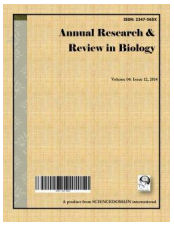


Annual Research & Review in Biology

15(4): 1-16, 2017; Article no.ARRB.35644
ISSN: 2347-565X, NLM ID: 101632869

Biodecolorization of Six Synthetic Dyes by *Pleurotus ostreatus* ARC280 Laccase in Presence and Absence of Hydroxybenzotriazole (HBT)

Ali M. Elshafei[1*], Maysa A. Elsayed[1], Mohamed M. Hassan[1], Bakry M. Haroun[2], Abdelmageed M. Othman[1] and Ayman A. Farrag[2]

[1]Department of Microbial Chemistry, National Research Centre, 33 El Bohouth St. (Former El Tahrir st.), Dokki, Giza, P.O. 12622, Egypt.
[2]Department of Botany and Microbiology, Faculty of Science, Al-Azhar University, Cairo, Egypt.

Authors' contributions

This work was carried out in collaboration between all authors. Author AME designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors MAE, MMH and AMO managed the analyses of the study. Author AMO managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/ARRB/2017/35644
Editor(s):
(1) George Perry, Dean and Professor of Biology, University of Texas at San Antonio, USA.
Reviewers:
(1) Abdul Kariem Arof, University of Malaya, Malaysia.
(2) Tatjana Radosavljević, University of Belgrade, Serbia.
(3) Hao Wang, Northeastern University, Massachusetts.
Complete Peer review History: http://www.sciencedomain.org/review-history/20411

Original Research Article

Received 23rd July 2017
Accepted 4th August 2017
Published 8th August 2017

ABSTRACT

Aim: The aim of the present study was to investigate the potential effect of laccase enzyme produced by the fungus *Pleurotus ostreatus* to decolorize six synthetic dyes in presence and absence of hydroxybenzotriazole (HBT) as a laccase mediator.
Study Design: Qualitative and quantitative evaluation for the efficiency of laccase produced by *Pleurotus ostreatus* to decolorize six tested dyes in the presence and absence of HBT.
Place and Duration of Study: Department of Microbial Chemistry, Genetic Engineering and Biotechnology Division, National Research Centre (NRC), Cairo, Egypt, between April 2016 and May, 2017.
Methodology: *Pleurotus ostreatus* ARC280 was maintained on Potato Dextrose Agar (PDA)

**Corresponding author: E-mail: alielshafei@yahoo.com, alielshafei48@yahoo.com;*

medium and kept at 4ºC. Production of laccase was preceded by growing *Pl. ostreatus* on liquid media under static conditions. The efficiency of laccase obtained from *P. ostreatus* ARC280 on dyes decolorization was determined by the decrease in absorbance at the maximum wavelength of each dye. The efficiency of decolorization was expressed in terms of decolorization percentage.

Results: Results obtained showed that the enzyme was efficient in decolorizing Acid-Green 27 (95.33%), Acid dye Lanapel Red BM 143-PL (100%) and Congo Red (72.76%) at dye concentration of 25 mg/l; enzyme units, 0.657 U and after 6 h of incubation at 30ºC. In the presence of HBT (1 mM), the time required for reaching 100% decolorization of Acid Green-27 and Acid dye Lanapel Red BM 143-PL was reduced to the half. Absorbance reduction at the maximum wavelength was recorded with all the tested dyes indicating the efficiency of the decolorization process.

Conclusion: The results obtained indicated the effectiveness of *P. ostreatus* ARC280 laccase and its mediated system in the decolorization of six structurally different dyes. Addition of HBT decreased the time required to reach the maximum decolorization percentage of Acid-Green 27 and Acid dye Lanapel Red BM 143-PL to the half. The decolorization percentage of Cibacron D-Blue was greatly affected by HBT-laccase mediator system by about 45% increase.

Keywords: Biodecolorization; laccase; Pleurotus ostreatus; Hydroxybenzotriazole (HBT); dye effluents.

1. INTRODUCTION

In the past few years great attention has been directed towards the treatment and decolorization of dye effluents due to the discharge of large colored synthetic dye effluents from industries into lakes and rivers, which is found to be harmful to the water bodies as it reduces sunlight penetration into water, decreasing both dissolved oxygen concentration and photosynthetic activity [1]. Another investigation has been reported that, the presence even a very small portion of dyes in water bodies is highly noticeable by human eye and alters the aquatic ecosystem by reducing the penetration of sunlight [2].

Many studies have been reported that discharging of these chemical pollutants in water streams cause potential health hazards because they are toxic or mutagenic to many living organisms [3,4]. From the different methods reported for the removal of dyes from industrial effluents, microbial decolorization is considered as the best one as an eco-friendly treatment for the removal of synthetic dyes besides the low cost of treatment, as compared with physical and chemical treatments [5,6].

Many investigations reported that efficient decolorization of dye effluents can be achieved by enzymatic oxidation using fungal oxido-reductases such as laccases which were found to be the most efficient treatment for dye decolorization [7]. Laccases (EC 1.10.3.2) are promising group of enzymes that could assist in treatment of wastewater effectively due to their capability to catalyze the oxidation of a broad range of substrates such as ortho- and para-diphenols, methoxy-substituted phenols, aromatic amines, phenolic acids and several other compounds coupled to the reduction of molecular oxygen to water with one electron oxidation mechanism [8]. Nowadays increasing attention for laccases produced from white-rot fungi as dye removal tool in different industrial applications [9]. Gentian Violet, Xylidine, Congo Red, Malachite Green, Remazol Brilliant Blue R, Indigo Carmine and Anthraquinone Blue are dyes could be decolorized by laccases produced by some white-rot fungi efficiently [10]. The present study investigates the potential effect of laccase enzyme produced by the fungus *Pleurotus ostreatus* to decolorize six synthetic dyes in presence and absence of hydroxybenzotriazole (HBT).

