Brazilian Journal
of Chemical
Engineering

ISSN 0104-6632
Printed in Brazil
www.scielo.br/bjce

Vol. 34, No. 03, pp. 701 – 707, July – September, 2017
dx.doi.org/10.1590/0104-6632.20170343s20160091

REMOVAL OF REACTIVE BLUE 21 AND REACTIVE RED 195 DYES USING HORSERADISH PEROXIDASE AS CATALYST

S. Farias[1], D. de Oliveira[1,*], A. A. Ulson de Souza[1],
S. M. A. Guelli U. de Souza[1] and A. F. Morgado[1]

[1]Chemical Engineering Department, Federal University of Santa Catarina, 88040-900 Florianopolis-SC, Brazil
*E-mail: debora.oliveira@ufsc.br

(Submitted: November 15, 2015; Revised: March 26, 2016; Accepted: April 7, 2016)

Abstract – Textile effluent is rich in hydrolyzed dyes that need to be removed. This study presents an evaluation of the potential of the enzyme horseradish peroxidase to remove the hydrolyzed dyes Reactive Blue 21 (RB 21) and Reactive Red 195 (RR 195) from cotton fiber and the effluent of the dyeing process. The parameters pH, dye concentration and temperature were evaluated to determine the optimal conditions to remove the dyes. The studies of removal of the dyeing effluent led to an increase of degradation for all tested colors. The use of the enzyme horseradish peroxidase as a biocatalyst can be a viable technological alternative to remove some hydrolyzed dyes.

Keywords: Reactive dye, dye, unfixed reactive dyes, horseradish peroxidase.

INTRODUCTION

Reactive dyes are extensively used in textile industries worldwide for dyeing of cotton fiber, mainly because of characteristics such as better dyeing processing, bright colors and reducing the energy consumption (Peralta-Zamora et al., 2003; Saeed et al., 2012). The dyes are adsorbed on cellulose by covalent bonding (Paul et al., 2013). However, 15% of them are discharged in the effluents (Abdel-Aty et al., 2013). The effluents with high concentration of dyes have the ability to change biological cycles when discharged into aquatic environments by affecting the process of photosynthesis, the oxygenation of the water, high toxicity and carcinogenic and mutagenic potentials (Nguyen and Juang, 2013; Solis et al., 2013; Cordoba et al., 2012).

Currently, there are a number of physical-chemical processes used to treat wastewater effluents; these processes involve methods such as adsorption, coagulation/flocculation, precipitation, activated carbon, ozonation, membrane filtration and ion exchange. However, most of these treatment processes are expensive and generate pollution by the use of many types of chemicals (Lade et al., 2012; Silva et al., 2013).

In this context, alternative methods to study the degradation of dyes with less environmental impact and high efficiency have become a challenge for further research. The use of the class of oxidoreductase enzymes such as peroxidases to remove a variety of organic compounds from wastewater is a promising route because these enzymes can catalyze the degradation of recalcitrant aromatic dyes (Silva et al., 2012). Peroxidases are produced by a large number of microorganisms and plants. They catalyze a variety of reactions, requiring the presence of hydrogen peroxide. There are many studies reported in the literature about the use of various types of peroxidases from different plant sources to remove reactive dyes, such as turnip peroxidase, soybean peroxidase, potato

* To whom correspondence should be addressed

peroxidase, and horseradish peroxidase (Silva et al., 2012; Loncar et al., 2012; Jiang et al., 2014). Soybean peroxidase was already used to remove Remazol Brilliant Blue R dye, evaluating the effect of some parameters such as dye concentration, amount of enzyme and hydrogen peroxide on the dye removal. The maximum removal was 86% after 13 min (Silva et al., 2013). In the last few years researchers have used peroxidases produced by white-rot fungi decomposition; these fungi are capable to degrade recalcitrant dyes with high rate of degradation from 84.9 to 99.6% (Hamedaani et al., 2007).

