Türk Biyokimya Dergisi [Turkish Journal of Biochemistry–Turk J Biochem] 2012; 37 (2) ; 200–206.
doi: 10.5505/tjb.2013.96636

Research Article [Araştırma Makalesi]

Yayın tarihi 30 Haziran, 2013 © TurkJBiochem.com

[Published online 30 June, 2013]

Determination of decolorization properties of Reactive Blue 19 dye using Horseradish Peroxidase enzyme

[Horseradish Peroksidaz enzimi kullanılarak Reactive Blue 19 boyasının renk giderilmesinin belirlenmesi]

[1,3]Mithat Celebi,
[2]Melda Altikatoglu,
[3]Zeynep Mustafaeva Akdeste,
[1,2]Hüseyin Yıldırım

[1]Yalova University, Faculty of Engineering, Department of Polymer Engineering, 77100 Yalova, [2]Yildiz Technical University, Faculty of Arts and Sciences, Department of Chemistry, Davutpasa Campus 34210 Esenler, Istanbul, [3]Technical University, Faculty of Chemical and Metallurgical Engineering, Department of Bioengineering, Davutpasa Campus 34210 Esenler, Istanbul, Turkey

Yazışma Adresi
[Correspondence Address]

Dr. Melda Altikatoglu

Yildiz Technical University, Faculty of Arts and Sciences, Department of Chemistry,
Davutpasa Campus 34210 Esenler, Istanbul, Turkey
Tel. + 90 212 383 4205
Fax. + 90 212 383 4134
E-mail. *maltikatoglu@yahoo.com*

Registered: 4 June 2012; Accepted: 3 March 2013

[Kayıt Tarihi : 4 Haziran 2012; Kabul Tarihi : 3 Mart 2013]

ABSTRACT

Aim: Fenolic compounds are major of pollutants in medicine, food and local matrixe. The enzyme peroxidase is known for its capacity to remove phenolic compounds and aromatic amines from aqueous solutions and also to decolorize textile effluents. In this study, decolorization of anthraquinone dye, Reactive Blue 19 (RB 19), large quantities of which used in textile and other industries was investigated using Horseradish peroxidase (HRP) at different conditions. In addition, decolorization efficiency of dye by enzyme was studied in the presence of denaturing agent urea and salt in synthetic wastewater.

Material and Methods: Horseradish peroxidase and Reactive Blue 19 were obtained by commercial way. The enzymatic decolorization of the dye was examined by UV-Vis spectrophotometer measurements. Decolorization studies were performed by varying parameters such as enzyme and dye concentrations, pH, temperature, incubation time, presence of H_2O_2, salt and urea.

Results: Optimum pH value for dye decolorization was determined as 5.0. Maximum dye was removed within 5 min after the beginning for every experiment. After 5 minutes of treatment, the color removal of dye was ca. 90-95% at pH 5.0 and all temperatures. Furthermore, the higher concentrations of dye, H_2O_2 and NaCl did not exhibit inhibition effect but the initial decolorization rate decreased with increasing the urea concentration. The kinetic constants of enzyme were determined for dye.

Conclusion: As a result, this study verifies the viability of the use of the horseradish peroxidase in the decolorization of Reactive Blue 19.

Keywords: Dye decolorization, Reactive Blue 19 (RB 19), Horseradish peroxidase (HRP), textile dye, industrial effluents

Conflict of Interest: Authors declare no conflict of interest.

ÖZET

Amaç: Fenolik bileşikler tıp alanında, besin ve çevresel matrislerde kirleticilerin başında gelmektedir. Peroksidaz enziminin, sulu çözeltilerden fenolik bileşikleri ve aromatik aminleri uzaklaştırma, aynı zamanda tekstil atıklarının rengini giderme kapasitesine sahip olduğu bilinmektedir. Bu çalışmada, tekstil ve diğer endüstrilerde geniş miktarda kullanılan antrakinon boyasının, Reactive Blue 19 (RB 19), Horseradish peroksidaz (HRP) enzimi ile renk giderilmesi farklı koşullarda incelendi. Ek olarak, enzimin boyayı giderme etkinliği sentetik atık suda denatürasyon ajanı olan üre ve tuz varlığında çalışıldı.

Gereç ve yöntemler: Horseradish peroksidaz ve Reactive Blue 19 boyası ticari olarak satın alındı. Boyanın enzimatik renk giderilmesi, UV-Vis spektrofotometre ile izlendi. Renk giderme çalışmaları enzim ve boya konsantrasyonu, pH, sıcaklık, inkübasyon süresi, ortamda H_2O_2, tuz ve üre varlığı gibi çeşitli parametrelere karşı yapıldı.

Bulgular: Boya giderme için optimum pH değeri 5.0 olarak belirlendi. Maksimum boya giderme, enzim ilave edildikten 5 dakika sonra elde edildi. Bütün sıcaklık değerleri için pH 5.0'de, uygulamanın 5. dakikasından sonra boyanın yaklaşık % 90-95 oranında renginin giderildiği görüldü. Ayrıca Reactive Blue 19 (RB 19), H_2O_2 ve tuzun yüksek konsantrasyonlarının renk giderme üzerine inhibisyon etki göstermediği ancak, üre konsantrasyonunun artması ile başlangıç boya giderme hızının azaldığı gözlendi. Enzim-boya reaksiyonunun kinetik sabitleri hesaplandı.