2. MATERIALS AND METHODS

2.1 Microorganism and Media

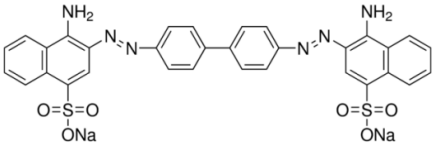
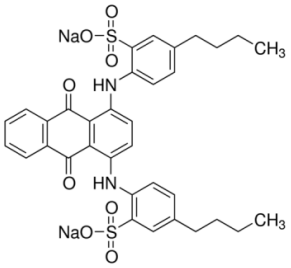
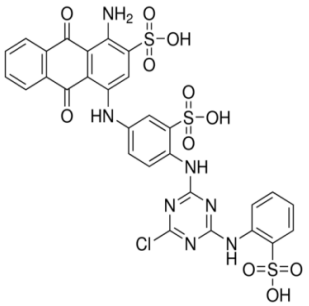
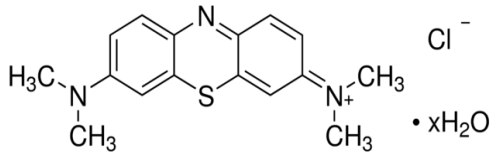
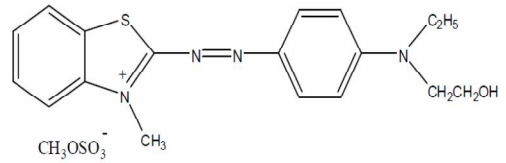
Pleurotus ostreatus ARC280 was maintained on Potato Dextrose Agar (PDA) medium and kept at 4ºC. Production of laccase was preceded by growing *Pl.ostreatus* on liquid media described by Tlecuitl-Beristain et al., 2008 [11]. Cell free filtrate produced after 26 days of incubation at 28ºC under static condition was used as crude enzyme preparation. Different solid media described by Munari et al., 2008 [12] were prepared to evaluate qualitatively the decolorization extent of some dyes by the fungus itself. The medium containing (g/L): dye, 0.1; glucose,10; agar, 30; 100 mL mineral solution

and 100 mL wheat bran washing water obtained by boiling 50 g of wheat bran in 1000 mL of distilled water. Mineral solution containing (g/L): $KH_2 PO_4$, 20; $(NH4)_2SO_4$, 14; $MgSO_4.7H_2O$, 3; urea, 3; $CaCl_2.2H_2O$, 4; $MnSO_4.7H_2O$, 0.0156; $FeSO_4.7H_2O$, 0.05; $ZnSO_4.7H_2O$, 0.014 and $CoCl_2$, 0.02. The dyes used are Congo Red, Acid Green-27, Cibacron D-Blue, Methylene Blue, Maxilon Blue and Acid dye Lanapel Red BM 143-PL. Each medium containing a specific dye was adjusted to an initial pH value of 5.0, sterilized by autoclaving at 1.5 atm and 121ºC for 20 min.

2.2 Chemicals

The enzyme substrate was supplied by Sigma-Aldrich, USA; Syringaldazine (4-hydroxy-3,5-dimethoxybenzaldehydeazine) (SGZ; Aldrich W404901). 1-Hydroxybenzotriazole hydrate (HBT) was supplied by Aldrich (HBT, 5482). Other chemicals used in this study were of analytical grade and higher purity. The dyes used were kindly obtained from Environmental Sciences Research and Textile Industries Research Divisions, National Research Centre, Egypt.

Table 1. Chemical structures and molecular formulae of the used dyes

Name	CAS No	Chemical structure	Molecular formula
Congo Red	**573-58-0**		**$C_{32}H_{22}N_6Na_2O_6S_2$**
Acid Green-27	**6408-57-7**		**$C_{34}H_{32}N_2Na_2O_8S_2$**
Cibacron D-Blue	**84166-13-2**		**$C_{29}H_{20}ClN_7O_{11}S_3$**
Methylene Blue	**122965-43-9**		**$C_{16}H_{18}ClN_3S \cdot xH_2O$**
Maxilon Blue			**$C_{20}H_{26}N_4O_6S_2$**

2.3 Qualitative Investigation of the Dyes Decolorization ability of *P. ostreatus* ARC280 on Dyes Decolorization

Congo Red, Acid Green-27, Cibacron D-Blue, Methylene Blue, Maxilon Blue (Table 1) and Acid dye Lanapel Red BM 143-PL dyes were subjected to decolorization test by using the filamentous fungus *Pl. ostreatus*. Each dye at a concentration of 100 mg/L was separately added to the solid medium described by Munari et al. 2008 [12]. The test was achieved by inoculation of Petri dishes (90 mm diameter) with plugs of 10 mm diameter of *P. ostreatus* ARC280 and follow up regularly the growth and decolorization process. Decolorization efficiency was considered by the disappearance of dyes' color around each plug throughout the incubation period.

