Biobleaching of loblolly pine kraft pulp with *Trametes trogii* culture fluids followed by a peroxide stage. Application of Doehlert experimental design to evaluate process parameters

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ABSTRACT

Loblolly pine kraft pulp was bleached in a totally chlorine-free sequence that involved treatment with culture supernatants from the white-rot fungus *Trametes trogii* followed by a peroxide stage. The whole process was performed at 28 °C, and did not require mediator addition in the enzymatic stage. Different operating conditions in the peroxide stage (pH, peroxide concentration and treatment time), were tested by using response surface methodology based on a Doehlert experimental design, in order to describe their effects and normalize a biobleaching protocol. The results showed that all three independent variables had significant effect on the luminance (L^*) and Chroma (C^*) of the enzyme-treated pulp. Best results were obtained after 1 h of enzyme incubation (352 U laccase, 2 U Mn-peroxidase per g of oven-dry pulp), followed by 96 h treatment with 2.5% hydrogen peroxide in sodium succinate buffer pH 6 (5% consistency). We obtained a noteworthy increase in $L^* = 94.45$ (compared with 94.5 of the white reference standard (titanium oxide), 69.94 of the initial pulp, and 83.11 of the peroxide-bleached control), a decrease in C^* (9.85), with minor pulp yield loss (less than 5%), under essentially mild conditions, using a low-cost source of enzyme.

1. Introduction

Removal of lignin from wood is the first step in the manufacturing of chemical paper pulps, kraft alkaline pulping being the most common process. Although most lignin is removed during cooking, some residual lignin remains in pulp that must be removed in oxidative bleaching reactions. Nowadays peroxide, oxygen and ozone, constitute environmentally friendly alternatives to develop totally chlorine-free (TCF) sequences. However, most TCF chemical reagents, due to their lower delignification power, are less efficient than chlorine reagents in attaining high and stable pulp brightness degrees [1]. The use of enzymes appears as a promising approach for chlorine-free pulp bleaching process. Xylanase prebleaching technology is now in use at several mills worldwide [2]. Some lignin-oxidizing enzymes, such as laccases and Mn-peroxidases (MnPs), also showed potential to perform biobleaching reactions by specific lignin oxidation and removal [1,3]. The results obtained with MnP in kraft pulp bleaching have been promising and the enzyme treatment has had only a minor effect on paper strength or yield [4,5]. Due to its rather low redox potential (0.5–0.8 V),

laccase is able to attack only the phenolic moieties in the lignin polymer, thus being less efficient in delignification and bleaching of pulp. The substrate range of laccases can be expanded to include non-phenolic compounds in the presence of synthetic or natural mediators such as ABTS, HBT and HBA [6,7]. Several aspects need to be solved before mill implementation of a ligninolytic enzymatic stage in TCF bleaching. One aspect to be addressed is the large-scale production and low price commercialization of suitable enzymes. A second aspect to be solved is the availability of efficient and economic mediators for the above applications. The eventual toxicity and inactivation effect [8] of most laccase mediators and their reaction products constitute an important issue that is still to be investigated [2].

Trametes trogii (BAFC 463), an Argentinean white-rot fungus, besides efficiently degrading lignin in wood [9], has been tested successfully in biomechanical pulping experiments [10] and also proved to be a good producer of xylanolytic [11] and ligninolytic enzymes (laccase and MnP) [12]. Recently, culture supernatants from *T. trogii* rendering high levels of MnP (0.25 U/mL) and laccase (44 U/mL), were assayed for the biobleaching of loblolly pine kraft pulp [13]. A strong brightness increase was attained after the enzymatic treatment followed by H_2O_2. Bleaching up to 82% ISO brightness (compared with 37% in the peroxide-bleached control) was obtained. Furthermore, after the enzymatic treatment,

hydrogen peroxide requirements decreased (as reflected by the higher residual peroxide), and the pulp luminance reached a value of 92.5. Culture supernatants offer several advantages over the use of purified enzymes: their obtention process is not expensive, and in addition, proteins or other factors present in the medium may stabilize crude enzymes. The fact that *T. trogii* produces high levels of both laccase and MnP is another advantage with respect to their possible synergism in biobleaching [14]. Moreover, the cost of the mediator is the determining factor for the commercialization of the laccase-mediator bleaching system, but *T. trogii* culture fluids do not require the addition of synthetic mediators for biobleaching [13].

