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ÖZFER YEŞİLADA

EMRE BİRHANLI

SİNEM ERCAN

NESRİN ÖZMEN

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Reactive dye decolorization activity of crude laccase enzyme from repeated-batch culture of *Funalia trogii*

Özfer YEŞİLADA[1,*], Emre BİRHANLI[1], Sinem ERCAN[1], Nesrin ÖZMEN[2]
[1]Department of Biology, Arts and Science Faculty, İnönü University, Malatya, Turkey
[2]Department of Science, Education Faculty, İnönü University, Malatya, Turkey

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Abstract: The effect of various factors on dye decolorization activity of crude laccase enzyme from repeated-batch culture of *Funalia trogii* ATCC 200800 to obtain rapid and high decolorization activity against Reactive Black 5 and Reactive Blue 171 dyes was investigated. All conditions used were important for dye decolorizing activity of this crude laccase enzyme. The optimum pH of decolorization was tested at 30 °C and it was around 3.0. This activity was highly reduced at pH 4.5–6.0. On the other hand, the optimum temperature for rapid and high decolorization was 50 °C. Importantly, the decolorization rate of crude laccase at pH 4.5 and pH 6.0 increased with the rise in temperature from 30 °C to 50 °C. Therefore, high decolorization could be obtained at pH 4.5 and even pH 6.0 by selecting the proper temperature for these pH values. Enzyme amount also affected the dye decolorization positively. This crude laccase could also decolorize the mixed dyes (Reactive Black 5 and Reactive Blue 171) and synthetic wastewaters. Native polyacrylamide gel electrophoresis showed the responsibility of the laccase enzyme in decolorization. Rapid and high textile dye decolorization through the selection of appropriate conditions could facilitate the development of more economical and environmentally friendly processes.

Key words: Crude laccase, decolorization, *Funalia trogii,* native gel electrophoresis, reactive dye

1. Introduction

Wastewaters from textile or dyeing industries contain various textile dyes. These dyes give these wastewaters their characteristic deep color. Because of their deep color, their disposal into waters may affect the ecosystem by reducing the photosynthetic activity and, therefore, affecting dissolved oxygen concentration (Robinson et al., 2002). Dyes may also affect the abiotic factors of the aquatic ecosystems by toxic and even genotoxic means (Hu and Wu, 2001; Dogan et al., 2005). Some of the industries mentioned above have no biological treatment systems. On the other hand, because some of the textile dyes are recalcitrant (Robinson et al., 2002), the industries with conventional secondary treatment systems such as activated sludge processes could not completely solve this pollution problem. Therefore, the effluents from conventional secondary treatment systems may also contain deep colors. Thus, these wastewaters need to be treated before being discharged into aquatic ecosystems. There are great attempts to develop new and effective environmentally friendly biological alternatives for decolorizing these dye-containing wastewaters (Yesilada, 1995; Murugesan et al., 2007b; Wang et al., 2009; Yesilada et al., 2010; Bonugli-Santos et al., 2012; Gül and Dönmez, 2012, 2013). The solution could be specific microorganisms or enzymes (Yeşilada and Özcan, 1998; Yesilada et al., 2010; Zeng et al., 2011; Hadibarata et al., 2012). The main advantages of using enzymes for decolorization of dyes may be their rapid decolorization activity and their ability to retain their activity even under unfavorable conditions (Murugesan et al., 2007b; Zeng et al., 2011).

Laccase (EC 1.10.3.2) is a biotechnologically important enzyme. Due to its low substrate specificity, it could be used in various biotechnological applications. Various organisms including white rot fungi, bacteria, and plants can produce this enzyme, but white rot fungi are the main and best laccase producers among them. White rot fungal species could produce several laccase isozymes. Culture conditions, fermentation mode, type and amount of nutrient, and inducers may affect the laccase production (Janusz et al., 2006; Birhanlı and Yeşilada, 2013) by influencing the expression of these isozymes (Giardina et al., 2010).

