AOX discharge, stipulating the AOX content of a bleaching effluent discharged to be no more than 12 mg/L (Zhang *et al.* 2012). To meet these limits, many techniques and process optimizations have been investigated to eliminate the discharge of AOX from laboratories and the papermaking industry, including end-of-pipe treatment techniques and modification of bleaching technologies (Savant *et al.* 2006). Unfortunately, the utilization of these techniques generates new environmental problems such as extra expenses for disposal of waste from treatment facilities (Savant *et al.* 2006). Reducing the use of chlorinated bleaching chemicals is crucial for decreasing the discharge of AOX in bleaching effluents; therefore, considerable attention has recently been shifted to exploring cost-effective and environment-friendly bleaching technologies for reducing AOX generation (Sharma *et al.* 2014).

In all of the environmentally friendly bleaching technologies, biobleaching with enzymes has shown tremendous potential for decreasing the use of chlorinated bleaching chemicals (Singh *et al.* 2008). Laccase is a representative biological enzyme for pulp bleaching and an effective lignin degrader (Knezevic *et al.* 2013a,b). It can selectively degrade the lignin of paper fibers (Jialin *et al.* 2006; Qiu and Chen 2012; Quintana *et al.* 2015), imposes little damage on the cellulose of paper fibers (Bourbonnais *et al.* 1995), and has been used in labs and at the pilot scale (Euring *et al.* 2011; Goncalves *et al.* 2014; Martin-Sampedro *et al.* 2015). Laccase-aided bleaching can reduce the use of chlorine dioxide in ECF bleaching and reduce the formation of organic chlorine compounds in the bleaching effluent (Bajpai *et al.* 2006). Other benefits of laccase-aided bleaching are an improved pulp yield and a lower capital investment (Bajpai *et al.* 2006). Baker *et al.* (2015) found that rot fungi able to secrete laccase showed higher delignification on pressure-refined *Miscanthus* than milled *Miscanthus* (Baker *et al.* 2015). Thaku *et al.* found a reduction in demand of chlorine dioxide by more than 35% during enzymatic pre-treatment on kraft pulps from wood and nonwood based raw materials (Thaku *et al.* 2012). Sharma *et al.* reported a 35% ClO_2 reduction and a 34% AOX content reduction in ECF bleaching during enzymatic pre-treatment of eucalyptus kraft pulp (Sharma *et al.* 2014). Another experiment obtained an increase in brightness of 40% ISO and 80% delignification in a laccase-aided bleaching of flax pup (Fillat and Roncero 2010). One experiment saw the kappa number reduced by 21.1% and increases in brightness, tear index, and burst index of 5.89%, 8%, and 18%, respectively, in laccase-aided bleaching of wheat straw-rich soda pulp (Singh *et al.* 2008).

Optimization through factorial design and response surface methodology (RSM) has been a common practice in biotechnology (Kalil *et al.* 2000). RSM is widely applied in chemical (Aljundi 2011), biology (Trawczynska and Wojcik 2015), enzyme, and other fields for the optimization of all kinds of biochemical, biotechnological, and microbiological products (Beg *et al.* 2003). It minimizes experimentation and time and is far more efficient than traditional methods for optimizing such a process (Nie *et al.* 2013). This technique to establish optimal process designs for the process of AOX generation in enzymatic ECF bleaching, however, is not available; therefore, the present work was an attempt to employ RSM to optimize the key parameters of enzymatic ECF bleaching of soda AQ bagasse. The parameters investigated include laccase enzyme dosage, HBT dosage, and reaction time, which are critical factors for the AOX content of the bleaching effluent, as well as brightness and kappa number of the pulp.

EXPERIMENTAL

Materials

Unbleached bagasse pulp (brightness 38.97% ISO, viscosity 1280.86 mL/g, and kappa number 12.51) used in the bleaching experiments was obtained from the Pumiao Paper Mill (Guangxi, China). The laccase was supplied by Shanghai Yongye Bio-Technology Co., Ltd. (Shanghai, China). The laccase had a laccase activity of 2000 U/g. Chlorine dioxide solution stored in a brown bottle was also collected from the Pumiao Paper Mill (Guangxi, China) (available chlorine concentration 21.58 g/L). Ceramic wool and activated carbon were procured from Analytik-Jena Instrument

Company (Jena, Germany), and all other chemicals used in this study were purchased from Aladdin Reagent (Shanghai) Co., Ltd. All of the chemicals were of analytical grade, unless otherwise mentioned.

Methods

The laccase activity was determined by monitoring the oxidation of 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) as increased absorbance at 420 nm. The reaction mixture contained 2.5 mL of sodium acetate buffer (pH 4.5), 0.1 mL of the enzyme sample, and 0.4 mL of 1 mM ABTS, in a final volume of 3.0 mL. In this study, 1 U is the amount of enzyme that converts 1 µmol of substance in one minute under the described conditions at 25 °C (Bajpai *et al.* 2006).