Sonuçlar: Sonuç olarak, bu çalışma Horseradish peroksidaz enzimi kullanılarak Reactive Blue 19 boyasının çeşitli faktörlere karşı renk giderilmesinin uygulanabilirliğini göstermektedir.

Anahtar Kelimeler: Boya giderme, Reactive Blue 19 (RB 19), Horseradish peroksidaz (HRP), tekstil boyası, endüstriyel atıklar

Çıkar Çatışması: Yazarlar çıkar çatışması olmadığını beyan ederler.

Introduction

Worldwide annual textile production is currently 30 million tons with expected growth of 3% per annum. Water consumption in the textile industry is on average 100 m^3 (per ton of product). The silk industry is the most water consuming (225 m^3/t), whereas the decorative fabric industry has four times less consumption (50 m^3/t). Besides, dye plants use great amounts of water – 225 m^3/t of dye [1, 2]. The losses of reactive dyes are about 2% in the production processes and about 9% through dying and finishing operations in the textile industry. As a result, from 40,000 to 50,000 tons of dye are discharged to surface water every year [1-4].

The textile industry usually produces vast quantities of wastewater that are characterized by strong color, high chemical oxygen demand (COD) and highly fluctuating pH. An estimated 1–15% of the dye is lost during dyeing and finishing processes and is released into wastewater. Accordingly, dye effluent may contain chemicals that are toxic, carcinogenic, mutagenic, or teratogenic to various fish species. Reactive dyes, which have good water solubility and are easily hydrolyzed into insoluble forms, are extensively used in dyeing processes and about 20–40% of these dyes remain in the effluent. Thus, an effective and economical technique for removing reactive dyes from textile wastewaters is needed [5].

The treatment of wastewaters containing dyes is complex and expensive. Methods such as chemical and electrochemical oxidation, membrane processes, coagulation-flocculation, adsorption or ion exchange are recommended [6, 7]. Some of these have already been used in practice, whereas others have only been tested in laboratories or applied on a small scale [4, 8]. Recently, researchers have been focusing their attention to enzymatic treatment. Many peroxidases such as lignin peroxidase, manganese peroxidase, soybean peroxidase, horseradish peroxidase (HRP) and laccase, etc., were applied to decolorize and degrade dye in industrial effluents [8, 9, 10-13].

Enzymes from various sources (fungus and plant based) are applied for the treatment of dye based compounds [14]. Horseradish peroxidase (HRP) is known to efficiently cleave aromatic azo compounds in the presence of H_2O_2 and to degrade and precipitate industrially important azo dyes [14-17]. Recently, the enzymatic approach has attracted much interest in the decolorization/degradation of textile and other industrially important dyes from wastewater [18]**.** Enzymatic approach has many advantages over conventional procedures (such as solvent extraction and adsorption onto activated carbon) which are effective but which suffer from high cost, incomplete purification, formation of hazardous by-products and applicability to only a limited concentration range [19]**.**

Currently, the world's production is over 40,000 structural dye units. Reactive dyes are one of the most common dyes. Due to the advantage of colouring and dyed fabric durability, reactive dyes have increasingly been used for dying and printing on both natural and regenerated cellulose fibres [7, 20]. Triazine and vinyl sulfone dyes are the most important moiety of reactive dyes. Vinyl sulfone reactive dyes contain a vinyl sulfone moiety -$SO_2CH=CH_2$, or more often a 2-sulfatoethylsulfone moiety -$SO_2CH_2CH_2OSO_2Na$ which is hydrolyzed into a previous one in the process of dying [20, 21]. Anthraquinone reactive dyes are one of the major groups among reactive dyes other than azo compounds, based on the classification of molecular structures. In general, they are commercially applied as primary or secondary dyes in trichromatic dyeing formulations [22]. Anthraquinone-based dyes are more resistant to biodegradation due to their fused aromatic structures compared to azo-based ones. In addition, they may cause acute toxicity or even mutagenic effects on exposed aquatic organisms. Therefore, anthraquinone reactive dyes have gradually attracted critical attention from the toxicological and environmental points of view, particularly in light of the current increase in their applications [23].

Reactive Blue 19 (RB 19) is an industrially important dye and is being used frequently as starting material in the production of polymeric dyes. RB 19, an anthraquinone derivative, represents an important class of often toxic and recalcitrant organopollutants. Therefore we used RB 19 for enzymatic decolorization [10]. It structurally resembles certain polycyclic aromatic hydrocarbons, which are substrates of ligninolytic peroxidases [24-26]. It has a structure consisting of azo, anthrazine, naphthalene and sulfonated groups (Figure 1). To our knowledge, RB 19 has not been investigated for degradation by HRP before for different conditions.