Enzymes from plant sources such the horseradish peroxidase (HRP, EC 1.11.1.7) have been used for the removal of aromatic-aqueous contaminants, primarily because it retains its activity over a wide range of pH and temperature. Once activated by hydrogen peroxide, HRP can catalyze the oxidation of 2O2, and a variety of toxic compounds including aromatic compounds, phenols, bisphenols, aniline, and heteroaromatic compounds like benzidines (Karam and Nicell, 1997; Buchanan and Nicell,1998; Wan et al., 2012). The discoloration of dyes by horseradish peroxidase has been employed to degrade synthetic and natural dyes with the obtained discoloration degrees higher for synthetic dyes (Abdel-Aty et al., 2013). The removal of the dyes Remazol Turquoise Blue G 133%, Lanaset Blue 2R and textile effluent by horseradish peroxidase was studied to determine the optimum conditions of degradation. Results showed 59%, 94% and 52% of removal, respectively (Guelli Ulson Souza et al., 2007).

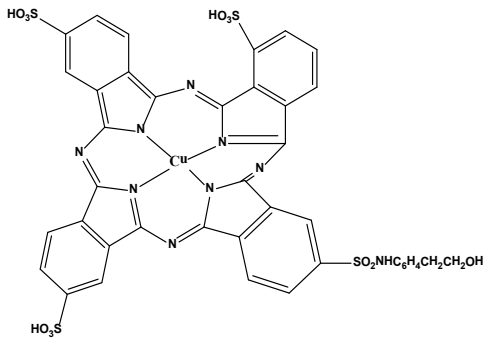
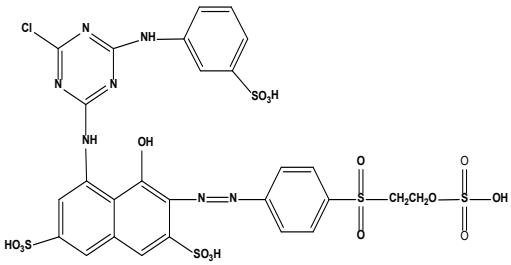
In the present study, the enzyme horseradish peroxidase was used to determine the degradation of the phthalocyanine dye Reactive Blue 21 and Reactive Blue 195.Subsequently, the optimum conditions were used to remove the dye unfixed on cotton fiber from the remaining effluent of the dyeing process.

EXPERIMENTAL

Materials

The enzyme horseradish peroxidase (HRP) was kindly donated by the company Toyobo from Brazil and has a specific activity of 8.8 MU/kg, molecular weight approximately 40,000. C.I Reactive Blue 21 (RB 21) and C.I. Reactive Red 195 (RR 195) dyes were donated by the company Karsten from Brazil. Table 1 presents the characterization of the reactive dyes used in this work.

Table 1. Characterization of the reactive dyes.

Structure of the reactive dye	Molecular weight (g/mol)	λmax[a] (nm)	λmax[b] (nm)
 C.I. Reactive Blue 21	1159.62	624	667
 C.I. Reactive Red 195	1021.41	541	541

a: Without chemical additives

b: With chemical additives (Sodium sulfate and Sodium Carbonate).

ABTS [2,20-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)], sodium phosphate dibasic were purchased from Sigma–Aldrich (São Paulo, Brazil). Potassium phosphate monobasic, sodium dithionite and sodium carbonate were purchased from Vetec (São Paulo, Brazil), sodium hydroxide was purchased from CRQ and hydrogen peroxide H2O2 (35% w/v) was purchased from Lafan (São Paulo, Brazil). Sodium sulfate was purchase from Quemis from Brazil.

Determination of horseradish peroxidase activity

The enzyme activity was determined through modified methodology, using 0.255 mL of ABTS solution 20 mM, 0.255 mL of hydrogen peroxide 10 mM, 0.300 mL of enzymatic extract, 2.19 mL of 0.05 mol.L^{-1} phosphate buffer pH 6.0 (Wu et al., 1997). The reaction was maintained at 30 °C in a thermostatic bath for 10 min and then monitored for 5 min by a spectrophotometer at 420 nm (ε420nm = 3.6.104 M^{-1}.cm^{-1}) (Johannes and Majcherczyk, 2000). The enzymatic activity (A) was calculated according to Equation 1.

$$A\left(\frac{U}{mL}\right) = \frac{\Delta_{Abs} \cdot V_{Total} \cdot 10^6}{\varepsilon_{ABTS} \cdot V_{extract} \cdot t} \quad (1)$$

where: A= enzymatic activity; ΔAbs = Absorbance final – Absorbance initial; ε = molar extinction coefficient for the ABTS (= 3.6.104 M^{-1}cm^{-1}); Vtotal = Total volume of solution (mL); V Extract = Total volume of extract enzymatic solution (mL); t= time of reaction in minutes.