One of the most promising approaches to improving the economics of Kraft pulp production consists of increasing overall pulp yield, while reducing operating cost requirements [15]. Many studies have focused on the delignification mechanism, enzyme capabilities, and the search for new mediators, but not on elucidating the influence of the operating conditions [3]. Traditional methods of optimization involved changing one independent variable while fixing the others at a certain level. Based on the principle of design of experiments, response surface methodology (RSM) encompasses the use of various types of experimental designs, generation of polynomial equations and mapping of the response over the experimental domain to determine the optimum product [16]. RSM requires minimum experimentation and time, and allows the investigation of the effect of all the parameters simultaneously. Different statistical designs have been recently employed for biobleaching optimization [16,17].

In the present study the whole biobleaching sequence was performed under essentially mild conditions (28 °C) to minimize pulp loss. A surface response methodology based on a Doehlert [18] design, was used to analyze the effects of various process parameters involved in the peroxide stage. The bleaching effect was evaluated based on the optical properties of the pulp as major consumer decision-making characteristics. The impact of three quantitative variables, namely pH, peroxide concentration and treatment time, on the luminance of the pulp, was investigated.

2. Materials and methods

2.1. Organism

T. trogii BAFC 463 (Polyporaceae, Aphyllophorales, Basidiomycetes) was obtained from the culture collection of: Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires.

2.2. Culture conditions

Medium for fungal cultures (GA medium) contained glucose, 10 g; MgSO$_4$·7H$_2$O, 0.5 g; KH$_2$PO$_4$, 0.5 g; K$_2$HPO$_4$, 0.6 g; MnCl$_2$·4H$_2$O, 0.09 mg; H$_3$BO$_3$, 0.07 mg; Na$_2$MoO$_4$·H$_2$O, 0.02 mg; FeCl$_3$, 1 mg; ZnCl$_2$, 3.5 mg; thiamine hydrochloride, 0.1 mg; asparagine monohydrate, 4 g; distilled water up to 1 L, supplemented with 1 mM copper sulphate, initial pH 6.5. Cultivation was carried out in 250-mL Erlenmeyer flasks with 50 mL of medium, inoculated with a 25-mm^2 surface agar plug from a 7-day-old culture grown on malt agar (1.3% malt extract, 1% glucose, agar 2%), and incubated statically at 28 ± 1 °C. Cultures were harvested at day 21 and filtered through a filter paper using a Büchner funnel; the culture supernatants were used as enzyme sources.

2.3. Enzymatic treatment of unbleached softwood kraft pulp (USKP) (L-stage)

An industrial unbleached softwood loblolly pine (*Pinus taeda*) kraft pulp (USKP) (Kappa number: 24.8), previously rinsed with distilled water, was treated with *T. trogii* culture fluids (consistency 12.5%), either at 28, 40 or 50 °C and 50 rpm, for different incubation periods (from 1 to 24 h). In the control samples, culture supernatants were replaced by distilled water. A medium-only control was also conducted.

2.4. Peroxide stage (P-stage)

One hour at 28 °C enzymatically treated pulps (ESKP) were bleached with H$_2$O$_2$. Different pulp consistencies were assayed (2.5, 5 and 10%). Increasing peroxide

concentrations (0–3%) were tested, diluted either in water or in different buffers (sodium phosphate, sodium succinate and Tris–malate 0.1 M, pH range from 4 to 8). Incubation was carried out at 28 °C and 50 rpm in 100 mL Erlenmeyer flasks. Different incubation periods were evaluated (12–120 h). The results reported are the mean of triplicate assays with a standard deviation of less than 5%.

2.5. Evaluation of pulp properties

Pulp sheets were prepared on a Büchner funnel to determine their luminance. Luminance (*L**) was measured against a white reference standard (titanium oxide *L** = 94.5) with a SP62 Portable Sphere Spectrophotometer (D65/10°). The color of the samples was described according to the CIE *L*a*b** color system, where *L**, *a** and *b** are the coordinates of the color in the cylindrical color space, based on the theory that color is perceived by black-white (*L** = lightness−darkness), red-green (*a**), and yellow-blue (*b**) sensations; and Chroma (*C**) is the perpendicular distance from lightness axis $C^* = (a^{*2} + b^{*2})^{1/2}$ [19]. The ideal bleach point is defined as *L** = 100 and *a** = *b** = 0. The measured responses were displayed as Δ*L** (luminance increments above the peroxide-bleached control value) and *C**. Pulp yield was determined gravimetrically.