In this work, we describe the decolorization of RB 19 as dye, large quantities of which are used in textile industry, by HRP. Effects of parameters such as aqueous phase pH, temperature, H_2O_2, HRP and dye concentration on the dye decolorization efficiencies were investigated. The kinetic constants for selected dye were also determined. Textile wastewater includes denaturing agent urea and salts caused from dying process of fabrics. Decolorization stability of Horseradish peroxidase enzyme was investigated in the presence of salt and inactivating agent urea at synthetic wastewater.

Materials and Methods

Horseradish peroxidase (EC 1.11.1.7) type VI (Mw ~ 40.000 Da) and C.I. Reactive Blue 19 were purchased from Sigma. General characteristics of dye are presented in Table 1. All chemicals are used in this study are obtained by commercial way. Other chemicals and reagents employed were analytical grade.

Decolorization of Reactive Blue 19 dye using Horseradish peroxsidase was carried out directly in the spectrophotometer cuvette. The reaction was started by adding buffer solution at different pHs (50 mM asetate buffer

in the pH range 3-5 and 50 mM phosphate buffer in the pH range 6-8), 10 µL dye (33.0 mg/mL RB 19 stock solution), 16.4 µL HRP solution (0.0033 mg/mL, 8440 U/mg) and finally 10 µL H_2O_2 (3 %) as the initiator in the reaction cuvette respectively. The total volume was 3.0 mL. Dye decolorization was measured with temperature controlling UV-vis spectrophotometer (Model UV-1700 Pharmaspec Shimadzu) based on the maximum absorbance at 594 nm in the visible range, at different pHs (3.0, 4.0, 5.0, 6.0, 7.0, 8.0), temperatures (25, 30, 35, 40, 45, 50 °C) and initial dye concentrations for 60 minutes. Experiments were performed in triplicate and results were given as the mean values. The efficiency of color removal was expressed as the percentage ratio of the decolorized dye concentration to that of initial one.

$$Dye\ Decolorization = \frac{A(i) - A(a)}{A(i)} \times 100$$

A(i) = initial dye absorbance at 594 nm

A(a) = dye absorbance after incubation at 594 nm

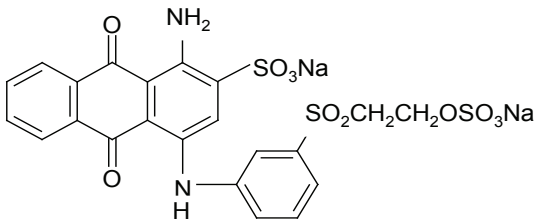

Figure 1. Chemical structure of Reactive Blue 19

The different reaction temperatures (25, 30, 35, 40, 45 and 50 °C) were used for the determination of maximum initial decolourisation rate. These reactions were conducted at pH 5.0 in the presence of 110 mg/mL RB 19 solution in cuvette. All assays were carried out with a minimum of three replicates.

Enzymatic decolorization was monitored in a medium containing 110 mg/L RB 19 solution in cuvette, at different pH values (4.0, 5.0, 6.0, 7.0 and 8.0) at 30 °C. All assays were carried out with a minimum of three replicates.

The influence of dye concentration on enzymatic colour removal was investigated by using different dye concentrations (28, 55, 110, 166, 263 mg/L) at 30 °C and pH 5.0. All assays were carried out with a minimum of three replicates.

The influence of enzyme concentration on decolorization was investigated by using different enzyme concentrations (0.082-13.2 µg/mL) at 30 °C and pH 5.0. All assays were carried out with a minimum of three replicates.

Effect of H_2O_2, urea and salt on decolorization of the dye with HRP

Different concentrations of H_2O_2, urea and sodium chloride were used to assess the effects in colour removal. The concentrations were, 0.5, 1.0, 1.5, 3.0 and 6.0 mM for H_2O_2; 0.05, 0.2, 1.0, 1.5, 2.0 and 2.5 M for urea; 0.05, 0.1, 0.25, 0.5, 1.0, 2.0 M for sodium chloride (NaCl). Reactions were conducted at 30 °C and pH 5.0 in the presence of 110 mg/L RB 19. All assays were carried out with a minimum of three replicates.

The kinetic experiments were performed by varying the concentration of substrate (0.004-0.042 mM) using constant enzyme and H_2O_2 concentration under the optimum conditions (at pH 5.0, 40 °C). The apparent kinetic constants Michaelise-Menten constants (K_m), V_{max} and k_{cat} of HRP were determined by linear regression and the Lineweaver – Burk plots. The values given in the paper represent the mean of three independent sets of experiments with S.D. of less than 5%.

Table 1 General characteristics of Remazol Brillant Blue R (RB 19)

Parameters	Characteristics
Color Index (C.I.)	Reactive Blue 19 (RB19)
Color Index No	61,200
Molecular weight (M_w)	624,54 g/mol
EC No	219-949-9
Maximum Absorption Wavelenght (λmax)	594 nm
Lineere formule	$C_{22}H_{16}N_2Na_2O_{11}S_3$

Table 2 The kinetic parameters of the decolorization of Remazol Brillant Blue R by HRP at pH 5,0.