2.4 Quantitative Determination of dyes Decolorization by *P. ostreatus* ARC280 Laccase

The decolorization of the previously mentioned dyes was investigated using the *P. ostreatus* ARC280 laccase. Stock solutions of the dyes were prepared in sterilized distilled water and diluted to the requested concentrations. During the investigation the reaction mixture with a total volume of 20 mL (dye solution, sodium citrate phosphate buffer 0.1 M (pH 6.0) and enzyme) was taken in 50 mL conical flasks and incubated in a horizontal shaker for a period of 6 h at 100 r/min and 30ºC. Decolorization of dyes was followed by measuring the absorbance at different optimum wavelengths of each dye at periodic intervals for 6 h. The absorption spectra of dyes between 400 and 800 nm were measured with spectrophotometer Cary 100 UV-Vis; Agilent Technologies (Germany). The efficiency of laccase obtained from *P. ostreatus* ARC280 on dyes decolorization was determined by the decrease in absorbance at the maximum wavelength of each dye. The decrease in absorbance at 505 nm (Congo Red), 605 nm (Acid Green 27), 620 nm (Cibacron D-Blue), 665 nm (Methylene blue), 610 nm (Maxilon Blue) and 510 nm (Acid dye Lanapel Red BM 143-PL) was then observed. The efficiency of decolorization was expressed in terms of decolorization percentage by Wang et al. 2011 [13] as follows:

$$\text{Decolorization (\%)} = 100 \times \text{Absorbance } t_0 - \text{Absorbance } t_f / \text{Absorbance } t_0$$

Where Absorbance t_0 is the absorbance at the optimum wavelength of the reaction mixture before incubation with the enzyme and Absorbance t_f is the absorbance at the optimum wavelength after incubation time [14]. Control reaction mixture by adding boiled enzyme solution was prepared in parallel under identical experimental conditions. A control reaction mixture without the crude enzyme was subjected to the same test.

2.5 Optimization of dyes Decolorization Process by Fungal Laccase

To obtain maximum degree of dyes decolorization by laccase of *P. ostreatus* ARC280, four parameters such as dyes concentration, enzyme concentration, reaction temperature and addition of HBT as a mediator were adjusted. Reaction mixtures were prepared as described before. During the incubation period, samples were withdrawn periodically and the remaining percentage of each dye was calculated. Optimization of the studied parameters was reached, as one of the parameters was optimized at a certain level it was then included at its optimized level in the subsequent experiments. The dyes decolorization efficiency of *P. ostreatus* ARC280 crude laccase was evaluated at its optimum pH value (6.0) and a model substrate of laccase (SGZ).

2.6 Evaluation of Bio-decolorization of Dyes by *P. ostreatus* ARC280 Laccase and Determination of their Absorption Spectra in Presence and Absence of HBT

The ability of the crude laccase of *P. ostreatus* ARC280 on dyes Decolorization was estimated by preparing the reaction mixtures each containing the tested dye (25 mg/L) at pH 6.0, 30ºC, and laccase (1.315 U), with and without HBT (1mM) as a mediator for 6 h on shaker at 100 r/min and follow up the change in the visible absorption spectra (400 – 800 nm).The reduction in the absorbance value of the tested dyes (25 mg/L) over the entire visible spectrum was recorded throughout the biodecolorization process of these dyes. The decrease in absorbance at the optimum wavelength of each dye as described previously was recorded.

2.7 Statistical Analysis

All the data were statistically evaluated according to the method described by Kenney and

Keeping, 1962 [15]. All data presented are the means of the results of different independent assays with a standard deviation of less than 5%. The Standard Deviation (SD) values have been displayed as Y-error bars in figures.

3. RESULTS AND DISCUSSION

3.1 Qualitative Assessment of dyes Biodecolorization by *P. ostreatus* ARC280

The aim of this experiment was to investigate the ability of *P. ostreatus* ARC280 to decolorize some synthetic dyes to share in finding out an eco-friendly method to reduce pollution. The results obtained revealed the ability of the fungus to decolorize Congo Red, Acid Green 27 and Acid dye Lanapel Red BM 143-PL completly after 20, 15 and 11 days of the fungal growth on solid medium. Methylene Blue (Basic) showed no decolorization even after 22 days that were sufficient to cover the plate with the fungus growth. Cibacron D-Blue and Maxilon Blue were not applied due to the interference of medium components with these two dyes. Following up the decolorization ability of the fungus and its growth appeared that the highest fungal mycelial growth rate was obtained with Acid dye Lanapel Red BM 143-PL where the mycelial growth covered the agar plate completely after 12 days of incubation with 100% decolorization zone after 11 days followed by Acid Green-27 where the mycelial growth that cover the plate completely after 17 days with 100% decolorization zone after 15 days. Congo Red showed maximum growth and decolorization at 25 and 20 days respectively. On the contrary with Methylene Blue (Basic) the fungus could grow perfectly without the ability of decolorization (Table 2 and Fig.1). This finding was in agreement with the results obtained by Zilly et al. 2002 [16] who reported that no decolorization of methylene blue by *Pleurotus pulmonarius.*