Textile dyes may be classified as reactive, direct, disperse, acid, basic, and vat dyes depending on the dyeing process used (Murugesan et al., 2007b). These dyes may

* Correspondence: ozfer.yesilada@inonu.edu.tr

also be classified depending on their structural varieties, such as acidic, basic, disperse, azo, diazo, anthraquinone-based, and metal complex dyes (Murugesan et al., 2007b). They may contain functional groups such as azo, anthraquinone, phthalocyanine, formazine, and oxazine as chromophores (Joo et al., 2007). Most of the reactive dyes are azo dyes having one or more azo groups. Some dyes are hardly removed by conventional secondary (biological) treatment systems and, therefore, most of them remain unaffected in treated wastewaters. Laccase is known as an effective enzyme for dye decolorization. However, this decolorization activity depends on the source of the enzyme and also on the chemical structure of the dye (Abadulla et al., 2000; Rodriguez-Couto, 2007; Michniewicz et al., 2008). Therefore, laccase enzymes obtained from different strains and also from different fermentation processes may have different dye decolorization activity. Textile dye decolorization activity of the crude laccase enzyme obtained from repeated-batch cultures of *Funalia trogii* ATCC 200800 was shown in our preliminary study (Birhanli and Yesilada, 2006), but the conditions for rapid and high decolorization activity have not been investigated. According to our literature knowledge, this is the first study for enhancing the reactive dye decolorization activity of this crude laccase enzyme, especially from repeated-batch cultures of this strain. Therefore, in this study, the effect of pH, temperature, initial dye concentration, and enzyme amount on dye decolorization activity of crude laccase enzyme from repeated-batch cultures of *F. trogii* ATCC 200800 was investigated. Hence, the main aim of this study is to enhance the dye decolorization activity of this crude laccase enzyme. The decolorization activity in mixed dyes and synthetic wastewaters of this crude laccase enzyme was also tested.

2. Materials and methods

2.1. Dyes

The diazo dyes Reactive Black 5 (RB 5) and Reactive Blue 171 (RB 171) were obtained from Pet-Sel Textile Chemistry Co. and were prepared as stock solutions (final concentration of 200 mg/L) by dissolving them in citrate phosphate buffer solutions with different pH values. These stock solutions were used according to the experimental design.

2.2. Organism used for laccase production

Funalia trogii ATCC 200800 was used to produce laccase enzyme. This fungus was subcultured at 30 °C every 2–3 weeks on Sabouraud dextrose agar (SDA) plates and maintained at 4 °C.

2.3. Laccase production by *F. trogii* pellets

F. trogii mycelium from slant SDA was cultured in 100 mL of Sabouraud dextrose broth (SDB) for 5 days at 30 °C and 150 rpm to produce preinoculum. After incubation, the preinoculum was homogenized and 7 mL of this homogenized preinoculum was used to inoculate 600 mL of fresh SDB. This culture was incubated for 5 days. The pellets obtained from this fermentation were then used to produce laccase enzyme during repeated-batch studies. In this process, 50 mL of stock basal medium (SBM) in 250-mL flasks was inoculated with pellets and this culture was incubated for 24 h. The culture medium was then completely removed and replaced with 50 mL of fresh SBM without removing the pellets, which was then incubated as stated above for the next cycle (Birhanli and Yesilada, 2010). This mode was repeated 5 times and then the culture was filtrated on fifth day and the obtained culture filtrate was used as crude laccase enzyme for decolorization experiments. The composition of SBM used was (g/L): KH_2PO_4 0.2; $CaCl_2.2H_2O$ 0.1; $MgSO_4.7H_2O$ 0.05; $NH_4H_2PO_4$ 0.5; $FeSO_4.7H_2O$ 0.035; glucose 2, yeast extract 1, and $CuSO_4.5H_2O$ 0.125.