	K_m (µM)	k_{cat} (min^{-1})	V_{max} (µM min^{-1})
HRP	454.5	21.84	1.813

Results and Discussion

Commercially available HRP was used in this study. There are several publications concerning degradation of RB 19 with ozone oxidation [23], using three white rot fungi named as *Pleurotus ostreatus* (*P. ostreatus*), *Coriolus versicolor* (*C. versicolor*) and *Funalia trogii* (*F. trogii*) [6], ozone-enhanced electrocoagulation [5], tropical Brazilian basidiomycetes fungi [27]. However, no publications were found enzymatically about RB 19 dye degradation by HRP enzyme at different circumstances before. In addition, dye decolorization experiments were applied in the presence of denaturing agent urea and salt in synthetic wastewater at different concentration. Dye decolorization values decrease at increasing concentration of denaturing urea because of inactivation of the enzyme. The objective of this study is to obtain the maximum decolorization percentage with the minimum quantity of inputs, minimizing the process costs. These are advantages of enviromental enzymatic approach.

In this paper, the parameters were optimized separately (pH, temperature, dye concentration, quantity of urea, salt and H_2O_2 and quantity of enzyme). These results found better than those reported earlier with respect to the concentration of RB 19, amount of catalyst, time, pH and temperature.

A series of experiments was carried out under the same operating conditions but varying reaction temperatures and the results are shown in Fig. 2. As clearly shown in Fig.2, the maximum amount of degradation (90-95%) in the first 5 minutes of treatment did not change with temperature at pH 5.0. It is evident from the figure that 5 min of the reaction time is sufficient for the dye degradation. Liu et al. reported that 5 min of reaction time was required for citraconic anhydride-modified HRP catalyzed bromophenol blue and methyl orange degradation [8]. After 5 min, the removal reaction followed by a very slow removal process. This slowdown can be attributed to the simultaneous decrease in the concentration of all the reacting species (phenol, HRP and H_2O_2). Subsequent experiments were conducted for 5 min of reaction time. In similar studies with the enzyme horseradish peroxidase, Bhunia et al. [11] evaluated that most of the degradation of the dye Remazol Blue, occurred within 3 h. Since the commercial dyes have a variety of colors and structures, the enzyme will act in a different way, both in relation to its removal capacity and the degradation rate [28].

Most enzymes have a characteristic pH value at which their activity is maximized. The interrelation of enzymatic activity with pH, for any enzyme, depends on the acidic–basic behavior of the substrate, as well as other factors which are, in general, difficult to analyze quantitatively [28]. The pH optimization for RB 19 was performed by measuring the initial rates of enzymatic dye degradation at different values of pH using 0.0033 mg/mL of HRP, 110 mg/L of dye and 3% of H_2O_2. The pH profiles show a maximum decolorization efficiency at pH 5.0 and 6.0. As clearly shown in Fig. 3, decolorization reached about 96 % at the 5th min. of treatment for pH 5.0. This was an extremely short period to achieve dye degradation. As for pH 6.0, decolorization reached 95 % at the 60 th min. of treatment at 30 °C. At pH values of greater than 6.0, the rates of enzymatic dye degradation were very low. At the two extreme pH values (i.e. pH 3.0 and 8.0), decolorization was not observed because of the activity of HRP was lost at this pHs [17]. No color change was observed in all control flasks containing the dye in aqueous solutions at different pH without enzyme. It has been reported that HRP showed the best activity at pH 5 [17, 28, 29]. The results of this study are in agreement with the ones obtained by Dong et al. [29], suggesting that decolorization might be due to the HRP activity. Bhunia et al. [11] studied the enzymatic decolorization of the dyes Remazol Blue and Red Cibacron, at different pH values, and itwas concluded that at pH values above 6.0, the HRP activity was inhibited. From these results it can be concluded that the ideal pH may vary for the same enzyme, highlighting the need to study the pH to be used.

Different RB 19 concentrations (28, 55, 110, 166, 263 mg/L) were used to determine the effect of dye concentration on enzymatic decolorization of RB 19. Dye decolorization was improved by increasing RB 19 concentrations; however, concentrations above 166 mg/L were inhibitory (Fig. 4).

Deveci et al. [10] investigated the effect of RB 19 concentration on decolorization with culture filtrates of *Funalia trogii* ATCC 200800. They obtained that concentrations above 100 mg/L were inhibitory [10].

The price of HRP was very high. The cost of enzyme had always been the bottleneck of application of enzymatic process on the treatment of wastewater. Thus, one could increase the reaction time to offset the reduction in decolorization efficiency at low enzyme concentration [8, 28].