3.2 Effect of Dyes Concentration on the Decolorization Process

Dye concentration is an important factor that affect the decolorization process, as high dyes concentration may cause toxic effect that restrict dye decolorization [17-19]. The six dyes under study were subjected to laccase action (1.315 U) at different concentrations of 10, 25 and 50 mg/L and following the decolorization extent of each dye at different time intervals from 1 to 6 hrs. Results obtained showed that Acid-Green 27 was completely decolorized with all tested dye concentrations after 6 hrs of incubation. Lanapel Red BM 143-PL, Congo Red, Cibacron D- Blue, Maxilon Blue and Methylene Blue at a concentration of 10 mg/L were affected by laccase after 6 hrs and give decolorization percentage of 85.19, 79.45, 44.19, 19.76 and 7.98% respectively (Fig.2). While as, increasing the dyes concentration to 50mg/L limits the decolorization process to give lower values of 81.32, 67.57, 28.47, 13.84 and 0% for the previously mentioned dyes respectively (Fig. 2). This finding is in accordance with Young and Yu, 1997 [20] who reported that high dye concentration is a reason of slower decolorization rate. Chakraborty et al. 2013 [21] reported that *Alternaria alternate* CMERI F6 decolorized

Table 2. Decolorization of different dyes by *P. ostreatus* ARC 280 growing on agar plates

Growth and decolorization characteristics	Growth colony diameter (mm)[a]				Growth (days)[b]	Decolorization zone diameter (mm)[c]				Complete decolorization (days)[d]
	Days					Days				
	7	9	15	22		7	9	15	22	
Control	75	90	90	90	9	-	-	-	-	-
Congo Red	40	44	69	82	25	40	46	72	90	20
Acid Green 27	59	62	85	90	17	59	70	90	90	15
Methylene Blue (Basic)	56	62	83	90	22	0	0	0	0	0
Acid dye Lanapel Red BM 143-PL	70	80	90	-	12	70	87	90	90	11

Control: colony diameter of P. ostreatus ARC 280 on the medium without dyes.

[a] *The number represents the diameter of the mycelia colony in millimeters.*

[b] *The number represents the day on which the Petri dish (diameter 90 mm) was completely colonized by the mycelia of the fungus.*

[c] *Diameter of the decolorized zone in mm.*

[d] *The number represents the day of cultivation on which the Petri dish was completely decolorized.*

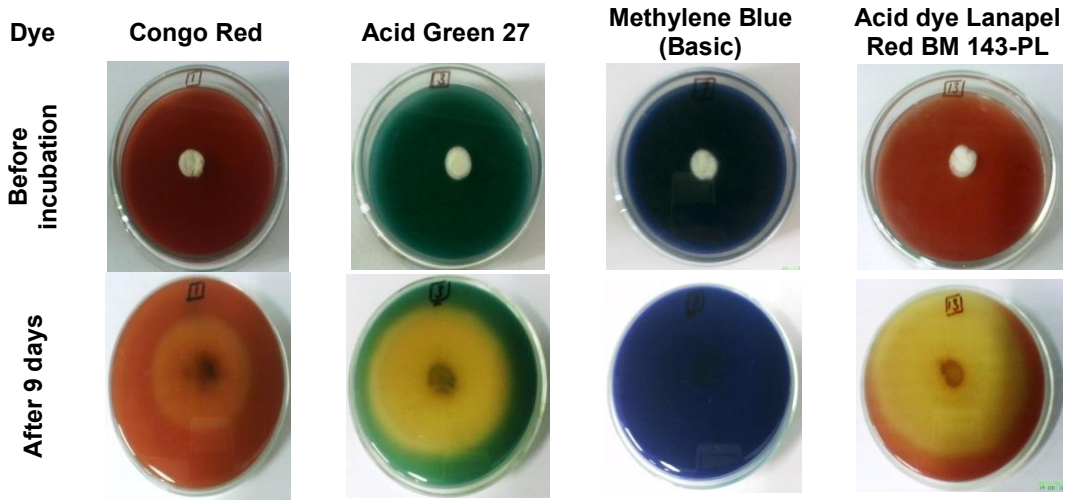


Fig. 1. Qualitative evaluation of dyes decolorization by *P. ostreatus* ARC280
N.B.: Cibacron D-Blue and Maxilon Blue were not applied due to the interference of medium components with these two dyes

99.99% and 78% of 600 and 800 mg/L Congo Red dye. The present results indicate that the concentration of 25 mg/L was intermediate in its response to degradation by laccase. In the following next experiments, the concentration of 25 mg/L will be used to study other factors that affect dyes decolorization by *P. ostreatus* ARC280 laccase. Roriz et al. 2009 [22] stated that the decolorization of Reactive Black 5 (RB5) by crude laccase from *Trametes pubescens* was decreased with increasing dye concentrations, even in the presence of high laccase concentrations.

3.3 Effect of Laccase Concentration on Dyes Decolorization

Determination of the amount of enzyme suitable for maximum decolorization of dyes is an essential role as a consequence of lowering the economics of the decolorization process. In this experiment, different laccase concentrations were used to determine how much of enzyme units from 0.657 to 2.629 U have the ability to obtain the maximum extent of decolorization of each dye under study. The results obtained revealed that the decolorization percentage using 0.657U of *P. ostreatus* ARC280 laccase after 6 h of incubation at 30ºC were 95.33, 100, 72.76, 25.78, 12.63 and 1.05% for Acid-Green 27, Acid dye Lanapel Red BM 143-PL, Congo Red, Cibacron D- Blue, Maxilon Blue and Methylene Blue (Basic) respectively (Fig. 3). However, increasing enzyme concentration to 1.315 U leading to decolorization percentage (after 6 h of incubation) of 100, 100, 78.07, 26.5, 15.16 and 1.15% for the previously mentioned dyes respectively, which is the same activity of dyes decolorization occurred at the concentration of 2.629 U in most cases and so the concentration of 1.315 U was used for the next set of experiments to reduce the cost of decolorization process. Hadibarata et al. 2012 [23] reported that, the decolorization rate of Remazol brilliant blue R (RBBR) increased with enzyme concentrations up to 0.75 U/L. The results obtained by Mogharabi et al., 2012 [24] demonstrated that, the minimum enzyme quantity to obtain maximum decolorization is 2.5 mg/mL. However, Soares et al. 2002 [25] reported that increasing the concentration of laccase up to 25 U/mL increased the rate of decolorization of Remazol Brilliant Blue R (RBBR) in the presence of 0.06% HBT. Maximum decolorization was obtained at laccase concentrations between 0.1 and 0.9 U/mL and 100 mg/L dye [26].