Laccase enzyme biobleching treatment of the unbleached bagasse pulp was carried out in a micro-reactor at 50 °C. The biobleaching treatment conditions and the variable levels are given in Table 1. Other constant parameters needed in this biobleching treatment were as follows: surfactant (Tween® 80) 0.05%, pulp consistency of 10% (buffer solution of sodium acetate and acetic acid), oxygen pressure 0.4 MPa, and rotating speed 60 rpm. After laccase enzyme biobleaching treatment, the pulps were washed to neutral pH with distilled water. The washed pulps were then used for chlorine dioxide bleaching.

Table 1. Range and Levels of the Three Independent Variables

Variables	Range and Levels						
	1	2	3	4	5	6	7
Laccase Enzyme Dosage (U/g)	0	5	10	15	20	25	30
HBT Dosage (%)	0	0.5	1.0	1.5	2.0	2.5	3.0
Treatment Time (min)	0	40	80	120	160	200	240

The washed pulps, chlorine dioxide solution, and sulfuric acid solution were mixed in a plastic bag with a pulp consistency of 10%. Other parameters used during chlorine dioxide bleaching were a chlorine dioxide dosage of 3.7% and a sulfuric acid solution pH value of 4.5. Then the bags were heated in a water bath at 70 °C for 1 h with kneading every 15 min. After bleaching, the pulps were washed to neutral pH with distilled water. The bleached pulps were placed in a lab with constant temperature and constant humidity for 24 h and then subjected to characterization. In the control experiment, the laccase enzyme biobleching treatment of the unbleached bagasse pulp was replaced with the buffer solution alone, and the subsequent conditions used for chlorine dioxide bleaching were the same as those for the experimental samples. All of the experiments were conducted in triplicate, and the mean values were recorded.

The brightness of the pulp was measured according to (TAPPI T452 om-02), and kappa number indicating the residual lignin in the pulp was determined according to (TAPPI T236 om-99).

The AOX content was measured by a Multi X 2500 halide analyzer (Jena, Germany). The process was as follows: first, the diluted bleaching effluent was passed through an activated carbon column and the organic chlorine of the bleaching effluent was adsorbed by the activated carbon; second, the adsorbed inorganic chlorine in the activated carbon column was washed with sodium nitrite; finally, the activated carbon

column was burned in a combustion furnace, after which the AOX content was calculated by the micro Coulomb titration method (Nie *et al.* 2013, 2014a,b).

According to the center combination experimental design theory of Box-Behnken (Box and Draper 1987), RSM was employed to optimize the bleaching process conditions of the unbleached bagasse pulp. In the current research, three variables, laccase enzyme dosage (X1), HBT dosage (X2), and pretreatment time (X3), were investigated at three levels (-1, 0, and +1) to assess the effects and interactions of the factors. The brightness and kappa number of the pulp and the AOX content of the bleaching effluent were chosen as the dependence response variables to assess the bleaching effect. With 5 experiments at the central level as replicates, the research included 17 total runs (Table 2).

RESULTS AND DISCUSSION

RSM Analysis

Table 2. Box-Benhnken Results and the Values of the Three Response Variables

Run	Laccase Dosage, X_1 (U/g)	HBT Dosage, X_2 (%)	Reaction Time, X_3 (min)	Brightness (%ISO)	Kappa Number	AOX (mg/L)
1	15	1.5	120	59.03	2.80	21.03
2	0	0	120	51.74	3.99	26.95
3	0	1.5	240	52.31	3.76	25.55
4	15	3	0	54.18	3.66	23.89
5	30	1.5	240	56.83	2.93	21.42
6	15	0	240	54.83	3.24	23.49
7	30	0	120	55.09	3.51	23.49
8	0	1.5	0	54.76	3.81	25.32
9	15	1.5	120	58.86	2.82	21.01
10	15	1.5	120	58.61	2.79	20.98
11	30	3	120	53.43	3.24	22.66
12	15	1.5	120	58.89	2.81	21.16
13	30	1.5	0	53.31	3.39	23.22
14	0	3	120	53.94	4.41	24.85
15	15	3	240	56.41	3.66	24.22
16	15	0	0	53.72	3.64	24.89
17	15	1.5	120	58.98	2.83	21.06

Based on the collected data (Table 2), regression analysis was used and was integrated into the aposteriori second-order polynomial model (Aljundi 2011) to

analyze whether there was correlation between the factors and the response variables (Trinh and Kang 2011). The second-order quadratic model is shown as Eq. 1,

$$Y_n = \beta_0 + \sum_{a=1}^{3} \beta_a X_a + \sum_{a=1}^{3} \beta_{(a+3)} X_a^2 + \sum_{b=2}^{3} \sum_{a=1}^{a<b} \beta_{(a+b+4)} X_a X_b \quad (1)$$

where Y_n is the predicted response variable (brightness, kappa number, and AOX content); n is the factor number; X_a and X_b are independent factors affecting Y_n; β_0 is the intercept; and β_a, $\beta_{(a+3)}$, and $\beta_{(a+b+4)}$ are the first-order coefficients for the linear term, the second-order quadratic term, and the interaction term (Trinh and Kang 2011). Using the Box-Benhnken design in Design Expert V8.0.6.1 (Statease Inc., Minneapolis, USA), the coefficients were evaluated by multiple linear regression analysis. Parameters with less than 0.95 significance ($p>0.05$) were added to the error term. The three-dimensional response surface and contour plots of the response models were plotted to characterize the effects and interactions of the invariables on brightness, kappa number, and AOX content. Additional experiments were carried out to validate the mathematical model.