2.4. Enzymatic dye decolorization

The crude laccase enzyme from repeated-batch culture of *F. trogii* was used as a biological system for dye decolorization. To this end, this crude enzyme sample was added into citrate phosphate buffer containing dye and incubated for 30, 60, and 300 s. Decolorization was determined by monitoring the absorbance changes at the maximum absorbance wavelength of the dyes used and was expressed in terms of percentage. Unless otherwise stated, the temperature and pH were 30 °C and 4.5, respectively.

2.5. Effect of pH on enzymatic dye decolorization

The effect of pH on decolorization activity was determined within the pH range of 2.5–6.0 at 30 °C. The pH of the reaction mixture was adjusted by citrate phosphate buffer. The dye concentration and enzyme amount used were 100 mg/L and 100 µL, respectively.

2.6. Effect of the enzyme amount on dye decolorization

Different amounts of enzyme (10–200 µL), from crude laccase enzyme source having 54 U/mL laccase activity, were used to test the effect of the amount of enzyme on dye decolorization. The pH, temperature, and dye amounts were 3.0, 30 °C and 100 mg/L, respectively.

2.7. Effect of initial dye concentration on enzymatic dye decolorization

The dye decolorization activity of crude laccase against the dye solutions with different initial dye concentrations (50–150 mg/L) was tested. The enzymatic dye decolorization studies were performed by using 100 µL of crude laccase at pH 3.0 and 30 °C.

2.8. Effect of temperature on enzymatic dye decolorization

The effect of temperature on dye decolorization activity was investigated within the temperature range of 30–70 °C at pH 3.0. The dye concentration and enzyme amount used were 100 mg/L and 100 µL, respectively.

2.9. Mixed dye and synthetic wastewater decolorization activity of crude laccase

Mixed dye (100 mg/L RB 5 and 100 mg/L RB 171) decolorization activity of this crude laccase enzyme was tested at pH 3.0 and 50 °C. Synthetic wastewater (100 mg/L RB 5, 90 g/L sodium sulfate, and 20 g/L sodium carbonate (A); 100 mg/L RB 171, 90 g/L sodium sulfate, and 20 g/L sodium carbonate (B); and 100 mg/L RB 5, 100 mg/L RB 171, 90 g/L sodium sulfate, and 20 g/L sodium carbonate (C)) decolorization ability of this crude enzyme was also investigated at 50 °C.

2.10. Native gel electrophoresis

To determine if the laccase was the enzyme responsible from dye decolorization, decolorization activity was also determined by a single-step detection method on native gels. Native polyacrylamide gel electrophoresis (PAGE) was performed on 10% separating and 4% stacking gels (Birhanli and Yesilada, 2010). After electrophoresis, one of the crude enzyme-loaded gels was incubated with a solution containing 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) for laccase activity determination. The other crude enzyme-loaded gels were stained with RB 5 and RB 171 separately. The dye solutions were then discarded and gels were examined for decolorization activity.

2.11. Laccase activity assay

Laccase (EC 1.10.3.2) activity was determined spectrophotometrically at 420 nm by detecting the oxidation of ABTS in the reaction mixture containing 100 mmol/L sodium acetate buffer, 0.5 mmol/L ABTS, and a suitable amount of crude enzyme. One unit was defined as the amount of enzyme that oxidized 1 μmol of substrate (ABTS) per minute at 30 °C. All values are the means of at least 3 replicates ± standard deviations (Birhanli and Yesilada, 2010).

3. Results

3.1. Laccase production

The repeated-batch method, which is a completely different fermentation method than the submerged and solid-state fermentation methods, was used to produce laccase enzyme. The culture filtrate obtained after 5 cycles of incubation was used as crude laccase enzyme to decolorize textile dyes. Only laccase enzyme was detected with this culture.

3.2. Enzymatic decolorization of reactive dyes

Enzymatic treatment may be a suitable environmentally friendly method for dye decolorization and laccase from white rot fungi may be used to decolorize textile dyes. To this end, firstly, the decolorization ability of crude laccase enzyme obtained from repeated-batch culture was tested using 2 reactive dyes (RB 5 and RB 171). The decolorization activity of crude laccase against these 2 dyes was different. Incubation time was detected as an important factor for decolorization. While the RB 5 and RB 171 decolorization values were 12% and 53% at pH 4.5 and 30 °C after incubation for 30 s, these values were 38% and 58% after 300 s of incubation, respectively.