3.4 Effect of Temperature

Enzymes catalyzed reactions are mostly affected by incubation temperature degrees [27], therefore studying this parameter is serving in detecting the suitable conditions to achieve the decolorization process. Three degrees of temperature were tested namely 30, 40 and 50°C. The results showed that at 30°C, a considerable decolorization rate was detected. With increase in aqueous phase temperature above 30°C, a gradual decrease in the dyes decolorization rate was observed depending on

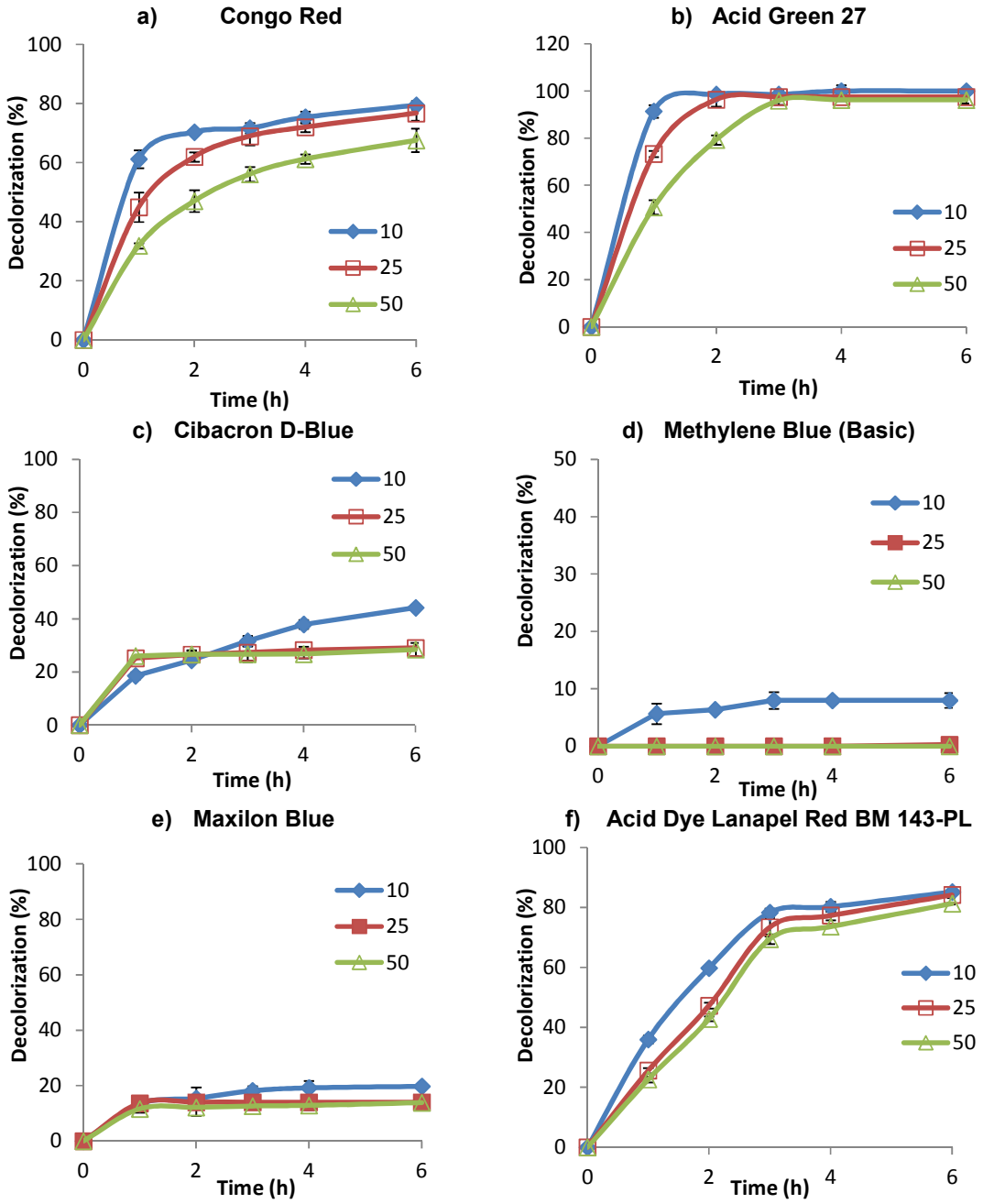


Fig. 2. Effect of dyes concentrations on the decolorization of different dyes by *P. ostreatus* ARC 280 laccase

Dye concentration: 10, 25 and 50 mg/l as indicated; dye solution: 20 ml; enzyme: 1.315 U; pH: 6.0; agitation: 100 rpm; contact time: 6 h and temperature: 30°C.