Assessment of Experimental Results for AOX Content of the Effluent

The second-order quadratic polynomial shown by Eq. 2 for AOX content was obtained by multiple linear regression analysis.

$$Y_1 = 21.04 - 1.49X_1 - 0.4X_2 - 0.33X_3 + 1.6X_1^2 + 1.84\,X_2^2 + 1.23\,X_3^2 + 0.32\,X_1\,X_2 - 0.51\,X_1\,X_3 + 0.43\,X_2\,X_3 \quad (2)$$

The polynomial shows that the quadratic model was in reasonable accordance with the experimental results ($R^2 = 0.9614$), where an appropriate model fit should be greater than 0.8 (Shukla *et al.* 2014). Figure 1 shows the close relationship between the actual values and the model values, which indicates a good fit of the second-order quadratic polynomial to the actual data.

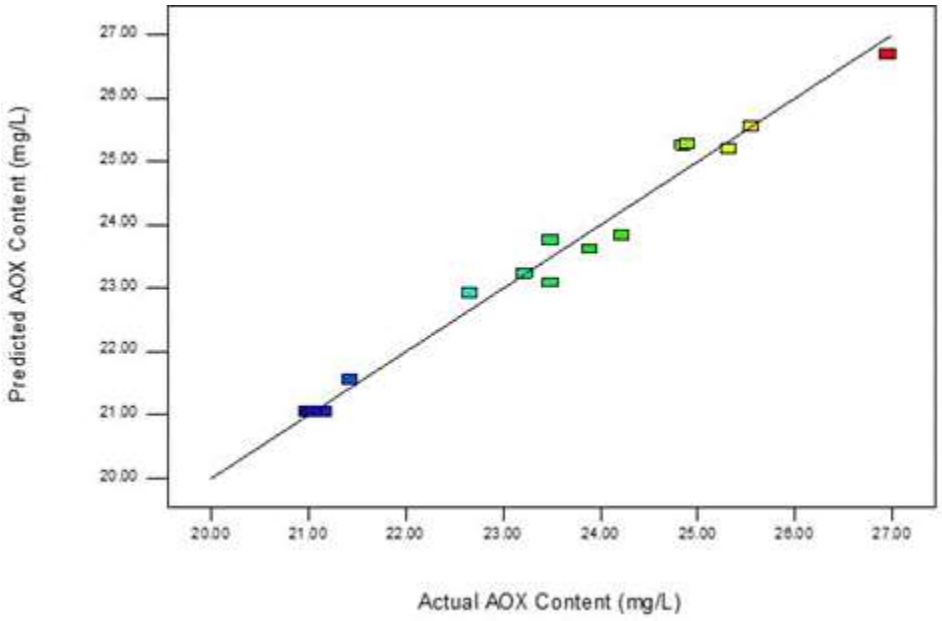

Fig. 1. Predicted *vs*. actual values for AOX content

Analysis of variance (ANOVA) results (Table 3) showed that the X_1, X_2, X_3, X_1X_3, X_1^2, X_2^2, and X_3^2 terms were significant ($p<0.05$). The p-value for lack of fitness was 0.0007. Lack of fit was significant ($p<0.05$) and the model equation was adequate. The predicted value of the sum of squares was 57.06, indicating that the model had a higher predictability and accuracy (Singh *et al.* 2008). The value of Prob > F, which infers significance, was < 0.0001, indicating that the model obtained was highly significant.

The noise ratio of 19.681 was greater than the standard value of 4, which indicates an adequate signal. Thus, the model can be used to navigate the design space. The best combination was a laccase enzyme dosage of 22.44 U/g, HBT dosage of 1.56%, and reaction time of 147.5 min. Under these conditions, the AOX content was 20.63 mg/L.

Table 3. ANOVA for Response Surface Quadratic Model of AOX Content

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob>F
Model	57.06	9	6.34	45.32	<0.0001
X_1	17.67	1	17.67	126.30	<0.0001
X_2	1.28	1	1.28	9.16	0.0192
X_3	0.87	1	0.87	6.19	0.0417
X_1X_2	0.40	1	0.40	2.88	0.1337
X_1X_3	1.03	1	1.03	7.36	0.0300
X_2X_3	0.75	1	0.75	5.36	0.0537
X_1^2	10.79	1	10.79	77.10	<0.0001
X_2^2	14.29	1	14.29	102.11	<0.0001
X_3^2	6.42	1	6.42	45.86	0.0003
Residual	0.98	7	0.14		
Lack of Fit	0.96	3	0.32	66.07	0.0007
Pure Error	0.019	4	4.844E-003		
Cor Total	58.04	16			

The three-dimensional response surface and contour plots for AOX yield are shown in Fig. 2. The responses for the variables were essentially in agreement with the models.