2.6. Enzyme activities

All the enzyme activities were determined at 50 °C (their optimal assay temperature). Laccase activity was measured using 2,2-azinobis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) in 0.1 M sodium acetate buffer (pH 3.4). Oxidation of ABTS was determined by the increase in A_{420} (ε_{420} = 36/mM cm). MnP activity was measured using phenol red as the substrate in 0.1 M sodium dimethylsuccinate buffer (pH 4.5) (ε_{610} = 22/mM cm). Enzyme activity is expressed in International Units (U), as the amount of enzyme needed to release 1 μmol of product per min [12].

2.7. Experimental design and statistical analysis

Different operating conditions (pH, peroxide concentration and treatment time) at the peroxide stage which followed the biobleaching of loblolly pine kraft pulp with crude extracts of *T. trogii*, were tested by using response surface methodology based on a Doehlert experimental design, in order to describe their effects and normalize a biobleaching protocol. The levels of the variables assayed were selected according to the preliminary studies. Enzymatically treated pulps (5% consistency) were bleached with H$_2$O$_2$ in sodium succinate buffer at 28 °C. To study the effect of three independent variables, Doehlert proposed an experimental design based on 13 combinations of the three variables studied. The equally spaced values of each independent variable and the combination between them were adopted and coded following the Doehlert design. The interrelationship between dependent and operational variables was established by a model including linear, interaction and quadratic terms. A quadratic polynomial model Eq. (1) was constructed for the description of the measured response as functions of the process variables:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3$$
$$+ b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 \tag{1}$$

where X_1, X_2 and X_3 are the values of the three factors studied (pH, peroxide concentration and treatment time), expressed as coded variables; *b* denotes the regression coefficients (calculated from experimental data by multiple regressions using the least-squares method) and *Y* represent the experimental responses measured (either *L** or *C**).

The coded and real values are shown in Table 1. Results were analyzed using the software STATISTICA 5.1 (StatSoft, Tulsa, Okla.).

3. Results and discussion

3.1. Influence of incubation temperature and treatment time in the enzymatic stage

The results obtained show the feasibility of bleaching pulp under essentially mild conditions in a chlorine-free sequence based on the use of *T. trogii* culture supernatants followed by H$_2$O$_2$ treatment as described in the following sections. Different incubation temperatures were assayed in the enzymatic stage. Although the optimum for *T. trogii* laccase activity is 50 °C [12], luminance obtained was similar when incubating the pulp with *T. trogii* fluids at 28 and 50 °C [352 U laccase, 2 U MnP per g oven-dry pulp (odp)]. Taking into account that culture supernatants of *T. trogii* retained 96–100% of their laccase and MnP activities for 12 h at 50 °C when incubated in the presence of loblolly pulp [13], different incubation periods (from 1 to 24 h) were assayed for the enzymatic stage. After a subsequent peroxide stage (consistency 5%, H$_2$O$_2$ 2% in buffer succinate

Table 1
Doehlert experimental design for process improvement and response variables [displayed as $C^* = (a^{*2} + b^{*2})^{1/2}$, and ΔL^* = luminance increments above the peroxide-bleached control value (L^* = 83.11)]. L^* of the original unbleached kraft pulp was 69.64 C^* = 30.59 (a^* = 12.15 and b^* = 28.08), while L^* value of the peroxide-bleached control was 83.11 and C^* = 28 (a^* = 9.29, b^* = 26.42). SD < 5%.