Decolorization efficiency of RB 19 at different HRP doses (0.082-13.1 µg/mL) are illustrated in Fig. 5, where the removal efficiency is plotted against time for both biocatalyst and different enzyme concentrations. The decolorization efficiencies increased with the increase in the concentrations of enzyme from 0.082 to 3.30 µg/mL. As clearly shown in Fig. 5, decolorization reached about 96% at the 5th min. of treatment. However, subsequent increase in enzyme dose up to 13.1 µg/mL had a significantly low impact on the dye degradation. The reason may be each peroxidase molecular catalyses fewer reactions under higher enzyme concentration, and decreasing the catalytic efficiency. The results indicate that the enzyme dose of 3.3 µg/mL was enough to the removal of RB 19 after 5 min. However, Ulson de Souza et al. [28] have reported that the decolorization of the dye was not significantly influenced by using a higher concentration of enzyme.

HRP require the presence of H_2O_2 [24, 25, 30]. In this study various H_2O_2 concentrations were used to determine the influence of H_2O_2 in RB 19 decolorization. The effects of H_2O_2 concentration on decolorization by HRP described in Fig. 6A. Decolorization did not occur without H_2O_2, however it was enhanced greatly with the addition of 0.05 mM and reached the its maximum (93%) in the first 5 minutes of incubation period. The decolorization was unchanged at higher concentrations. Vyas and Molitoris [25] used the extracellular ligninolytic enzyme of *Pleurotus ostreatus* for decolourisation of RB 19 and showed that H_2O_2 was needed to initiate the enzyme activity. Similarly, Young and Yu used LiP for decolourisation of dyes and they could only initiate enzyme activity in the reaction by adding peroxide ions to the medium [24, 25]. Ulson de Souza et al. [28] have observed that in the absence of this coadjutant there was no decolorization, and the concentration which showed a better enzyme performance 2×10^{-3} mmol/L (59% of

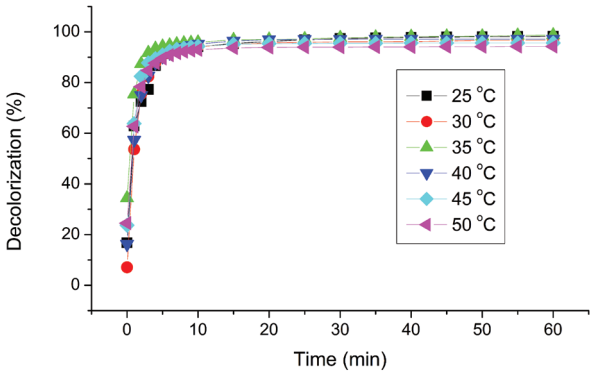

Fig. 2 Time course of RB 19 (110 mg/L) decolorization with HRP (0.0033 mg/mL) for different temperatures at pH 5.0. Each data point represents the average value of three independent experiments.

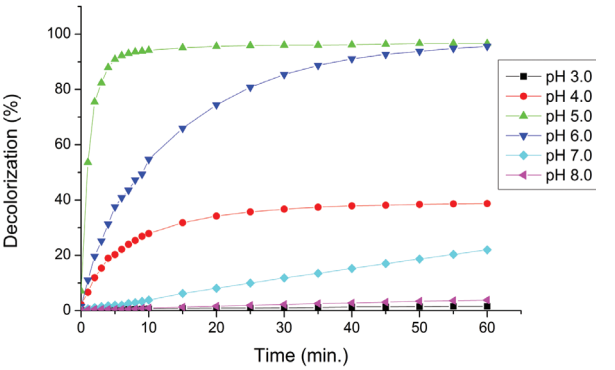

Fig. 3 Time course of RB 19 (110 mg/L) decolorization with HRP (0.0033 mg/mL) for different pHs at 30 °C. Each data point represents the average value of three independent experiments.

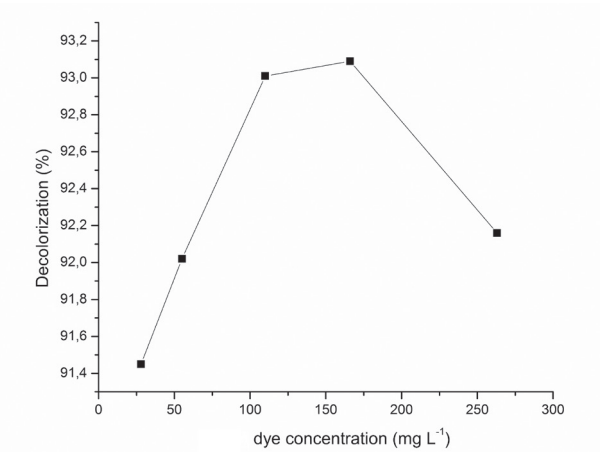

Fig. 4 Influence of dye concentration on decolorization of RB 19 by HRP (0.0033 mg/mL) at the 5th min. of treatment at 30 °C and pH 5.0. Each data point represents the average value of three independent experiments.