According to the plots, at the best laccase enzyme dosage, the AOX content was relatively lower than those for the other values of laccase enzyme dosage. At the best HBT dosage, the AOX content was also relatively lower. The content of HBT was too low to degrade the residual lignin; however, the content of HBT was too high and inhibited the combination of laccase and residual lignin, leading to the increased content of AOX in the bleaching effluent.

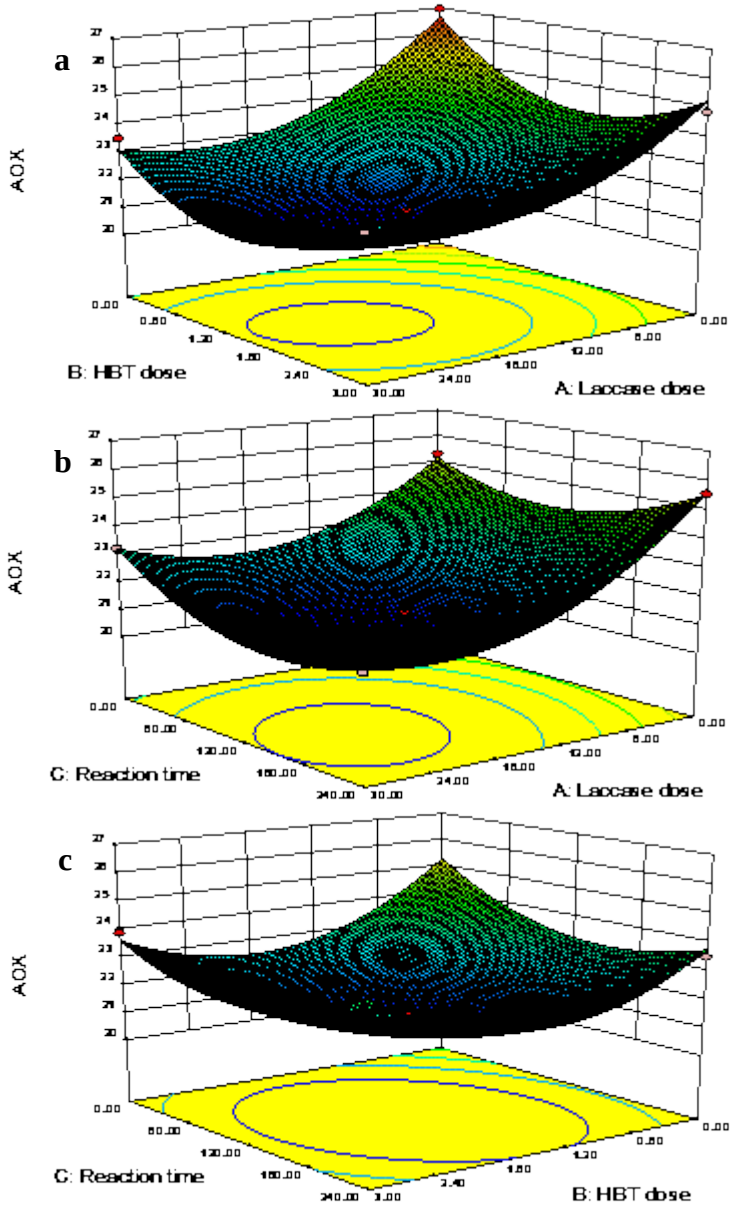

Fig. 2. Three-dimensional response surface plots for AOX yield: (a) laccase dose and HBT dose, (b) laccase dose and reaction time, and (c) HBT dose and reaction time

At the best reaction time, and under the chosen laccase enzyme dosage and HBT dosage, the AOX content was slightly lower than with the increase or decrease in reaction time. This was expected, as phenolic moieties of residual lignin could not be degraded as much as possible when the reaction time was too short; however, the reaction would result in dehydrogenetive polymerization of the degraded residual lignin molecules if the reaction time was too long (Balakshin *et al.* 2001). This was due to the disappearance of β-O-4´, and β-β´ and β-5´ bonds of lignin that were present in original residual lignin. Similar to these results, the sequential enzymatic treatment studied by Sharma resulted in a reduced AOX content of 34% in bleach effluents (Sharma *et al.* 2014).

Similar to the assessment of AOX content, a corresponding model for brightness is given by Eq. 3:

$$Y_2 = 58.87 + 0.74X_1 + 0.32\ X_2 + 0.55\ X_3 - 2.9\ X_1^2 - 2.42\ X_2^2 - 1.67\ X_3^2 - 0.97\ X_1\ X_2 + 1.49\ X_1\ X_3 + 0.28\ X_2\ X_3 \quad (3)$$

The model is in strong accordance with the experimental results, with R^2 = 0.9766. Figure 3 shows that the predicted values and the actual values were distributed uniformly.