Trial	Factor						Response			
	Time (h)		H$_2$O$_2$ (%)		pH		ΔL^*	C^*	a^*	b^*
1	(0)[a]	**84**	(0)	**2**	(0)	**5**	4.440	21.354	6.47	20.35
2	(0)	**84**	(1)	**3**	(0)	**5**	7.545	17.804	5.29	17.00
3	(0.866)	**120**	(0.5)	**2.5**	(0)	**5**	8.620	15.304	4.29	14.69
4	(0.289)	**96**	(0.5)	**2.5**	(0.816)	**6**	11.340	9.849	2.41	9.55
5	(0)	**84**	(−1)	**1**	(0)	**5**	0.000	26.434	8.79	24.93
6	(−0.866)	**48**	(−0.5)	**1.5**	(0)	**5**	−0.260	28.004	9.51	26.34
7	(0.866)	**120**	(−0.5)	**1.5**	(0)	**5**	4.800	21.928	6.73	20.87
8	(−0.866)	**48**	(0.5)	**2.5**	(0)	**5**	1.840	25.473	8.28	24.09
9	(−0.289)	**72**	(−0.5)	**1.5**	(−0.816)	**4**	−3.840	30.494	10.95	28.46
10	(0.289)	**96**	(−0.5)	**1.5**	(0.816)	**6**	9.260	14.525	3.76	14.03
11	(−0.289)	**72**	(0.5)	**2.5**	(−0.816)	**4**	−2.010	29.500	10.98	27.38
12	(−0.577)	**60**	(0)	**2**	(0.816)	**6**	9.200	14.699	4.12	14.11
13	(0.577)	**108**	(0)	**2**	(−0.816)	**4**	−2.560	29.681	10.53	27.75

[a] Coded (in parenthesis) and real (in bold) values of the experimental variables analized.

pH 6, 96 h at 28 °C), the differences in luminance attained (L^* after 1 h incubation: 93.8, after 24 h: 94.1) were too small to justify using long treatment times. Similar results were obtained by García et al. [20], Ibarra et al. [21], Moldes and Vidal [3] and Sigoillot et al. [7]. The highest effect of laccase on pulp was produced during the initial hours of treatment. Therefore, the enzymatic stage can be shortened, if required for process development.

Although Moldes and Vidal [3] observed a protective effect of pulp on *Trametes villosa* laccase activity, when assaying it for biobleaching, only 38% of residual activity was detected after 2 h at 50 °C in the presence of pulp, where the temperature was the main cause of deactivation. In the presence of HBT, residual activity was 1%. Enzyme inactivation by the oxidized species of some mediators is a general drawback of the laccase-mediator systems [8,21]. On the contrary, *T. trogii* laccase and MnP activities demonstrated high stability at 50 °C when incubated in the presence of pulp, laccase retained 100% of its activity after 72 h and MnP 90%. Without pulp, laccase and MnP exhibited only 1/2 and 1/3 of their initial values. *T. trogii* culture supernatants did not required mediator addition and thus correspondingly, did not significantly lose their bleaching capacity even after successive recovery and reapplication to unbleached pulp [13].

3.2. Preliminary experiments on the impact of pulp consistency, pH, buffer and hydrogen peroxide dosage in the P-stage

While pulp consistency at the enzymatic stage was 12.5%, different consistencies were assayed in the peroxide stage (2.5, 5 and 10%). Luminance obtained was similar when using 2.5 and 5% of pulp, but it decreased notably at 10%. Pulp consistency, as a mass fraction, should be as high as possible in order to minimize the amount of water used in a mill application. The absence of significant differences in the results suggests that the two consistency levels (2.5 and 5%) provide a similar fiber environment and that dissolved reactants have unrestricted access to the pulp fibers. The influence of pulp consistency in softwood kraft pulp laccase bleaching, was previously studied by Geng et al. [22], they found minor differences among 2.5 and 10% consistency. Moldes and Vidal [3], with hardwood pulp, found virtually identical pulp properties when using 5% or 10% consistency.

Enzymatically treated pulps were bleached at 5% consistency for 96 h at 28 °C, with increasing H$_2$O$_2$ concentrations (from 0.2 to 2%), diluted in different 0.1 M buffers [sodium phosphate (pH range from 6 to 8), sodium succinate (pHs from 4 to 6) and Tris–malate (pHs from 5 to 8)]. Results varied when using different buffers even at the same pH, best luminance values were obtained with buffer

succinate and 2% H$_2$O$_2$. Therefore this buffer was selected for the subsequent experiments.