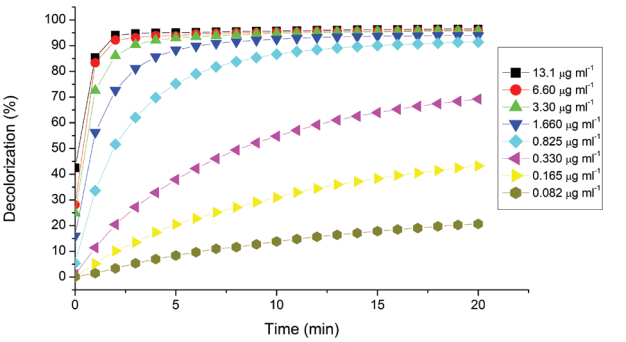

Fig. 5 Time course for decolorization of RB 19 (110 mg/L) with eight different enzyme concentrations at 30 °C and pH 5.0. Each data point represents the average value of three independent experiments.

decolorization) for Remazol Turquoise Blue G 133% and Lanaset Blue 2R. dyes, began to have an inhibitory effect.

The catalytic cycle of the peroxidases consists of a two-electron oxidation of the native ferric enzyme to compound I by hydrogen peroxide, followed by two single electron reductions via intermediate compound II to its resting state by appropriate reducing substrates (redox mediators or target compounds) [31, 32]. Compound II may be further oxidized to an inactivated form, compound III, in the presence of excess hydrogen peroxide [33].

In addition, Fig. 6B and 6C shows the effect of urea and salt (NaCl) concentrations, respectively. Real dyehouse wastewater consist of high COD, colour, salts, unfixed dyestuffs and little soluble O_2. In addition, in several dying processing and printing process urea is using to increase dissolving of dye molecules and other purposes. However, it forms contamination in wastewater [35]. In dying several dying process, inorganic salts decrease dissolving of dye molecules but, they improve affinity of dyes to fabric. For these reasons, decolorization effiency of Reactive Blue 19 dye by using Horseradish peroxidase enzyme was investigated against to inactivating agent urea and salt in synthetic wastewater.

The initial decolorization rate decreased with increasing the urea concentration from 0 to 2.5 M (Fig. 6B). The adding urea exhibited an inhibitory influence on the enzyme activity. The increasing concentrations of urea also inhibited RB 19 decolorizing peroxidase activity. Asgher et al. [34] investigated the effect of the nitrogen additives on decolorization of vat dyes by laccase. They also observed higher dye removal rates in nitrogen limited medium. Sodium chloride usually comes out in the effluent along with sectional wastes of textile mills. Fig. 5C reports the effect of sodium chloride on the enzymatic decolorization of RB 19. The addition of NaCl (to presence of up to 2.0 M) did not cause any alteration in the decolorization by HRP, had no effect on the enzymatic activity.

To investigate the mechanism of enzymatic conversion, a kinetic model has been used to fit the experimental data. The correlation between specific decolorization rate and dye concentration can be described by a Mic-