3.3. The effect of pH on enzymatic dye decolorization

Because reaction conditions are important for enzymatic dye decolorization, the optimum pH necessary for high decolorization ability of crude laccase was investigated at 30 °C. This investigation was carried out at different pH values during 300 s of incubation. Table 1 shows the variations in dye decolorization at different pH values. The crude laccase tested in our study showed much higher decolorization activity against the 2 dyes at pH 2.5–4.0. At pH 4.5–6.0, this activity was highly reduced (Table 1). While RB 5 and RB 171 were enzymatically decolorized at about 10% and 56% at pH 6.0, respectively, these decolorization activities increased to 78% and 75% at pH 3.0.

Table 1. The effect of different pH values on decolorization of RB 5 and RB 171 by crude laccase at 30 °C.

pH values	Decolorization (%) of RB 5 after:			Decolorization (%) of RB 171 after:		
	30 s	60 s	300 s	30 s	60 s	300 s
pH 2.5	44.78 ± 1.74	54.67 ± 0.67	76.85 ± 1.27	64.69 ± 1.53	67.08 ± 1.44	71.80 ± 1.10
pH 3.0	34.44 ± 1.83	52.22 ± 0.45	78.14 ± 1.50	65.48 ± 0.53	68.26 ± 1.56	74.96 ± 1.27
pH 3.5	31.09 ± 1.23	47.68 ± 2.01	68.81 ± 1.91	58.47 ± 3.12	60.85 ± 2.75	66.83 ± 1.37
pH 4.0	20.64 ± 2.80	32.91 ± 2.69	59.67 ± 3.63	58.76 ± 1.39	62.40 ± 0.37	66.55 ± 0.51
pH 4.5	12.04 ± 3.89	19.96 ± 3.62	37.50 ± 3.66	52.88 ± 0.39	56.36 ± 1.07	58.22 ± 1.06
pH 5.0	10.65 ± 2.47	15.84 ± 3.50	29.17 ± 1.44	48.83 ± 2.84	53.50 ± 2.70	55.19 ± 2.49
pH 6.0	3.22 ± 2.38	4.51 ± 2.17	10.35 ± 1.82	48.18 ± 3.85	53.71 ± 1.90	55.50 ± 1.66

3.4. The effect of enzyme amount on enzymatic dye decolorization

To test the effect of enzyme amount on dye decolorization, different amounts of enzyme from a crude laccase enzyme source having 54 U/mL enzyme activity were used. Enzyme amount affected the dye decolorization positively and decolorization increased with increase in enzyme amounts. Because the decolorization changed very little above an enzyme amount of 100 µL, this amount was determined as the effective amount of laccase activity for dye decolorization (Figure 1).