Table 4. ANOVA for Response Surface Quadratic Model of Brightness

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob>F
Model	100.37	9	11.15	75.23	<0.0001
X_1	4.37	1	4.37	29.45	0.0010
X_2	0.83	1	0.83	5.61	0.0497
X_3	2.43	1	2.43	16.40	0.0049
X_1X_2	3.72	1	3.72	25.13	0..0015
X_1X_3	8.91	1	8.91	60.11	0.0001
X_2X_3	0.31	1	0.31	2.12	0.1891
X_1^2	35.49	1	35.49	239.41	<0.0001
X_2^2	24.67	1	24.67	166.44	<0.0001
X_3^2	11.72	1	11.72	79.05	<0.0001
Residual	1.04	7	0.15		
Lack of Fit	0.93	3	0.31	11.75	0.0188
Pure Error	0.11	4	0.026		
Cor Total	101.41	16			

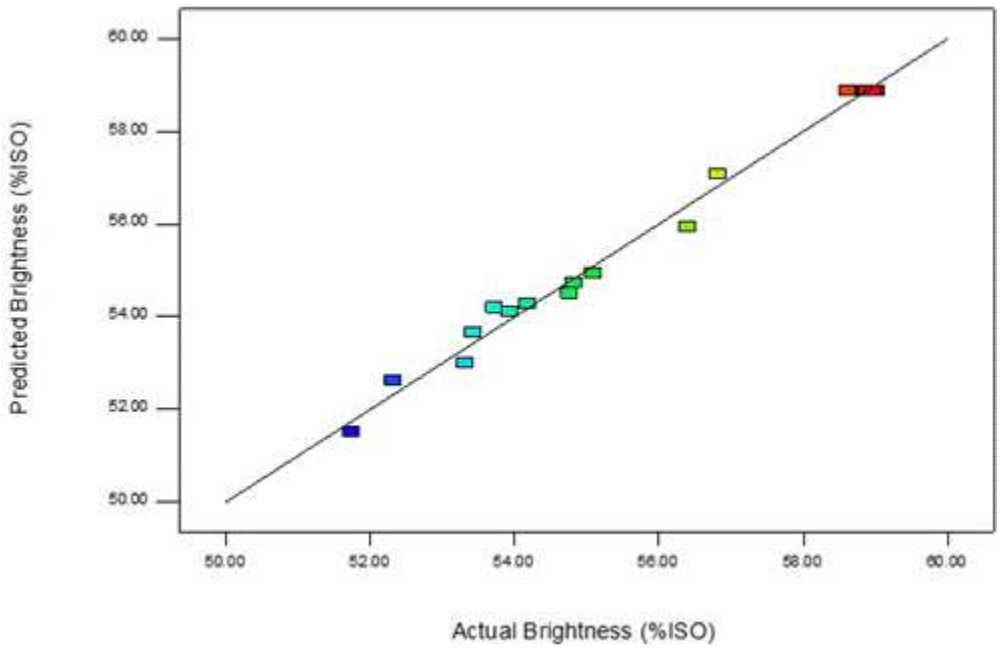

Fig. 3. Predicted *vs*. actual values of brightness

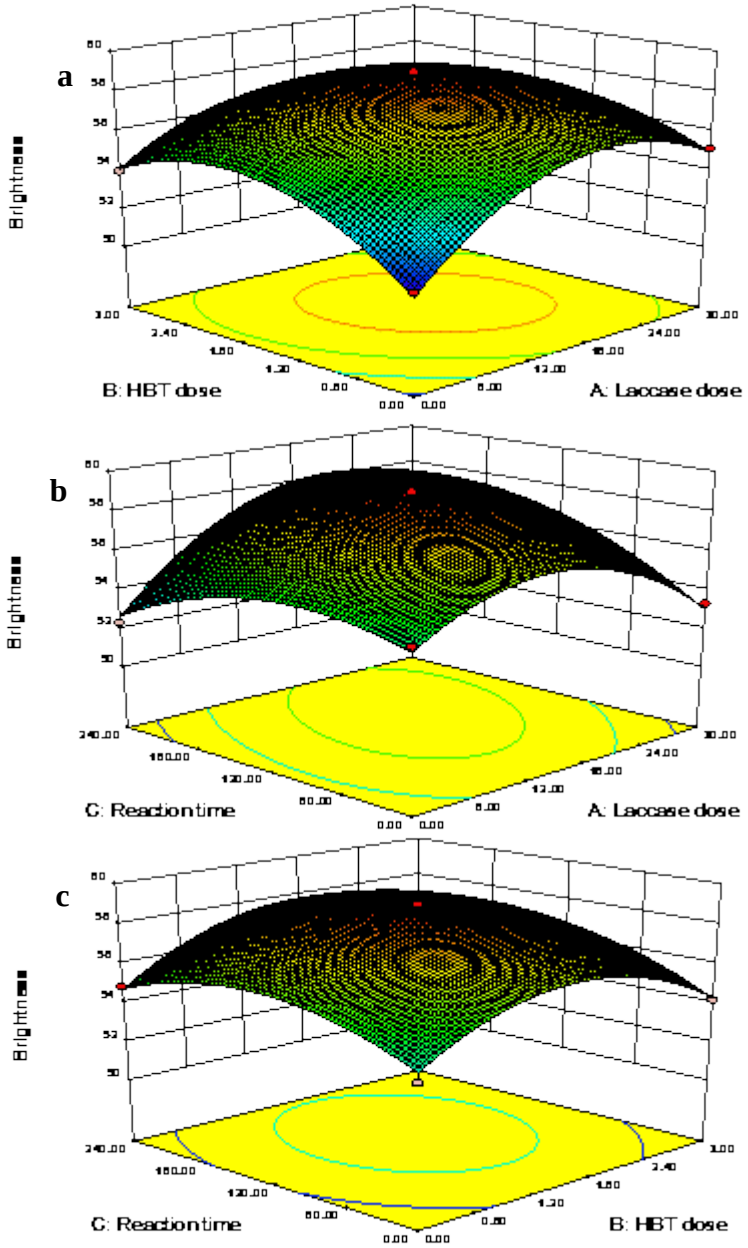

Fig. 4. Three-dimensional response surface drawings for brightness: (a) laccase dose and HBT dose, (b) laccase dose and reaction time, and (c) HBT dose and reaction time

Analysis of variance (ANOVA) results (Table 4) showed that the value of Prob > F was <0.0001, indicating that the model obtained was highly significant. The ANOVA result showed that the X_1, X_2, X_3, X_1X_2, X_1X_3, X_1^2, X_2^2, and X_3^2 terms were significant ($p<0.05$). The p-value for lack of fitness was 0.0188. Lack of fit was significant ($p<0.05$) and the model equation was adequate. The value of the sum of squares was 100.37, indicating that the model had a higher predictability and accuracy. The noise ratio (24.891) was greater than the standard value of 4, which indicates an adequate signal; thus, the model can be used to navigate the design space. The optimal conditions for a higher brightness of the bleached pulp were a laccase enzyme dosage of 17.77 U/g, HBT dosage of 1.57%, and reaction time of 150.12 min. Under these conditions, the brightness was 59.02% ISO.

The three-dimensional response surface and contour plots for brightness are shown in Fig. 4. The contour and the peak in the response surface demonstrate that the extreme value was situated in the center. In other words, the optimal condition occurred in the experimental range. There were obvious interactions between laccase enzyme dosage and HBT dosage, laccase enzyme dosage and reaction time, and HBT dosage and reaction.