3.3. Modeling

A surface response methodology based on a Doehlert experimental design was applied to study the effects of pH at the peroxide stage, H$_2$O$_2$ concentration and time (Table 1). Those factors resulted the most promising for optimizing the process on the basis of the preliminary research. pH was assayed within the limits of the buffer succinate (from 4 to 6), considering that best biobleaching results were obtained with this buffer in the previous step. Some optical properties, such as L^* and C^* showed to be a useful indication for the biobleaching process efficiency, were directly related to the degree of delignification, and could further provide more complete information than the conventionally used brightness measurements [19]. Taking into account that the determination of structural changes on pulp after biobleaching with laccase-mediator systems could be accurately correlated with changes in optical properties of pulp in order to obtain a simple tool to asses the bleaching reaction [19], the outcome of the treatments was compared via L^* and C^*, according to the CIE color system. A full quadratic model containing ten coefficients was used to describe the response observed to fit Eq. (1). Only the linear coefficients of the variables under study were found to be significant at $P < 0.05$, none of the interaction and quadratic terms were statistically significant (Tables 2 and 3). Thus, since we were not applying an orthogonal regression, new regres-

Table 2
Quadratic model coefficients for the response of luminance (ΔL^*) versus the coded levels, and their statistical significance estimated by multiple nonlinear regression. R^2 91.1%.

Term	Coefficient	SE coefficient	t	p
Constant	4.440	1.548	2.868	0.064
Linear				
b_1 (time)	2.775	0.774	3.585	0.037
b_2 (peroxide)	3.113	0.774	4.023	0.028
b_3 (pH)	7.803	0.774	10.075	0.002
Interactions				
b_{12}	0.993	1.787	0.556	0.617
b_{13}	0.511	1.999	0.256	0.815
b_{23}	−0.198	2.000	−0.099	0.927
Quadratic				
b_{11}	−0.696	1.896	−0.367	0.738
b_{22}	−0.670	1.896	−0.353	0.747
b_{33}	−0.972	1.844	−0.527	0.635

Table 3
Quadratic model coefficients for the response of Chroma (C^*) versus the coded levels, and their statistical significance estimated by multiple nonlinear regression. R^2 93.8%.

Term	Coefficient	SE coefficient	t	p
Constant	21.35	1.722	12.400	0.001
Linear				
b_1 (time)	−3.93	0.861	−4.559	0.020
b_2 (peroxide)	−4.01	0.861	−4.658	0.019
b_3 (pH)	−10.33	0.861	−11.994	0.001
Interactions				
b_{12}	−2.36	1.988	−1.188	0.320
b_{13}	−1.29	2.224	−0.581	0.602
b_{23}	−1.42	2.224	−0.638	0.569
Quadratic				
b_{11}	1.51	2.109	0.716	0.526
b_{22}	0.77	2.109	0.363	0.741
b_{33}	−0.41	2.052	−0.201	0.854

Table 4
Regression coefficients for ΔL^* based on the reduced linear models and native variables. R^2 96.4%.

Term	Coefficient	SE coefficient	t	p
Constant	−39.96	2.474	−16.148	0.00000
b_1 (time)	0.07	0.011	5.614	0.00033
b_2 (peroxide)	3.11	0.494	6.299	0.00014
b_3 (pH)	6.37	0.403	15.779	0.00000

$\Delta L^* = -39.96 + 0.07{*}\text{time} + 3.11{*}\text{peroxide} + 6.37{*}\text{pH}.$

Table 5
Regression coefficients for C^* based on the reduced linear models and native variables. R^2 94.9%.

Term	Coefficient	SE coefficient	t	p
Constant	80.048	3.91807	20.431	0.000
b_1 (time)	−0.094	0.01883	−5.015	0.001
b_2 (peroxide)	−4.011	0.78271	−5.124	0.001
b_3 (pH)	−8.434	0.63908	−13.197	0.000

$C^* = 80.048 - 0.094{*}\text{time} - 4.011{*}\text{peroxide} - 8.434{*}\text{pH}.$

Fig. 1. (A and B) Contour plots based on the equations shown in Tables 4 and 5, for the effects of H_2O_2 and incubation time at the peroxide stage (at pH 6.0) on ΔL^* (A) and C^* (B). ΔL^* = luminance increments above the peroxide-bleached control value ($L^* = 83.11$).

the pulp obtained. These positive linear coefficients indicate that luminance raised with increasing time, H_2O_2 and pH in the range tested. From these coefficient values, it can be seen that pH is the factor with the most positive influence on the luminance achieved.

sions with only the linear terms were conducted (Tables 4 and 5). These new regressions were carried out with the native dependent variables to make the equations easier to use. The quality of the model fit was evaluated by the coefficient R^2. R^2 represents the proportion of variation in the response data that can be explained by the fitted model. We considered the high R^2 as an evidence for the applicability of the model in the range of variables included. Based on the reduced equations, the iso-response curves of ΔL^* (luminance increments above the peroxide-bleached control value) and C^*, at pH 6, were represented as contour plots (Fig. 1A and B respectively). Analysis of the iso-response surfaces at pHs 4 and 5 rendered similar results, but less luminance was attained. ΔL^* and C^* experimental data and those predicted by the model are shown in Fig. 2A and B, respectively. The validity of the model was confirmed by fitting different values of the variables in the model equation and by actually carrying out the experiment at those values of the variables. The differences between actual and predicted responses were always <10%, thus proving its validity.