One enzyme activity unit (U) was defined as the amount of enzyme which converts 1 µM of ABTS into its radical cation per minute.

Enzymatic degradation studies

The reactions were conducted according to a modified methodology, described as: using a thermostatic bath at 130 rpm with 0.4 mL of hydrogen peroxide 100 µmol.L^{-1}, 0.1 mL of enzyme extract 29.85 U.mL^{-1}, 1.5 mL of the dye concentrations studied and citrate or phosphate buffer 0.05 mol.L^{-1} (1.2 mL) at different pH values (Silva et al., 2012). In control reactions all reagents were employed except for H2O2, which was replaced by buffer. The experiments were performed by varying the dye concentration (from 20 to 60 mg.L^{-1}), pH (from 3 to 7), and temperature (from 20 to 50 ºC). The decrease of the color of solution was monitored in a spectrophotometer during 1 h. Table 1 presents the wavelengths used to calculate dye decolorization. The dye decolorization percentage was determined by Equation 2.

$$D\ (\%) = \frac{Abs_{initial} - Abs_{final}}{Abs_{initial}} .100 \quad (2)$$

Removal of the effluent of the dyeing process

The dyeing was carried out using each dye alone and a mixture of the two dyes. Initially we used 2% by weight of dye (and in the case of mixing 1 % mass of each dye, total 2% by mass) and 50 g / L of sodium sulfate. The removal of the effluent from the dyeing process was performed together with the removal of unfixed dye for cotton fiber in the dyeing process. To quantify the dye solution removed, the spectral analysis was performed in the wavelength range from 400 to 800 nm where quantification was done based on the decrease of the concentration of the chromophore peak identified before and after the reaction.

RESULTS AND DISCUSSION

Effect of pH on the removal of dyes

The effect of pH on the removal of dyes RB 21 and RR 195 by the HRP enzyme is shown in Figure 1.

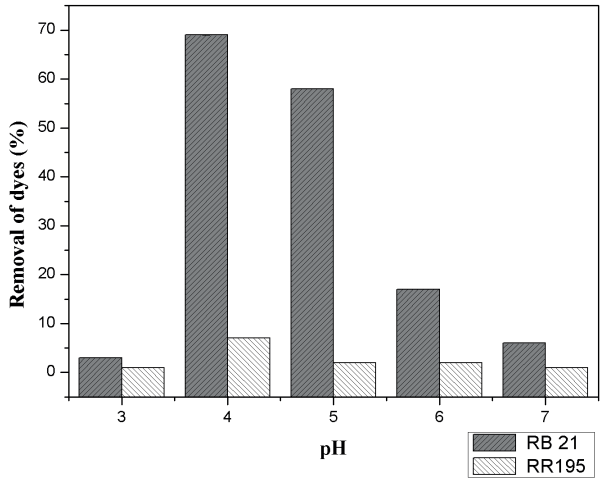


Figure 1. Effect of pH on the removal of dyes RB 21 and RR 195 by the HRP enzyme. Conditions: temperature 30 ºC; reaction time 1 h; dye concentration 50 mg L^{-1}; H2O2 concentration 100 µmol L^{-1}; enzyme activity 29.85 U mL^{-1}.

The maximum decolorization was 69.0±0.005% for RB 21 and 7.00±0.002% for RR 195 in 1 h and pH 4. Similar results were obtained for the study of degradation of dye RB 21 by HRP, a removal of 59% was obtained at pH 4

after 50 min of reaction time (Guelli Ulson Souza et al., 2007).

The dye RR 195 showed low efficiency of degradation; in general for most of the red reactive dyes low degradation is observed. The degradation of the dye Remazol Red Ultra RGB was performed using turnip peroxidase and a degradation of 5.5% was obtained in 1 h of reaction time (Silva et al., 2012). The effect of Trichosanthes diocia peroxidase in the removal of disperse dyes insoluble in water was investigated. The disperse red dyes and disperse black had a percentage decolorization of 79 and 60%, respectively, at pH 4 for 60 min of reaction (Saeed et al., 2012).