The brightness increased when the laccase enzyme dosage was insufficient (less than 15 IU/g), and decreased a little thereafter. The dosage of laccase was not sufficient enough (less than 15 IU/g) to degrade the residual lignin, leading to a low brightness. Similar to previous results, a laccase enzyme dosage of 20 U/g maximized the brightness of the pulp (Fillat and Roncero 2009). Ravalason *et al.* (2012) also observed the potential of laccase for increasing pulp brightness. It seems that mediators are required for laccase biobeaching because of the large size of laccase (Singh *et al.* 2007). Therefore, a mediator such as HBT may be beneficial for the enhancement of brightness, but only a small amount of HBT is necessary. A low dosage of HBT did not effectively promote the degradation of lignin. However, further increases in the dosage of HBT beyond the optimal amount also did not promote the delignification. A quick treatment resulted in an inadequate reaction between laccase and lignin; however, a long treatment time led to brightness reversion. As reported by another work, an appropriate enzymatic reaction time enhanced the brightness by approximately 5.89%, without further alkaline extraction (Singh *et al.* 2008).

Assessment of Experimental Results for Kappa Number of Pulp

The same analysis that was used for AOX content and brightness was applied to kappa number. The regression model for kappa number of the pulp is shown by Eq. 4:

$$Y_3 = 2.81 - 0.36X_1 + 0.074X_2 - 0.11X_3 + 0.45X_1^2 + 0.53X_2^2 + 0.21X_3^2 - 0.17\,X_1X_2 - 0.1X_1X_3 + 0.1X_2X_3 \quad (4)$$

The regression coefficient was close to 1 (R^2 = 0.9805), showing that the quadratic model was in accordance with the experimental results. Figure 5 shows a strong correlation between the predicted values and the actual values.