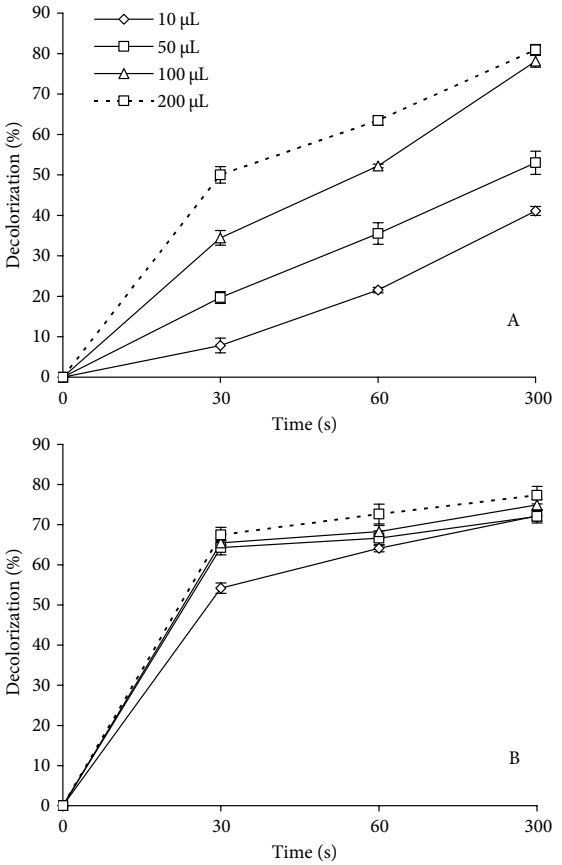

Figure 1. The effect of different amounts of crude laccase on decolorization of RB 5 (A) and RB 171 (B) at 30 °C.

3.5. The effect of initial dye concentration on enzymatic dye decolorization

The dye decolorization activity of crude laccase was tested in dye solutions containing different initial dye concentrations (50–150 mg/L). The results showed that crude laccase could effectively decolorize these 2 dyes, especially at a concentration of 100 mg/L (Table 2).

3.6. The effect of temperature on enzymatic dye decolorization

Firstly, the effect of temperature on dye decolorization activity of crude laccase was investigated at pH 3.0. As shown in Figure 2, crude laccase decolorized the 2 dyes at different rates. Decolorization of RB 5 increased with increase of temperature after 30 s of incubation. However, the decolorization was very similar at 300 s of incubation. Although RB 5 decolorization was only 34% after 30 s of incubation at 30 °C, the decolorization of RB 5 increased to 62% at 50 °C with the same incubation period. At 300 s, these values were 78% and 81%, respectively. Therefore, its maximum decolorization at pH 3.0 was obtained at 50 °C. On the other hand, the decolorization values of RB 171 at all temperatures were above 60% in 30 s. This dye was also decolorized to a maximum at 50 °C. The crude laccase decolorized RB 171 more rapidly than RB 5.

The decolorization efficiency of the crude laccase enzyme at 30 °C decreased above the pH value of 3.0. Therefore, the effect of the optimum temperature (50 °C, determined for pH 3.0) on decolorization activity of crude laccase enzyme at pH values of 4.5 and 6.0 was also investigated. As shown in Table 3, 50 °C induced the decolorization ability of this crude laccase. At 30 °C, while the RB 5 decolorization values were 38% and 10% at pH 4.5 and pH 6.0 after 300 s of incubation, respectively, these values reached 68% and 56% when 50 °C used. Although RB 5 was decolorized to a limited extent at pH 4.5 (12%) and pH 6.0 (3%) at 30 °C after 30 s of incubation, it was highly decolorized at 50 °C (Table 3). At 50 °C, RB 171 decolorization values were 80% and 85% at pH 4.5 and pH 6.0 after 300 s of incubation, respectively.

Table 2. The effect of initial dye concentration on decolorization of RB 5 and RB 171 (pH 3.0) by crude laccase at 30 °C.

Initial dye concentrations	Decolorization (%) after:		
	30 s	60 s	300 s
50 mg/L RB 5	27.77 ± 1.02	34.49 ± 0.91	40.10 ± 1.06
100 mg/L RB 5	34.44 ± 1.83	52.22 ± 0.45	78.14 ± 1.50
150 mg/L RB 5	30.17 ± 1.84	36.43 ± 0.98	42.84 ± 1.02
50 mg/L RB 171	44.00 ± 0.80	45.06 ± 0.64	46.98 ± 0.90
100 mg/L RB 171	65.48 ± 0.53	68.26 ± 1.56	74.96 ± 1.27
150 mg/L RB 171	63.59 ± 1.23	64.99 ± 0.91	68.32 ± 1.76