Effect of dye concentration on the removal of dyes

The dye concentration was studied ranging in concentration from 20 to 60 mg.L^{-1}, as shown in Figure 2.

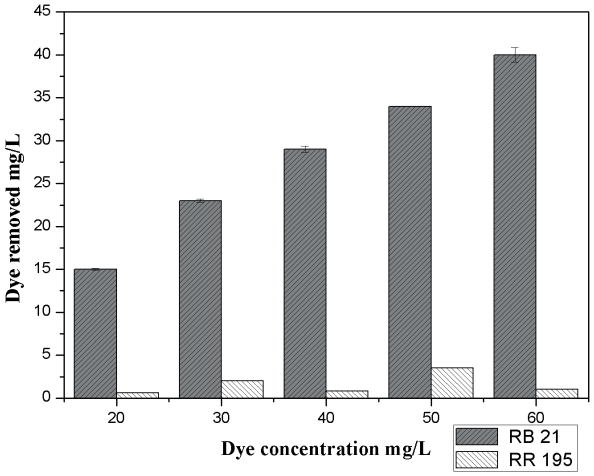


Figure 2. Effect of dye concentration on the removal of dyes RB 21 and RR 195 by the HRP enzyme. Conditions: temperature 30 ºC; reaction time 1 h; pH 4; H2O2 concentration 100 µmol L^{-1}; enzyme activity 29.85 U mL^{-1}.

The optimum removal for RB 21 was obtained when a concentration of 60 mg.L1 was used, 40 ± 0.18 mg.L^{-1} of removal. At the concentration of 50 mg.L^{-1} a decolorization of 34 mg.L^{-1} was obtained. The studies with RB 21 showed that there was an increase in the enzyme-mediated decolorization efficiency, when more dye was present (Mohan et al., 2005). If the enzyme concentration is kept constant and the substrate is gradually increased, the reaction rate will increase up to a maximum value and remain constant after this point. In the degradation of the dye RR 195, the maximum removal was obtained when a concentration of 50 mg.L^{-1} was used, 3.5±0.0005 mg.L^{-1} of removal; with 30 mg.L^{-1} a removal of 2 mg.L^{-1} was obtained. The dye RR 195 did not show the same increase of decolorization observed for dye RB 21.

Effect of temperature on the removal of dyes

The enzymatic reactions are strongly temperature dependent. In enzyme catalyzed reactions the velocity increases to a maximum within an optimum temperature range in which the enzyme is stable and retains its activity (Nelson and Cox, 2000). The effect of temperature was studied by varying the temperature between 20 and 30 ºC, as shown in Figure 3.

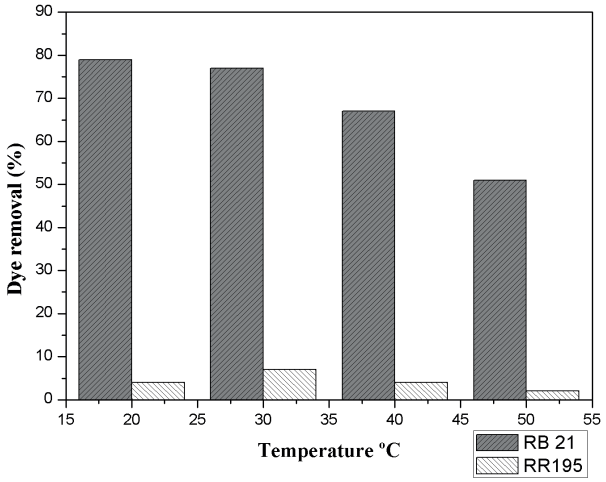


Figure 3. Effect of temperature on the removal of dyes RB 21 and RR 195 by the HRP enzyme. Conditions: reaction time 1 h; pH 4; dye concentration 50 mg L^{-1}; H2O2 concentration 100 µmol L^{-1}; enzyme activity 29.85 U mL^{-1}.

In the studies with dye RB 21 the highest removal was obtained at 20 °C, 79.0±0.007%; however, at 30 °C the removal wa 77.0%±0.0016. The maximum dye degradation for RR 195 was obtained at 30 °C, 7.00±0.05%. The variation of the temperature from 30 to 80 °C was studied for the decolorization of bromophenol blue and methyl orange in the presence of modified peroxidase (Liu et al., 2006). The optimum temperature for both dyes was 30 °C and the efficiency decreased after this value.