the individual dye. In case of Maxilon Blue and Methylene Blue, the decolorization process was completely inhibited at 50°C. While as a decrease in decolorization percentage was detected at 50°C with 27.35, 31.03, 80.44 and 10.05% in case of Congo Red, Acid-Green 27, Acid dye Lanapel Red BM 143-PL and Cibacron-Blue respectively (Fig.4). Chakraborty et al. 2013 [21] reported that *Alternaria alternata* CMERI F6 has the ability to decolorize Congo

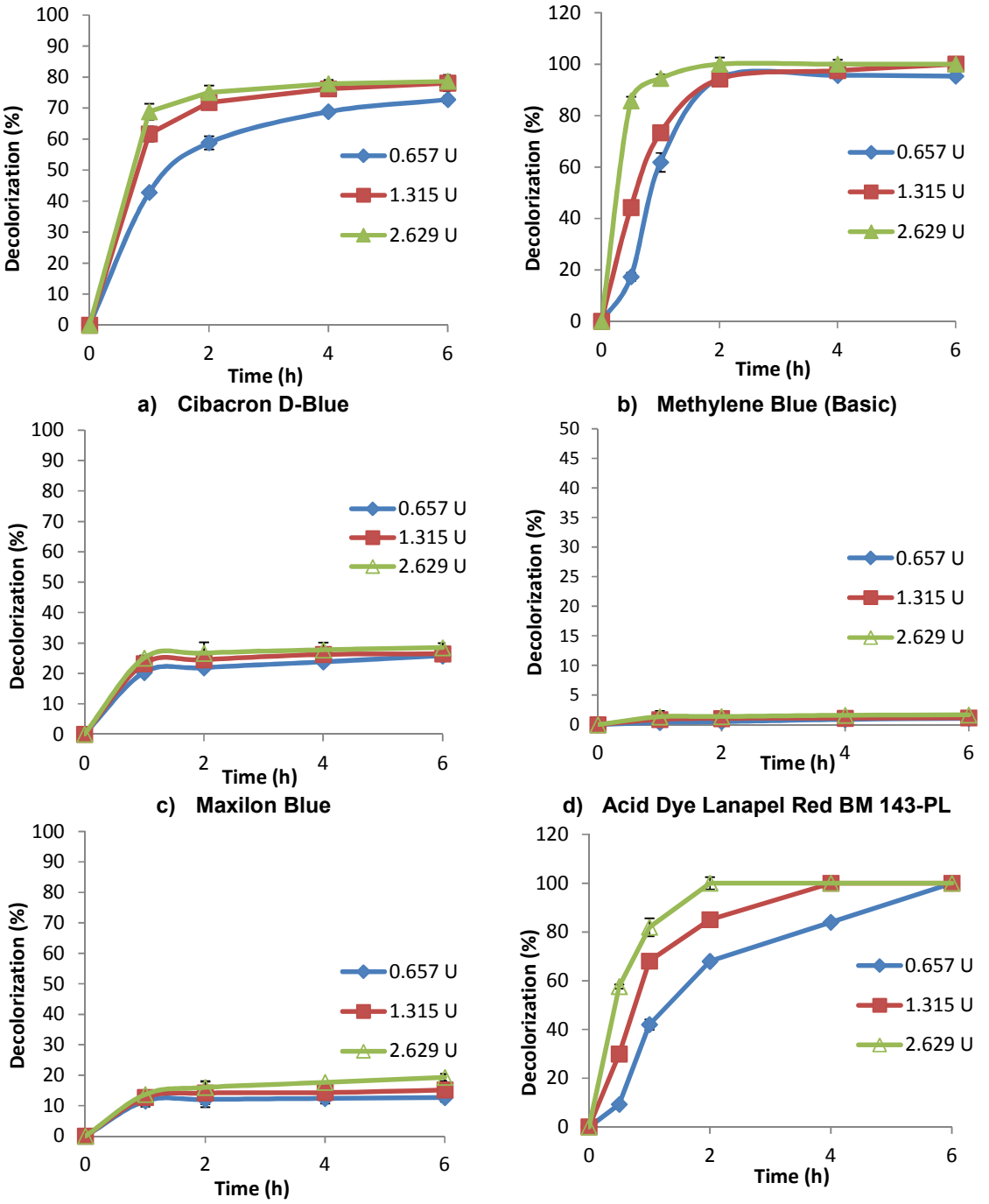


Fig. 3. Effect of enzyme concentration on the decolorization of synthetic dyes by *P. ostreatus* ARC 280 laccase

Dye concentration: 25 mg/l; dye solution: 20 ml; enzyme concentration: as indicated; pH: 6.0; agitation: 100 rpm; contact time: 6 h and temperature: 30°C

Red dye at 25°C as a mild treatment despite different harsh treatments such as acid-alkali treatment and exposure to high temperature degrees. On the other hand, the white-rot fungi, *Polyporus sp*. S133 was able to avoid the decrease of decolorization rate after temperature increased to 60ºC [23]. These results are in agreement with those of

Zouari-Mechichi et al. 2006 [28] who revealed the stability of laccase at high temperature. Another report by Kunamneni et al. 2008 [29] revealed the capability of immobilized laccase from the ascomycetes *Myceliophthora thermophila* to decolorize six dyes including acid green 27 dye. The immobilized laccase showed higher stability towards heat denaturation at 60 and 70°C. This application is very attractive on the decolorization of dyes in the textile industry.