The present model and data analysis allowed us not only to define the best levels, among the assayed, of the selected factors for the bleaching process; but also showed that all three independent variables had a significant positive effect on the luminance (L^*), and negative on the Chroma (C^*) of the enzyme-treated pulp. Moreover we could also demonstrated that there were not combined effects among the three variables studied. For luminance, the positive values for the three variables in the linear term (Table 2) illustrate the significant, positive effect of all of them on the luminance of
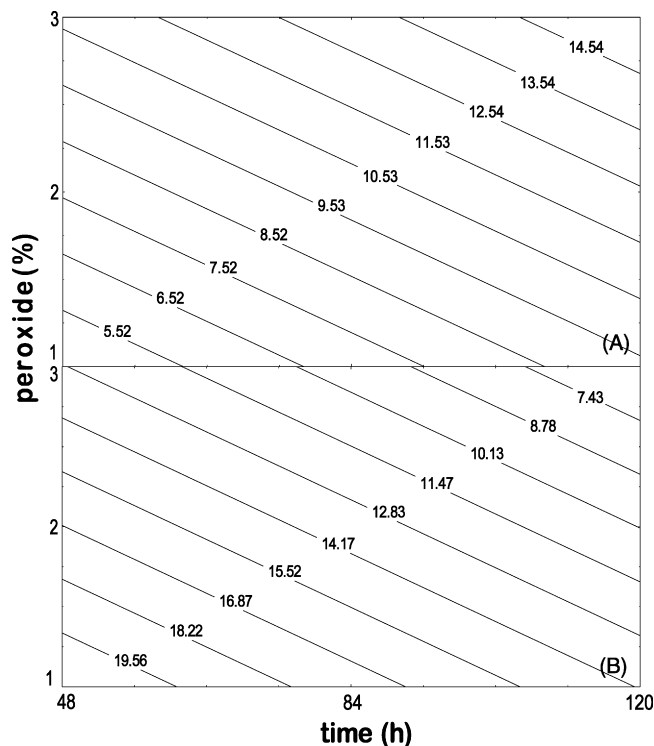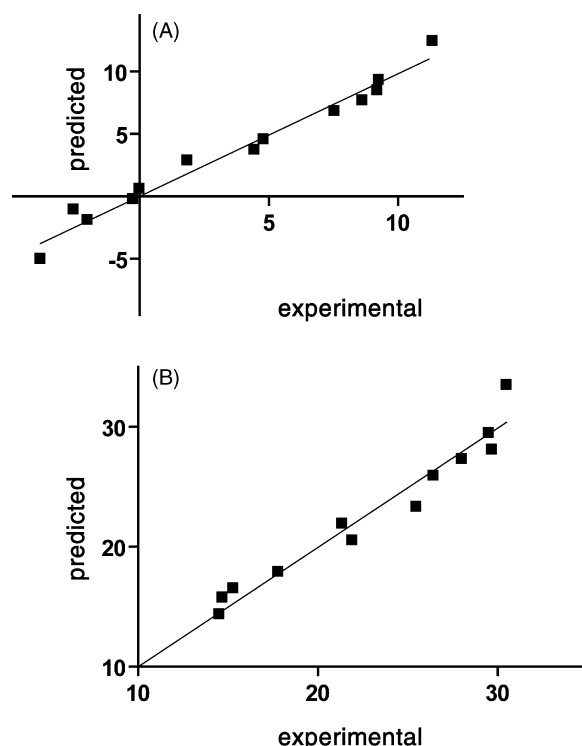