Decoloration using a dye mixture

The best conditions for the removal of each dye found in the studies using simulated effluent were applied to the mixture RB21+RR195. The studies were carried out using dye (30 mg L^{-1}), enzyme (29.85 U mL^{-1}), and hydrogen peroxide (100 µmol L^{-1}), at pH 4 and 30 ºC with 1 h of reaction (Figure 4). The absorption spectrum for the dye mixture shows a decrease in the chromophore peaks for each dye, after the enzymatic reaction. It can be observed that the maximum absorbance peaks were at approximately 541 and 624 nm for RR195 and RB21, respectively. Removals of 23% for RR195 and 45% for RB21 were obtained. Some authors performed the enzymatic degradation with a mixture of Reactive Black 5,

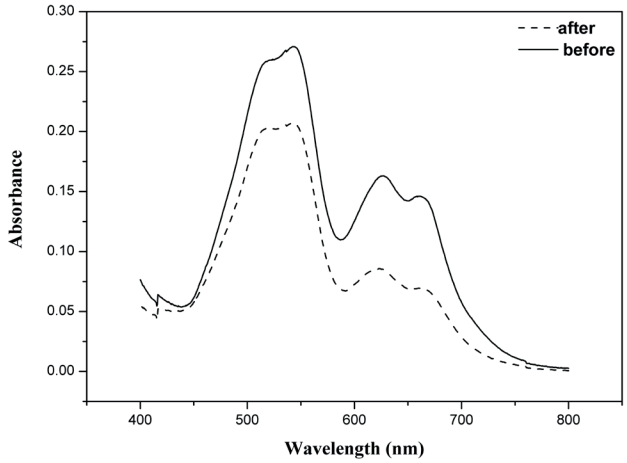


Figure 4. Absorption spectra of the mix of dyes RB21+RR195 before and after enzymatic treatment with horseradish peroxidase, without chemical additives. Conditions: dye concentration 30 mgL^{-1}; reaction time 1 h; pH, 4; H2O2 concentration 100 μmol L^{-1}; enzyme concentration 29.85 U mL^{-1}.

Reactive Yellow 15 and Reactive Red 239 dyes, using turnip peroxidase, and the total color removal was 49% (Silva et al., 2012).

Removal of the effluent from the dyeing process

The studies of removing effluent from the dyeing process were carried out in a single step with the removal of dyes unfixed to fiber. The absorption spectra of dye RB 21 before and after enzymatic treatment with horseradish peroxidase are shown in Figure 5.

The removal of effluent from the dyeing process with RB 21 dye by the enzyme HRP, showed 79% decolorization, verified by the decrease of the chromophore peak after enzyme treatment. It is observed that the effluent from the dyeing process had a significant amount of electrolytes remaining from the dyeing step. The large amount of electrolytes can cause an inhibition at the removal efficiency. This inhibition was observed in the degradation of dyes such as turquoise remazol G 133% (CTR) and remazol brilliant blue R (RBBR) by radish peroxidase; the decrease of removal was from 47 to 37% for CTR and 69.5 to 58% for the dye RBBR (Silva et al., 2012). The removal of the dyes becomes more difficult when it comes to real industrial effluent, where several electrolytes and chemical auxiliaries are present together, as well as a range of dyes. The degradation of the dye turquoise blue G 133% was performed and the authors obtained 52% of removal and the formation of a new peak

The use of immobilized enzyme to remove dyes has been studied by many researchers. The degradation of real effluent using polyphenoloxidase was studied (Khan et al., 2006). An increase in the decolorization was observed when immobilized enzyme was used, from 82 (for soluble enzyme) to 95%.

The absorption spectra of dye RR 195 before and after enzymatic treatment with horseradish peroxidase after dyeing are shown in Figure 6.