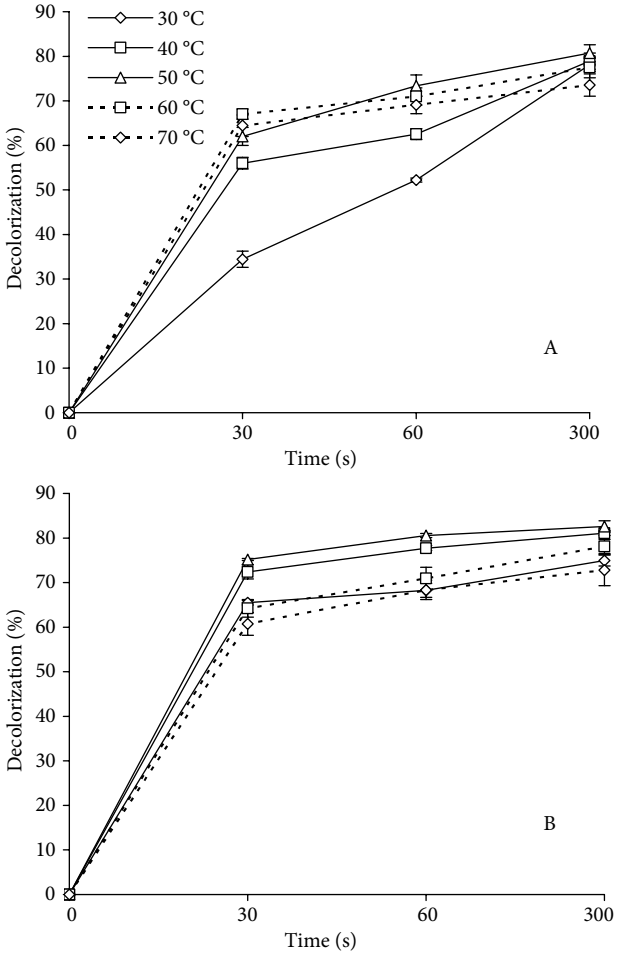

Figure 2. The effect of different temperatures on decolorization of RB 5 (A) and RB 171 (B) by crude laccase at pH 3.0.

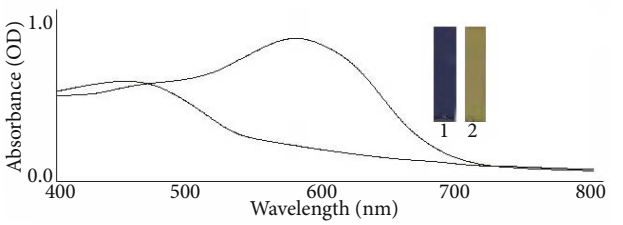

Figure 3. Spectra and color difference of untreated (1) and treated (2) mixed dyes after incubation with crude laccase at pH 3.0 and 50 °C.

3.7. Enzymatic decolorization of mixed dyes and synthetic wastewaters

Because textile and dyeing effluents generally contain a mixture of dyes (mixed dyes), the decolorization ability of crude laccase enzyme against mixed dyes was tested at pH 3.0 and 50 °C. As shown in Figure 3, this crude laccase enzyme showed high and rapid decolorization performance against the mixed dye solution under the optimized conditions. The decolorization effect of crude laccase against synthetic wastewaters was also investigated (Nilratnisakorn et al., 2008). The results showed that the crude laccase enzyme could also decolorize these synthetic wastewaters (Table 4).

3.8. Detection of the enzyme responsible for dye degradation

As stated above, laccase was the only enzyme detected in culture filtrate during the spectrophotometric activity determinations. Even so, native PAGE was used to show that the enzyme responsible for decolorization was laccase. Only a single laccase activity band against ABTS was detected on the gel. The other crude enzyme-loaded gels stained with dyes gave one decolorized band at the same point with laccase (Figure 4).