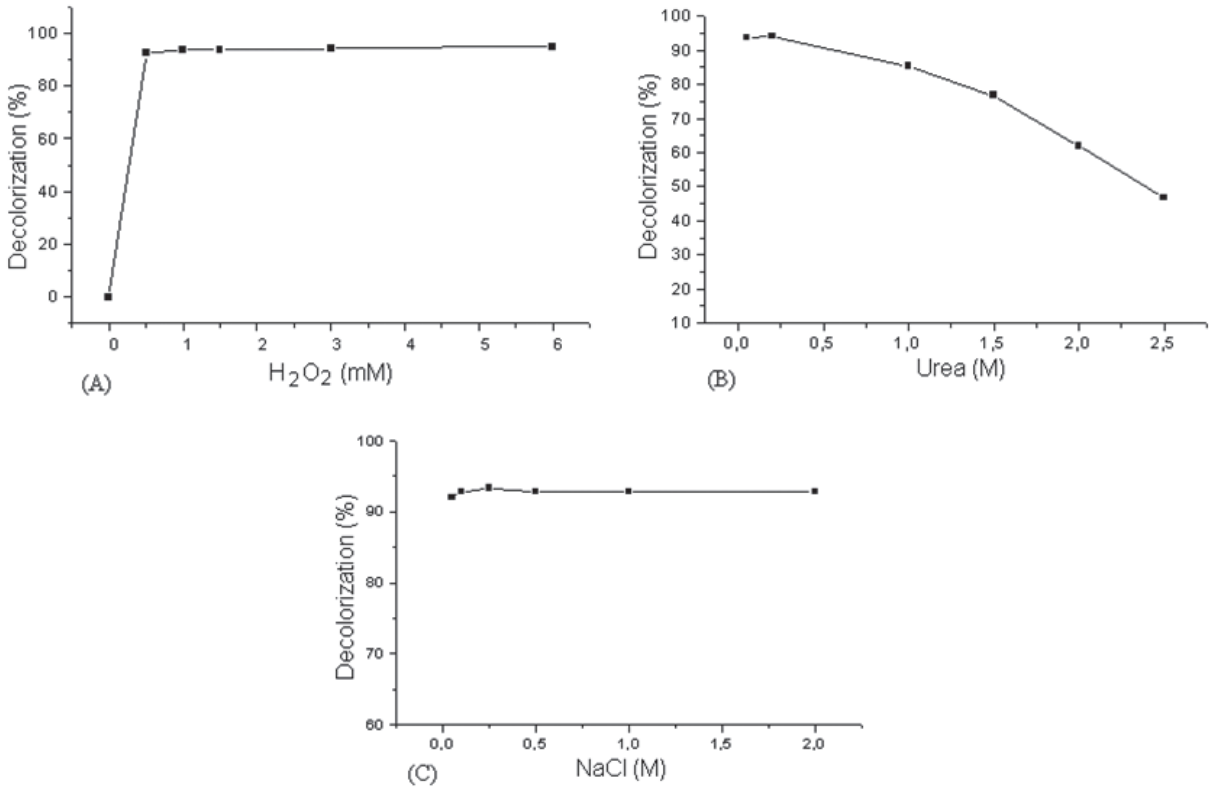

Fig. 6 Effects of (A) H2O2; (B) urea; (C) NaCl addition on decolorization of RB 19 (110 mg/L) by HRP (0.0033 mg/mL) at the 5th min. of treatment at 30 °C and pH 5.0. Each data point represents the average value of three independent experiments.

haelise Menten kinetics. Lineweaver – Burk plots were made from the initial rates obtained at varying dye concentrations while the amount of enzyme was held constant. The kinetic constants, Michaelise Menten constant (K_m), maximum decolorization rate (V_{max}) and catalytic constant (K_{cat}) of HRP were determined for RB 19 (Table 2). Vyas and Molitoris [25] suggested that there were RB 19 decolorizing enzyme proteins which possess different catalytic properties.

Conclusion

This paper reported some detailed characteristics of decolorization of one common industrial dye, RB 19, by the HRP enzyme. Decolorization of dye was dependent on initial pH and temperature of the reaction medium, the concentrations of dye and enzyme. HRP was found to be effective in decolorization of the tested dye, resulting in almost complete color removal. The decolorization that was seen in the absence of peroxide ions, was very fast and was complete within a few minutes after the mixing of reaction components at pH 5.0. Furthermore, the decolorization activity was not affected by temperature changes (25-50 °C), addition of high concentrations of H_2O_2 and salt. Also, decolorization of dye was reduced in the presence of urea. As a result, it was optimized the conditions for obtaining high dye decolorization. The use of the HRP may conceivably be extended to other anthraquinone-type textile dyes, indeed suggesting a potential application field for the removal of dyes in industrial effluents. In this study, enzymatic dye decolorization have benefits such as short reaction time, less chemical usage end little toxic compound production, more enviromental method.

Acknowledgments

This research was supported by the Scientific Research Fund of Yildiz Technical University (29-01-02-GEP01). We thank to 11th Committee of Istanbul Chamber of Industry for financial support to PhD student M. Çelebi.

Conflict of Interest: Authors declare no conflict of interest.

References

[1] Parvathi C, Maruthavanan T. Adsorptive removal of *Megenta MB* cold brand reactive dye by modified activated carbons derived from agricultural waste. Indian J Sci Tech 2010; 3 (4): 408–410.

[2] Yasin Y, Hussein MZ, Ahmad FH. Adsorption of activated carbon. Malaysian J Anal Sci 2007; 11: 400–406.

[3] Reddy SS, Kotaiah B, Velu M. The removal of composite reactive dye from dyeing unit effluent using sewage sludge derived activated carbon. Tur J Engg Env Sci 2006; 4:367–373.

[4] Shin KS, Oh I.K, Kim CJ. Production and purification of Remazol brilliant blue R decolorizing peroxidase from the culture filtrate of *Pleurotus ostreatus*. App Environment Microbiol 1997; 63 (5):1744–1748.

[5] Song S, Yao J, He Z, Qiu J, Chen J. Effect of operational parameters on the decolorization of C.I. Reactive Blue 19 in aqueous solution by ozone-enhanced electrocoagulation. J Hazard Mat 2008; 152: 204–210.

[6] Erkurt EA, Unyayar A, Kumbur H. Decolorization of synthetic dyes by white rot fungi, involving laccase enzyme in the process. Process Biochem 2007; 42: 1429–1435.

[7] Kara S, Aydinera C, Demirbas E, Kobyaa M, Dizge N. Modeling the effects of adsorbent dose and particle size on the adsorption of reactive textile dyes by fly ash. Desalination 2007; 212: 282–293.

[8] Liu JZ, Wang TL, Ji LN. Enhanced dye decolorization efficiency by citraconic anhydride-modified horseradish peroxidase. J Mol Catal B Enzymatic 2006; 41: 81–86.

[9] Levin L, Papinutti L, Forchiassin F. Evaluation of Argentinean white rot fungi for their ability to produce lignin-modifying enzymes and decolorize industrial dyes. Biores Tech 2004; 94: 169–176.

[10] Deveci T, Unyayar A, Mazmanci MA. Production of Remazol brilliant blue R decolourising oxygenase from the culture filtrate of *Funalia trogii* ATCC 200800. J Mol Catal B Enzymatic 2004; 30: 25–32.

[11] Bhunia A, Susheel D, Wangikar PP. Horseradish peroxidase catalyzed degradation of industrially important dyes. Biotech Bioeng 2001; 72: 562–567.