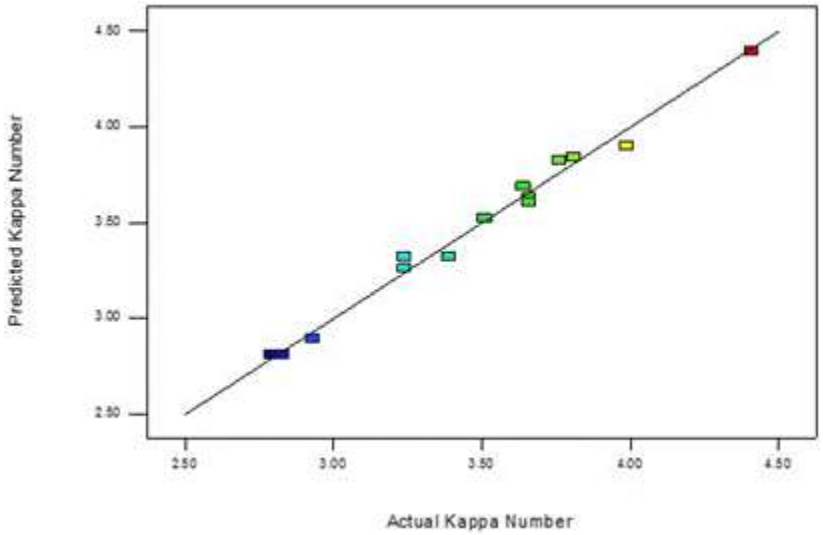

Fig. 5. Predicted *vs*. actual values of kappa number

Table 5. ANOVA for Response Surface Quadratic Model of Kappa Number

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob>F
Model	3.84	9	0.43	90.35	<0.0001
X_1	1.05	1	1.05	222.82	<0.0001
X_2	0.044	1	0.044	9.22	0.0189
X_3	0.10	1	0.10	21.94	0.0023
X_1X_2	0.12	1	0.12	25.23	0.0015
X_1X_3	0.042	1	0.042	8.91	0.0204
X_2X_3	0.040	1	0.040	8.48	0.0226
X_1^2	0.85	1	0.85	180.72	<0.0001
X_2^2	1.17	1	1.17	248.33	<0.0001
X_3^2	0.19	1	0.19	40.30	0.0004
Residual	0.033	7	4.718E-003		
Lack of Fit	0.032	3	0.011	42.70	0.0017
Pure Error	1.000E-003	4	2.500E-004		
Cor Total	3.87	16			

Similar to the analysis results for the other factors, the ANOVA results (Table 5) for kappa number showed that X_1, X_2, X_3, X_1X_2, X_1X_3, X_1^2, X_2^2, and X_3^2 were significant model terms, as their values were less than 0.05 ($p<0.05$). The p-value for lack of fitness was 0.0017. Lack of fit was significant ($p<0.05$) and the model equation was adequate. The Prob > F was <0.0001, indicating that the model for kappa number was highly significant. A value of Prob > F greater than 0.1 indicates that the model terms were not significant. The value of the sum of squares was 3.84, indicating that the model fitted each point in design. The value of Prob>F was 0.0001, which indicates that the model obtained was highly significant. The noise ratio (30.111) was greater than the standard value of 4, indicating an adequate signal. Thus, the model can also be used to navigate the design space. The optimal conditions for a lower kappa number of the bleached pulp were a laccase enzyme dosage of 21.59 U/g, HBT dosage of 1.45%, and reaction time of 165.89 min. Under these conditions, the value of the kappa number was 2.71. The three-dimensional response surface and contour plots for kappa number are shown in Fig. 6.

The optimal condition occurred within the experimental range. There were obvious interactions between laccase enzyme dosage and HBT dosage, laccase enzyme dosage and reaction time, and HBT dosage and reaction time. The kappa number obviously declined when the laccse enzyme dosage was insufficient, or the HBT dosage was too high or too low, or the reaction time was too long or too short. As shown before, the accumulation of degraded residual lignin was the principal reason for the delignification limit, which resulted in the difficulty of the chemicals diffusing into fibers (Balakshin *et al.* 2001). Similar results have been reported for *Eucalyptus* kraft pulp, with a 43% reduction in kappa number after treatment of laccase followed by alkaline extraction (Fu *et al.* 2000). The optimum conditions with

laccase preparation achieved a 21.1% reduction in kappa number (Singh *et al.* 2008). Another work reported that an appropriate time of the laccase-mediator system treatment caused a significant kappa number reduction (Bajpai 2004); therefore, an appropriate laccase enzyme dosage, HBT dosage, and reaction time are essential for decreasing the kappa number.