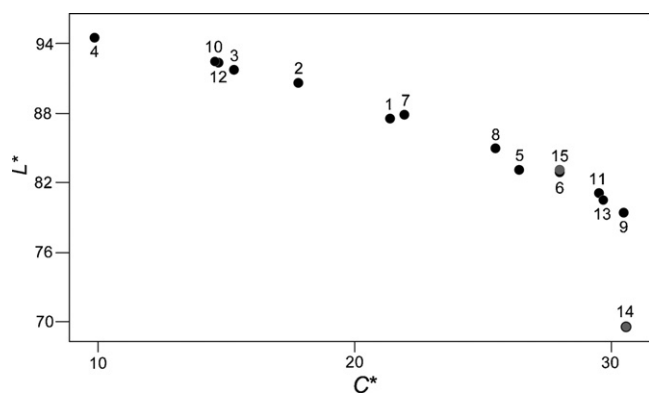**Fig. 2.** (A and B) Predicted versus experimental values for ΔL^* (A) and C^* (B).

Fig. 3. Chromatic values (L^* versus C^*) of loblolly kraft pulp treated under the different assayed conditions displayed in Table 1. 14: unbleached kraft pulp and 15: peroxide-bleached control.

The primary reaction of laccase is the oxidation of phenolic hydroxyls to phenoxy radicals in the presence of oxygen [23]. Treatment conditions such as pH were reported to influence the formation of radicals in laccase treatment [24]. pH may also affect the reactivity of the phenoxy radicals formed. When Moldes and Vidal [3] analyzed eucalypt pulp bleaching by laccase–HBT, they also found that brightness increased with increasing concentration of H_2O_2; similar results (ISO brightness 66.5%) were obtained in that work with odp treated with 0.5% H_2O_2, if the enzymatic stage was performed with the laccase–HBT system, and with 2% H_2O_2 if no HBT was used in such stage. Maximum brightness of 68.0% was obtained in control tests with 3% H_2O_2.

Best results among the assayed were obtained at pH 6, with H_2O_2 2.5%, after 96 h of incubation L^* of the pulp was 94.45 with a C^* of 9.85 ($a^* = 2.41$ and $b^* = 9.55$). Bleached softwood pulps usually have a brightness of about 70–90% and a yellowness (b^*) of 8 or more. There is an ongoing need for improved but inexpensive pulps having enhanced brightness and decreased b^* values, and with greater stability of the optical properties (i.e. decreased reversion) [25]. The negative linear coefficients indicate that C^* values decreased with increasing time, H_2O_2 and pH in the range tested (Table 3). pH is the factor with the most negative influence on both responses. C^* (Chroma) decreased notably after the best treatment (9.85), in comparison with the C^* of the unbleached kraft pulp (30.59) and that of the peroxide-bleached control (28) (Fig. 3). L^* and C^* values are in the range obtained previously while biobleaching eucalyptus kraft pulp with a laccase-mediator system [19].

Taking into account that one of the most promising approaches to improve the economics of kraft pulp production consists in increasing overall pulp yields [15], an additional advantage of the current process is its minor pulp yield loss (less than 5%). Different laccase biobleaching protocols involved a subsequent peroxide stage at 80–90 °C [3,7], and as a result a high energy demand. In the present work very high luminance values were attained (L^*) = 94.45 (compared with L^* = 94.5 of titanium oxide, the white reference standard), under mild conditions (28 °C) thus reducing energy consumption.

4. Conclusions

The results obtained in this work showed that efficient bleaching of loblolly pine kraft pulp can be achieved in a chlorine-free sequence, with culture supernatants from *T. trogii*, which combine high titers of laccase (with high redox potential and thermal stability) accompanied by significant levels of MnP; followed by a peroxide stage. With a Doehlert experimental design we

demonstrated that all three independent variables (pH, peroxide concentration and treatment time at the peroxide stage) had a noteworthy effect on the luminance (L^*), and Chroma (C^*) of the enzyme-treated pulp, and that there were not combined effects among the three variables studied. Positive and negative linear coefficients for L^*, and C^* respectively indicated that luminance raised while Chroma decreased, with increasing time, H_2O_2 and pH in the range tested. Best L^* and C^* values were in the range obtained previously while biobleaching with laccase-mediator systems, but *T. trogii* fluids did not require purification or mediator addition. Moreover the whole process including a subsequent peroxide stage may be performed under mild conditions (28 °C) thus reducing energy consumption and mass yield loss. Therefore, pulp biobleaching by *T. trogii* culture supernatants, should be positioned as a promising alternative to be used in further studies focused on environmentally friendly paper making.

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