In studies to degrade the dye colorant RR 195 by the enzyme horseradish peroxidase 31% degradation was obtained calculated from the decrease of the chromophore peak after the enzymatic treatment. An increase in the degradation of 7% could be observed awhen only the dye was present in solution and 31% when the electrolytes were present in solution. Sodium chloride was used as donor of chloride to verify the effect on the activity of a fungal peroxidase from Perenniporia subacida. An increase in activity of enzyme of 130% was obtained ain the presence of 100 mM sodium chloride. In kinetic tests it was observed

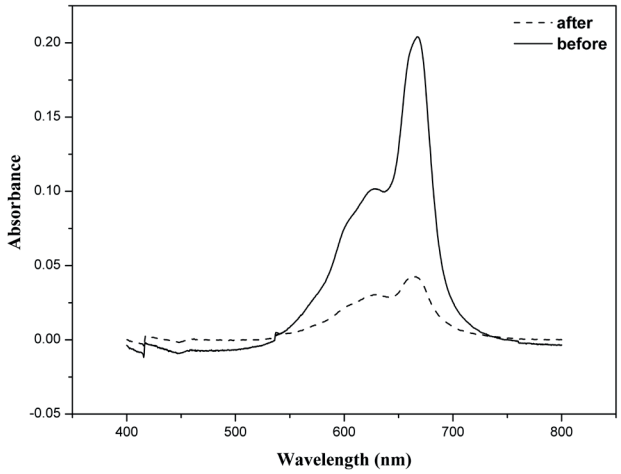


Figure 5. Absorption spectra of dye RB 21 before and after enzymatic treatment with horseradish peroxidase after dyeing. Conditions: temperature 30 oC; reaction time 45 min; dye concentration 2% (wt/v); H2O2 concentration 2 mmol L^{-1}; enzyme activity 29.85 U.mL^{-1}, pH 4.

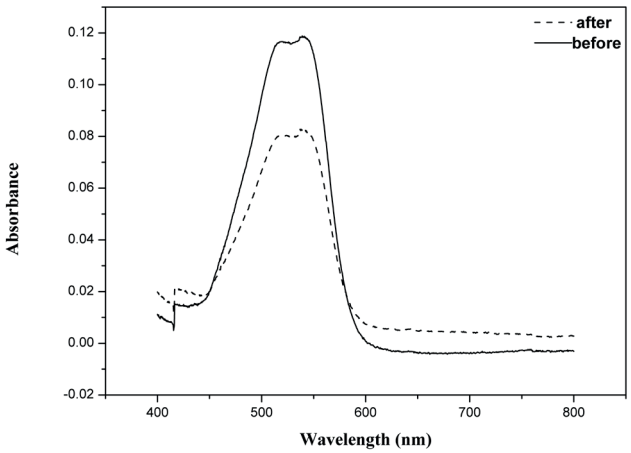


Figure 6. Absorption spectra of dye RR 195 before and after enzymatic treatment with horseradish peroxidase after dyeing. Conditions: temperature 30 oC; reaction time 45 min; dye concentration 2% (wt/v); H2O2 concentration 2 mmol L^{-1}; enzyme activity, 29.85 U mL^{-1}, pH 4.

that in small quantities chloride can increase the affinity between enzyme and substrate, and this could be explained by changes in the conformation of the enzyme (Si and Cui, 2013). The degradation of the direct red dye 23 (DR 23) and mixture of direct dyes was performed, obtaining 86% degradation for the dye DR 23 and 73% for a mixture of direct dyes in the presence of the heavy metal salt, zinc chloride (Loncar et al., 2012).

After the analysis of the separated dyes, they were mixed to verify whether the presence of another dye would have some effect when two dyes were present. The absorption spectra of the mixture of dyes RB 21 and RR 195 before and after enzymatic treatment with horseradish peroxidase after dyeing are shown in Figure 7.

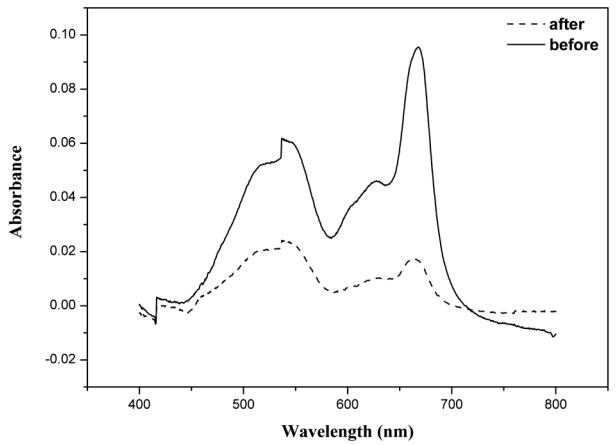


Figure 7. Absorption spectra of mixture dyes RB 21 and RR 195 before and after enzymatic treatment with horseradish peroxidase after dyeing. Conditions: temperature 30 oC; reaction time, 45 min; dye concentration 2% (wt/v); H2O2 concentration 2 mmol L^{-1}; enzyme activity 29.85 U mL^{-1}, pH 4