4. Discussion

In this study, enzymatic decolorization of reactive dyes was tested. Because laccase is a primary enzyme with the ability of textile dye decolorization, culture filtrate containing a high amount of laccase enzyme was obtained. Various factors such as culture conditions, fermentation mode, type and amount of nutrient, and inducers may affect the laccase production by influencing the expression of laccase isozymes (Giardina et al., 2010; Parenti et al., 2013). Therefore, laccase production ability of *F. trogii* was investigated under repeated-batch method and culture filtrate with 54 U/mL laccase was obtained under these

Table 3. The effect of different pH values on decolorization of RB 5 and RB 171 by crude laccase at 50 °C.

Dyes at pH:	Decolorization (%) after:		
	30 s	60 s	300 s
RB 5 at pH 4.5	17.34 ± 1.15	34.76 ± 0.61	67.70 ± 0.93
RB 5 at pH 6.0	3.68 ± 0.87	12.75 ± 1.56	55.95 ± 1.31
RB 171 at pH 4.5	61.80 ± 1.42	65.62 ± 1.24	79.89 ± 1.29
RB 171 at pH 6.0	59.67 ± 0.93	65.56 ± 1.69	84.80 ± 1.38

Table 4. Decolorization of synthetic wastewaters (A, B and C) by crude laccase at 50 °C.

Synthetic wastewaters	Decolorization (%) after:		
	30 s	60 s	300 s
A	55.93 ± 0.71	62.53 ± 0.19	67.09 ± 0.54
B	48.20 ± 2.83	64.89 ± 1.31	82.54 ± 0.65
C	30.37 ± 1.90	53.85 ± 1.69	77.21 ± 1.19

A: 100 mg/L RB 5, 90 g/L sodium sulfate, and 20 g/L sodium carbonate.
B: 100 mg/L RB 171, 90 g/L sodium sulfate, and 20 g/L sodium carbonate.
C: 100 mg/L RB 5, 100 mg/L RB 171, 90 g/L sodium sulfate, and 20 g/L sodium carbonate.

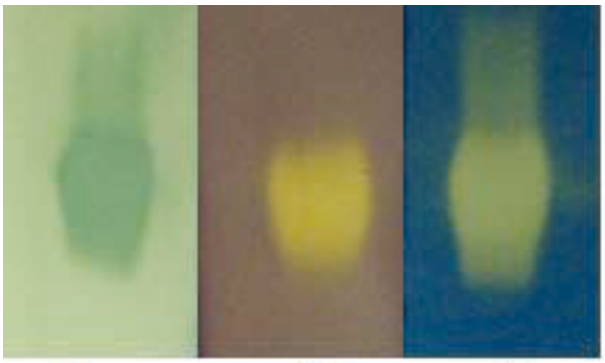

Figure 4. Decolorization of RB 5 and RB 171 dyes on native gel loaded with crude laccase from repeated-batch culture of *F. trogii*. Zymogram of laccase (1) and RB 5 dye (2) and RB 171 dye (3) decolorization zones on native gel.

conditions. This culture, containing high amounts of laccase, was used as crude laccase enzyme to decolorize reactive textile dyes.