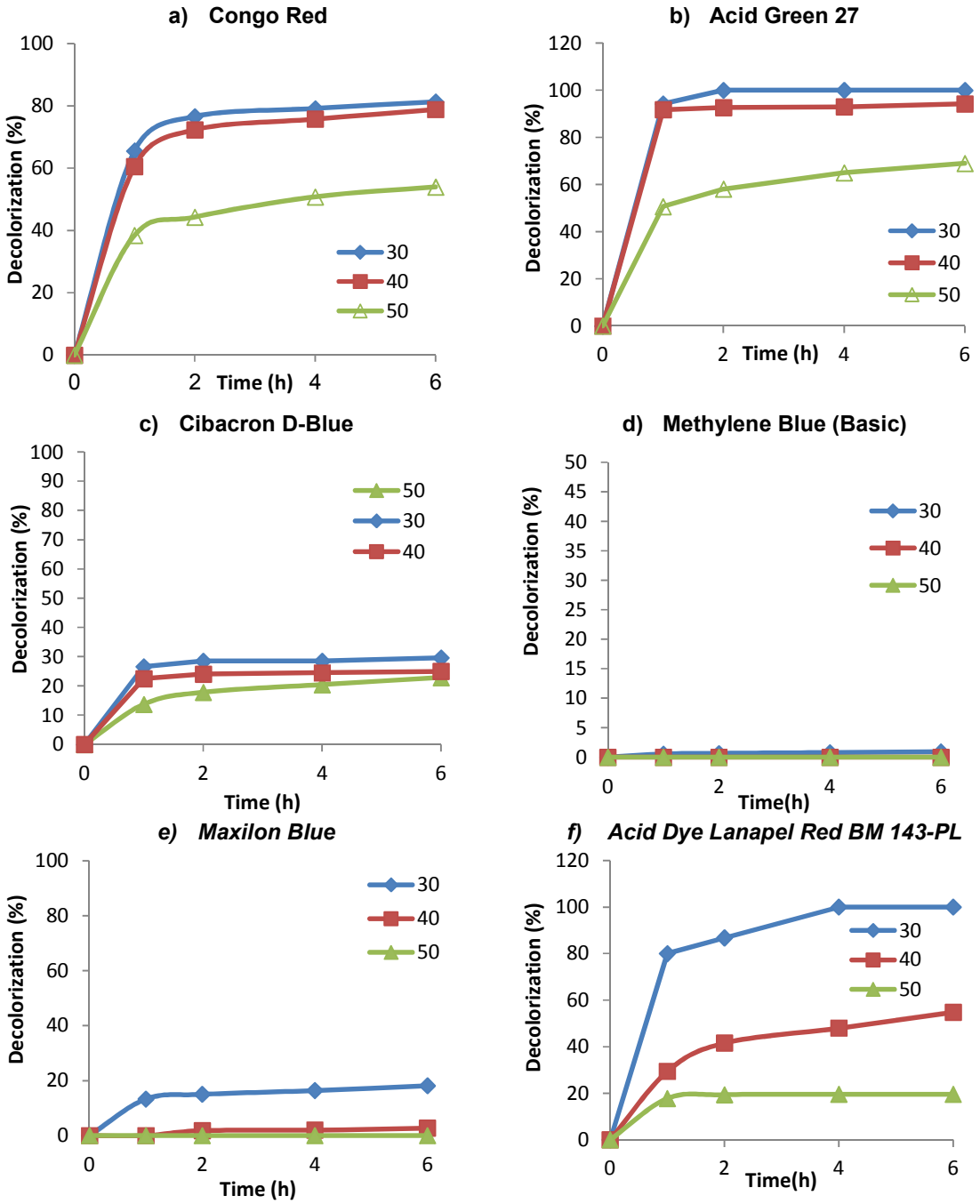


Fig. 4. Effect of incubation temperature on the decolorization of dyes by *P. ostreatus* ARC280 laccase

Dye concentration: 25 mg/l; dye solution: 20 ml; enzyme: 1.315U; pH: 6.0; agitation: 100 rpm; contact time: 6 h and temperature: as indicated