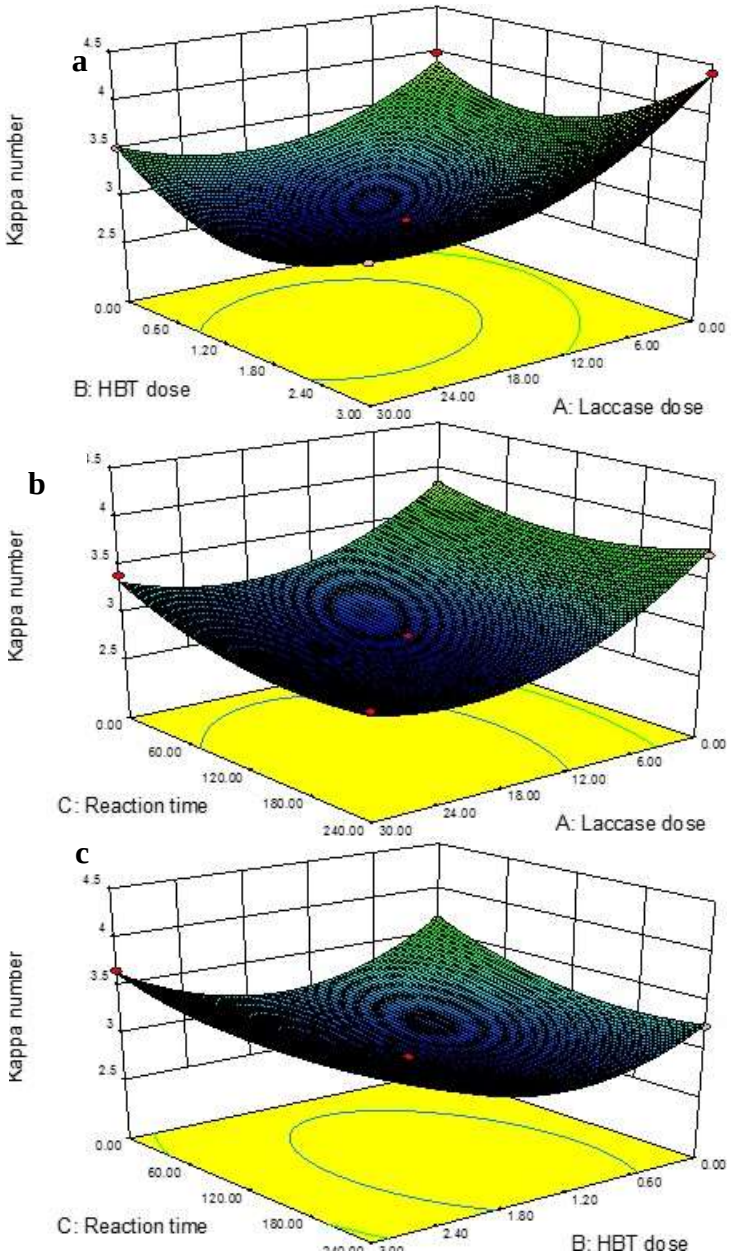

Fig. 6. Three-dimensional response surface drawings for kappa number: (a) laccase dose and HBT dose, (b) laccase dose and reaction time, and (c) HBT dose and reaction time

Validation Experiments

To verify the statistical model, three checkpoint experiments were carried out. The brightness, kappa number, and AOX content of the tested values and the predicted

values are listed in Table 6. The tested values and the predicted values were in good agreement, suggesting the validity of the statistical model.

Table 6. Predicted and Tested Values of Variables During the Validation Experiment

Laccase Dosage (U/g)	HBT Dosage (%)	Reaction Time (min)	Parameter	Predicted	Tested	Relative Error (%)
22.41	1.56	147.50	AOX (mg/L)	20.63	20.73	-0.47%
17.77	1.57	150.12	Brightness (%ISO)	59.02	58.74	+0.48%
21.59	1.45	165.89	Kappa number	2.71	2.70	+0.37%

Integration of the Optimization Results

The three different independent responses of AOX content of the effluent, kappa number, and brightness of the pulp obtained their best levels under different reaction conditions. Because of the importance of these three different responses in practice, the professional software Design Expert 8.0.6.1 was employed to confirm the best conditions for the aim of obtaining a higher brightness, a lower kappa number, and a lower AOX content. The laccase enzyme dosage of 20.30 U/g, HBT dosage of 1.51%, and reaction time of 154.51 min were obtained as the best conditions by Design Expert V8.0.6.1. Under the best conditions, an AOX content of 20.67 mg/L, brightness of 58.94% ISO, and kappa number of 2.71 were predicted. To verify the veracity of the best conditions predicted by Design Expert V8.0.6.1, three additional validation experiments were carried out under the best conditions in triplicate.

Table 7. Predicted and Tested Values of the Best Conditions

No.	Brightness (%ISO)		Kappa Number		AOX (mg/L)	
	Predicted	Tested	Predicted	Tested	Predicted	Tested
1	58.94	58.11	2.71	2.71	20.67	20.64
2	58.94	58.72	2.71	2.75	20.67	20.72
3	58.94	58.95	2.71	2.72	20.67	20.66
Average	58.94	58.59	2.71	2.73	20.67	20.67
Control	-	49.72	-	4.27	-	26.84

As shown in Table 7, a mean AOX content of 20.67 mg/L, mean brightness of 58.59% ISO, and mean kappa number of 2.73 were obtained, corresponding to the predicted values for AOX content, brightness, and kappa number of 20.67 mg/L, 58.94% ISO, and 2.71, respectively. Therefore, enzymatic bleaching of the unbleached bagasse pulp with laccase followed by chlorine dioxide bleaching resulted in a 17.84% increase in brightness, 36.07% reduction in pulp kappa number, and 23% reduction of AOX content in the bleaching effluent compared with the control. Compared with the 23% reduction in AOX content in the present research, Sharma *et al.* (2014) reduced the AOX content by 34% in bleaching effluent with a bleaching sequence of $D_0E(p)D_1D_2$.

CONCLUSIONS

1. A RSM approach was used to optimize the enzymatic treatment of laccase-aided chlorine dioxide bleaching of bagasse pulp. The optimal conditions to achieve a balance of lower AOX content, higher brightness, and lower kappa number were as follows: laccase enzyme dosage of 20.3 U/g, HBT dosage of 1.51%, and reaction time of 154.5 min. These conditions resulted in a 17.84% increased brightness, 36.07% reduced kappa number, and 23% reduced AOX content.
2. This research shows potential benefit to paper mills as well as the natural environment and therefore provides a theoretical foundation for the industrial application of laccase enzyme in bleaching processes.

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