Textile dyes are responsible for the deep color of the effluents of textile and dyeing industries. Because color negatively affects the photosynthetic activity and dissolved oxygen concentration of the ecosystem, these dyes have serious negative impacts on aquatic systems. Therefore, there is a need to decolorize textile dyes. In this study, textile dyes RB 5 and RB 171 were decolorized to different extents by crude laccase. The difference of the redox potentials of these reactive dyes and the suitability of the steric structure of these dyes with the active site of the enzyme may affect decolorization (Tavares et al., 2008). Decolorization activity may depend on the source of the enzyme and also on the chemical structure of the dye (Abadulla et al., 2000; Rodriguez-Couto, 2007; Michniewicz et al., 2008). Therefore, the crude laccase obtained from repeated-batch culture and different strains may have different dye decolorization activities. It was reported in some studies that laccase could not decolorize some reactive dyes without mediators (Tavares et al., 2008). The crude laccase from solid-state culture of *T. trogii* SYBC-LZ and *G. lucidum* could decolorize RB 5 only in the presence of a mediator (Murugesan et al., 2007b; Zeng et al., 2011). Murugesan et al. (2007a) reported the same observation about the dye decolorization activity of purified laccase of *P. sajor caju*. Camarero et al. (2005) also reported that RB 5 and Azure B could be decolorized only in the presence of mediators. However, mediators have some disadvantages. Because they can inactivate the laccase enzyme, mediator concentration may inhibit the decolorization (Roriz et al., 2009; Daâssi et al., 2012). More importantly, some of mediators such as hydroxybenzotriazole may also be toxic or hardly biodegradable (Camarero et al., 2004). Its high cost is also disadvantageous for application (Camarero et al., 2005). Therefore, biological systems without mediators could be an ecofriendly solution for pollution. Hence, we tested whether it is possible to obtain high levels of decolorization by obtaining the proper conditions without using any mediator. Redox potential of laccases is also important for dye decolorization and laccases obtained from different fungi may have different redox potentials (Li and Eriksson, 1999; Soares et al., 2001; Nyanhongo et al., 2002). The decolorization with laccase enzymes depends on the enzyme source (the species and even the strain of the enzyme obtained), the chemical structure of the dyes used, and the reaction conditions (Abadulla et al., 2000; Claus et al., 2002; Nyanhongo et al., 2002; Rodriguez-Couto, 2007; Michniewicz et al., 2008; Zeng et al., 2011). The optimum pH of decolorization was around 3.0 at 30 °C and dye decolorization activity was highly reduced at pH 4.5–6.0. The pH and time of incubation were detected as the important parameters for obtaining high decolorization values (Kokol et al., 2007). Michniewicz et al. (2008) reported an optimum pH value of around 3.5 for decolorization of azo and anthraquinone dyes by crude laccase from *C. unicolor*. Daâssi et al. (2012)

reported the optimum pH for RB 5 decolorization of crude laccase from *T. trogii* CLBE 55 as 4.5. It was reported that laccase hardly decolorized RB 5 without a mediator and the decolorization of this dye with crude laccase from *T. pubescens* was only 10% at pH 4.0 in 24 h (Roriz et al., 2009). Temperature is also an important parameter for enzymatic dye decolorization (Nyanhongo et al., 2002; Murugesan et al., 2007b; Zeng et al., 2011). The reactive dyes used in our study were decolorized to a maximum at 50 °C. Daâssi et al. (2012) reported the optimum temperature of RB 5 decolorization by crude laccase from *T. trogii* CLBE 55 as 60 °C. The decolorization efficiency of the crude laccase enzyme at 30 °C decreased above the pH value of 3.0. The decolorization rate of crude laccase at pH values of 4.5 and 6.0 increased with the rise in temperature from 30 °C to 50 °C. This showed that high decolorization could be obtained at pH 4.5 and even pH 6.0 by selecting the proper temperature for these pH values. Therefore, the proper temperature of laccase for dye decolorization was significantly related to the pH of the medium. The crude enzyme-loaded native gels stained with dyes gave one decolorized band at the same point with laccase. Murugesan et al. (2007b) reported no RB 5 decolorization with crude laccase from *Ganoderma lucidum* KMK2, and it needs a mediator for decolorization of RB 5 on native gel. In our study, dye decolorization was obtained without any mediator. Because some of the mediators have high prices or toxicity problems (Johannes and Majcherczyk, 2000), dye decolorization without any mediators has a great advantage (Moilanen et al., 2010).

In conclusion, it was possible to enhance the decolorization activity of this crude laccase from repeated-batch culture of *F. trogii* ATCC 200800 by optimizing the conditions without a mediator. Because the high price and toxicity of some of the mediators may affect application, decolorization potential without any mediator may be advantageous for application of this crude laccase to biological dye treatment systems. This crude laccase source showed high decolorization potential in a short time. It could also decolorize the mixed dyes (RB 5 and RB 171) and synthetic wastewaters. This could be an alternative environmentally friendly solution to solve the textile dye pollution problem.

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