In the studies of decolorization of the dye mixture, a removal of 60% was obtained for the dye RR 195 and 81% for the dye RB 21. An important effect observed in this study was the synergistic effect of enzyme action when the two dyes were present. The removal of the RR 195 dye increased from 31 to 60% and RB 21 from 79 to 81%. A similar result was obtained for the degradation of a mixture of reactive dyes and an increase observed in removal efficiency (Silva et al., 2012). The degradation of various reactive dyes by phenoloxidase enzymes has been described. The removal of 93-99.9% was obtained in 1 h of reaction (Loncar et al., 2012). The removal of the dye direct yellow 106 (DY106) by immobilized C-peroxidase was studied and a the degradation of 87% was obtained after 3 cycles of use of the catalyst (Boucherit et al., 2013).

CONCLUSIONS

The enzyme horseradish peroxidase has proved viable to remove certain types of reactive dyes and a dye mixture. The degradation is strongly influenced by reaction parameters such as pH, temperature and dye concentration. The studies of removing the effluent from the dyeing process showed an increase of removal when electrolytes were present in solution. The use of enzymes to remove textile effluents appears as a possible technological alternative to chemical processes.

ACKNOWLEDGEMENTS

The authors would like to thank CAPES for the financial support given by way of the scholarship, and Karsten and Toyobo do Brasil for supplying the dyes and HRP enzyme, respectively.

REFERENCES

Abdel-Aty, A., Hamed, M. B., Fahmy, A. S., Mohamed, S. A. Comparison of the potential of Ficus sycomorus latex and horseradish peroxidases in the decolorization of synthetic and natural dyes. Genet Eng Biotechnol J., 11, 95-102 (2013).

Boucherit, N., Abouseoud, M., Adour, L.. Degradation of direct azo dye by Cucurbita pepo free and immobilized peroxidase. Boucherit N[1], Abouseoud M, Adour L. J Environ Sci., 25, 1235-1244 (2013).

Buchanan, I. D. and Nicell, J. A., Kinetics of peroxidase interactions in the presence of a protective additive. J Chem Technol Biotechnol., 72, 23-32 (1998).

Cordoba, A., Magario, I., Ferreira, M. L., Evaluation of hematin-catalyzed Orange II degradation as a potential alternative to horseradish peroxidase Int Biodeterior Biodegradation,73, 60-72 (2012).

Guelli Ulson Souza, S. M. A., Forgiarini, E., Ulson de Souza, A. A., Toxicity of textile dyes and their degradation by the enzyme horseradish peroxidase (HRP). J Hazard Mater., 147, 1073-1078 (2007).

Hamedaani, H. R. K., Sakurai, A., Sakakibara, M., Descolorization of synthetic dyes by a new manganese peroxidase-producing white rot fungus. Dyes Pigm., 72,157-162 (2007).

Karam, J. and Nicell, J., Potential Applications of Enzymes in Waste Treatment. J Chem Tech Biotechnol., 69, 141-153 (1997).

Khan, A. A., Akhtar, S., Husain, Q., Direct immobilization of polyphenol oxidases on Celite 545 from ammonium sulphate fractionated proteins of potato (Solanum tuberosum). J Mol Catal B: Enzym., 40, 58-63 (2006).

Jiang, Y., Tang, W., Gao, J., Zhou, L., He, Y., Immobilization of horseradish peroxidase in phospholipid-templated titania and its applications in phenolic compounds and dye removal. Enzyme Microb Technol., 55, 1-6 (2014) doi: 10.1016/j.enzmictec.2013.11.005

Johannes, C. and Majcherczyk, A., Laccase activity tests and laccase inhibitors. J Biotechnol., 78, 193-199 (2000).