[12] Knutson K, Kirzan S, Ragauskas A. Enzymatic biobleaching of two recalcitrant paper dyes with horseradish and soybean peroxidase. Biotech Lett 2005; 27: 753–758.

[13] Mohan SV, Prasad KK, Rao NC, Sarma PN. Acid azo dye degradation by free and immobilized horseradish peroxidase (HRP) catalyzed process. Chemosphere 2005; 58: 1097–1105.

[14] Novotny C, Rawal B, Bhatt M, Patel M, Susek V, *et al.* Irpex lactless and *Pleatotus ostreatus* for decolourization of chemically different dyes. J Biotech 2001; 89: 113–122.

[15] Aitken MD. Waste treatment applications of enzymes: opportunities and obstacles. Chem Eng J 1993; 52: 49–58.

[16] Nam S, Tratnyek PG. Reduction of azo dyes with zero-valent iron. Water Research 2000; 34 (6):1837–1845.

[17] Onder S, Celebi M, Altikatoglu M, Hatipoglu A, Kuzu H. Decolorization of naphthol blue black using the horseradish peroxidase. Appl Biochem Biotechnol 2011; 163: 433–443.

[18] Husain Q. Potential applications of the oxidoreductive enzymes in the decolorization and detoxification of textile and other synthetic dyes from polluted water. Critical Rev in Biotechnol 2006; 26: 201–221.

[19] O'Brien AM, O'Fagain C. Dye bleaching and phenol precipitation by phthalic anhydride-modified horseradish peroxidase. J Chem Technol Biotechnol 2000; 75: 363-368.

[20] Filipkowska U, Klimiuk E, Grabowski S, Siedlecka E. Adsorption of reactive dyes by modified chitin from aqueous solutions. Polish J Environ Studie 2002; 11(4): 315–323.

[21] Epolito WJ, Lee YH, Bottomley LA, Pavlostathis SG. Characterization of the textile anthraquinone dye Reactive blue 4. Dyes Pigments 2005; 67: 35–46.

[22] Fanchiang JM, Tseng DH. Degradation of anthraquinone dye C.I. Reactive Blue 19 in aqueous solution by ozonation. Chemosphere 2009; 77: 214 -221.

[23] Young L, Yu J. Ligninase-catalysed decolorization of synthetic dyes. Water Research 1997; 31(5):1187–1193.

[24] Vyas BRM, Molitoris HP. Involvement of an extracellular H_2O_2-dependent ligninolytic activity of the white rot fungus *Pleurotus ostreatus* in the decolorization of Remazol brilliant blue R. Appl Environ Microbiol 1995; 61(11): 3919–3927.

[25] Klibanov AM, Tu TM, Scott KP. Peroxidase-catalyzed removal of phenols from coal-conversion waste waters. Science 1983; 221: 259–261.

[26] Tatsumi K, Wada K, Ichikawa H. Removal of chlorophenols from wastewater by immobilized horseradish peroxidase. Biotech Bioeng 1996; 5:126–130.

[27] Ulson de Souza SMAG, Forgiarini E, Ulson de Souza AA. Toxicity of textile dyes and their degradation by the enzyme horseradish peroxidase (HRP). Journal of Hazard Mater 2007; 147: 1073–1078.

[28] Dong Y, Chen J, Li C, Zhu H. Decoloration of three azo dyes in water by photocatalysis of Fe (III)eoxalate complexes/H_2O_2 in the presence of inorganic salts. Dyes and Pigments 2007; 73: 261-268.

[29] Heinfling A, Martine AJ, Martinez AT, Bergbauer M, Szewzyk U. Transformation of industrial by manganese peroxidases from *Bjerkandera adusta* and *Pleurotus ostreatus* in a mangenese independent reaction. Appl Environ Microbiol 1998; 64(8): 2788–2793.

[30] Tien M, Kirk TK. Lignin-degrading enzyme from *Phanerochaete chrysosporium*: purification, characterization, and catalytic properties of a unique H_2O_2-requiring oxygenase. Proceeding of the National Academy of Sciences USA 1984; 81: 2280–2284.

[31] Wariishi H, Akilesvaran L, Gold MH. Manganese peroxidase from the basidiomycete *Phanerochaete chrysosporium*: spectral characterization of oxidation state and the catalytic cycle. Biochem 1988; 27: 5365–5370.

[32] Cai D, Tien M. Kinetic studies on the formation and decomposition of compounds II and III. Reactions of lignin peroxidase with H_2O_2. J Biol Chem 1992; 267: 11149–11155.

[33] Asgher M, Batool S, Bhatti HN, Noreen R, Rahman SU, *et al.* Laccase mediated decolorization of vat dyes by *Coriolus versicolor IBL–04*. Inter Biodeter Biodegrad 2008; 62: 465–470.

[34] Lau WJ, Ismail AF. Polymeric nanofiltration membrane for textile dyeing wastewater treatment: preparation, performance evaluation, transport modelling, and fouling controls. Desalination 2009; 245: 321–348.