Lade, H. S., Waghmode, T. R., Kadam, A. A., Govindwar, S. P., Enhanced biodegradation and detoxification of disperse azo dye Rubine GFL and textile industry effluent by defined

fungal-bacterial consortium. Int Biodeterior Biodegradation, 72, 94-107 (2012).

Liu, J-Z., Wang, T-L., Ji, L-N., Enhanced dye decolorization efficiency by citraconic anhydride-modified horseradish peroxidase. J. Mol. Catal. B: Enzym., 41, 81-86 (2006).

Loncar, N., Janovi, B., Vujcic, M., Vujcic, Z., Decolorization of textile dyes and effluents using potato (Solanum tuberosum) phenoloxidase. Int Biodeterior Biodegradation, 72, 42-45 (2012).

Marchis, T., Avetta, P., Bianco-Prevot, A., Fabbri, D., Viscardi, G., Laurenti, E., Oxidative degradation of Remazol Turquoise Blue G 133 by soybean peroxidase. J. Inorg. Biochem., 105, 321-327 (2011).

Matto, M. and Husain, Q., Decolorization of direct dyes by salt fractionated turnip proteins enhanced in the presence of hydrogen peroxide and redox mediators. Chemosphere, 69, 338-345 (2007).

Mohan, S.V., Prasad, K. K., Rao, N. C., Sarma, P. N., Acid azo dye degradation by free and immobilized horseradish peroxidase (HRP) catalyzed process. Chemosphere, 58, 1097-1105 (2005).

Nelson, D. L. and Cox, M. M.; Lehninger Principles of Biochemistry, 4th ed., Freeman and Company: New York, 2000.

Nguyen, T. A. and Juang, R-S., Treatment of waters and wastewaters containing sulfur dyes: A review. J Chem Eng., 219, 109-117 (2013).

Paul, J., Kadam, A. A., Govindwar, S. P., Kumar, P., Varshney, L., An insight into the influence of low dose irradiation pretreatment on the microbial decolouration and degradation of Reactive Red-120 dye. Chemosphere, 90, 1348-1358 (2013).

Peralta-Zamora, P., Pereira, C. M., Tiburtius, E. L. R., Moraes, R. A. M., Minussi, R. C., Duran, N., Decolorization of reactive dyes by immobilized laccase. Appl. Catal. B., 42, 131-144 (2003).

Saeed, Q., Batthi I. A., Zuber, M., Nosheen, S., Zia, M. A.,Abbas, M., Study of application of mono azo reactive dyes on cotton by exhaust method and printing properties. J. Basic Appl. Sci., 12, 191-197 (2012).

Si, J. and Cui, B-K., A new fungal peroxidase with alkaline-tolerant, chloride-enhancing activity and dye decolorization capacity. J. Mol. Catal. B: Enzym., 89, 6-14 (2013).

Solis, M., Solis, A., Perez, I. H., Manjarrez, N., Flores, M., Discoloration of Indigo Carmine Using Aqueous Extracts from Vegetables and Vegetable Residues as Enzyme Sources. Process Biochem., 47, doi: 10.1155/2013/250305 (2013).

Silva, M. C., Correa, A. D., Torres, J. A., Amorim, M. T. S. P., Descoloração de corantes industriais e efluentes têxteis simulados por peroxidase de nabo (Brassica campestre). Quim Nova,35, 889-894 (2012). (In Portuguese)

Silva, M. C., Torres, J. A., Sa, L. R.V., Chagas, P. M. B., Ferreira-Leitão, V. S., Correa, A., The use of soybean peroxidase in the decolourization of Remazol Brilliant Blue R and toxicological evaluation of its degradation products. J. Mol. Catal. B: Enzym., 89, 122-129 (2013).

Wan, M. M., Gao, L.,Chen, Z., Wang, Y.K., Wang, Y., Zhu, J. H., Facile synthesis of new periodic mesoporous organosilica and its performance of immobilizing horseradish peroxidase. Microporous Mesoporous Mater.,155, 24-33 (2012).

Wu, Y., Tayor, K.E., Biswas, N., Bewtra, J. K.,Comparison of additives in the removal of phenolic compounds by peroxidase-catalyzed polymerization. Water Res., 31, 2699-2704 (1997).