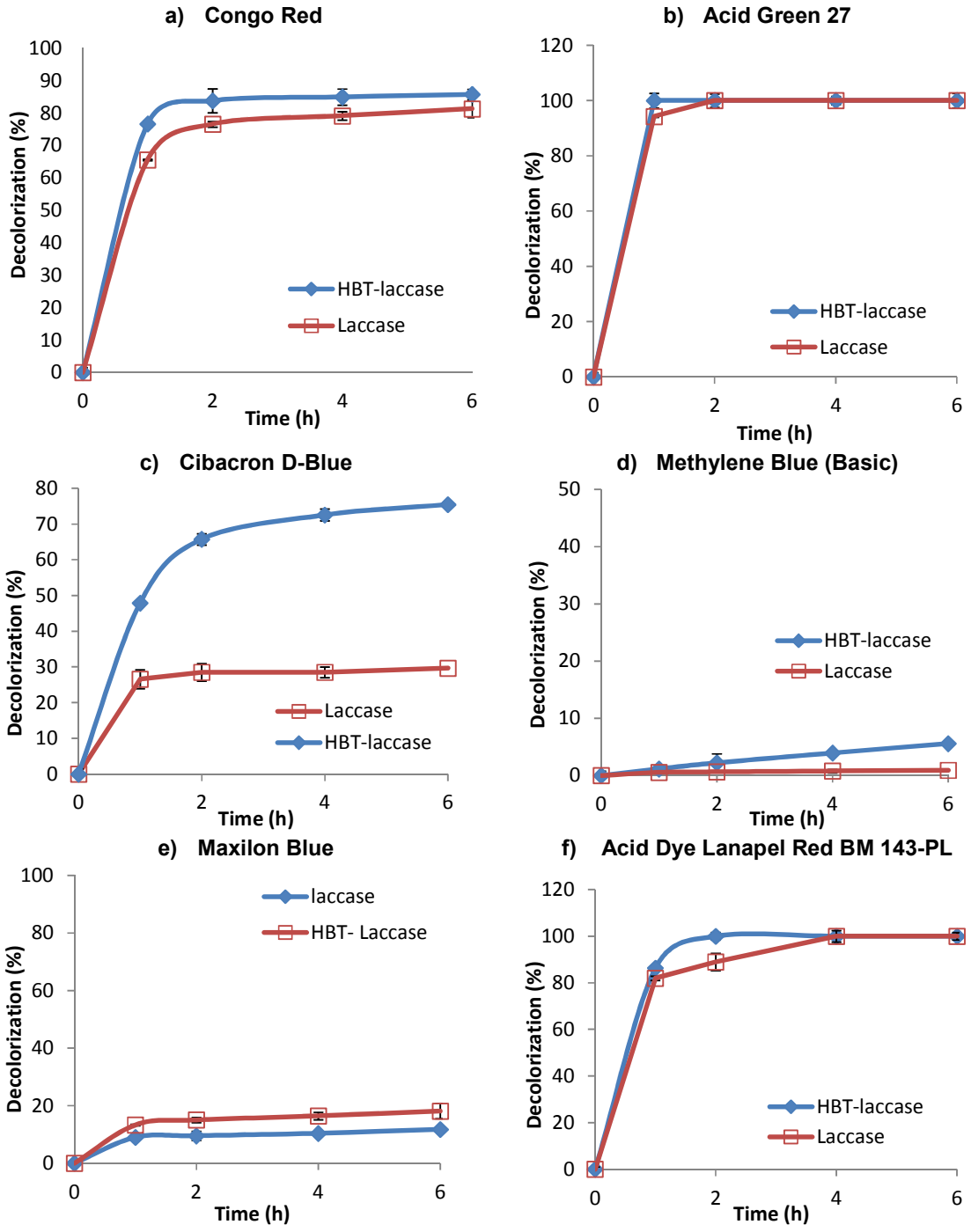


Fig. 5. Effect of HBT-laccase mediator system on the decolorization of different dyes
Dye concentration: 25 mg/l; dye solution: 20 ml; enzyme: 1.315 U; HBT: 1 mM; pH: 6.0; agitation: 100 rpm; contact time: 6 h and temperature: 30°C.

3.5 Effect of HBT-laccase Mediator System

Some dyes under study were not decolorized by laccase enzyme alone, therefore adding redox mediator is important to expand the catalytic activity of laccase towards many recalcitrant compounds [30,31]. HBT (1-hydroxybenzotriazole) is commonly used as laccase mediator and has been reported to be

capable in improvement the decolorization process of different dyes [32]. The decolorization rate depends on the structure and the redox-potential of the enzyme as well as the dye structure [25]. Results showed that Acid-Green 27 and Acid dye Lanapel Red BM 143-PL were completely decolorized after two and four hours respectively on subjecting to laccase enzyme alone but addition of HBT decrease the time required to reach the maximum decolorization percentage (100%) to the half (Fig.5). Addition of HBT to the reaction mixtures containing Congo Red, Methylene Blue and Maxilon Blue had minor effect on the decolorization process where an increase in decolorization percentage by 4, 5, 6% were obtained respectively in comparison with the addition of laccase only. On the other hand the decolorization of Cibacron D-Blue was greatly affected by HBT-laccase mediator system which increase the decolorization percentage by about 45% (Fig. 5).

In accordance with our results, the importance of presence of hydroxybenzotriazole (HBT; 5 mM) as the laccase mediator was established by Forootanfar et al. 2012 [33] who revealed that laccase from *Paraconiothyrium variabile* could catalyze the decolorization of bromophenol blue (100%), Commassie Brilliant blue (91%), panseu-S (56%), Rimazol brilliant blue R (RBBR; 47%), Congo Red (18.5%), and Methylene Blue (21.3%) after 3h of incubation in presence of hydroxybenzotriazole (HBT; 5 mM) as the laccase mediator. It was also observed that decolorization efficiency of all dyes was enhanced by increasing of HBT concentration from 0.1 mM to 5 mM. Studies of Moreira-Neto et al. 2013 [34] indicated that seven fungi related to the two genera *Pleurotus* and *Trametes* showed high capability for decolorization of the reactive dyes Cibacron Brilliant blue H-GR and Cibacron Red FN-2BL. Laccase was found to be the main lignolytic enzyme responsible for dye decolorization. Amoozegar et al. 2011 [35] studied the capability of different microorganisms to degrade azo dyes including Maxilon Blue. They found that halotolerent and halophilic microorganisms are the best candidates for bio-decolorization process as they are able to tolerate the stress factors and harsh conditions of dye polluted environment such as heavy metals. In this respect Asad et al. 2007 [36] reported that the percent of Maxilon Blue removal time by the three isolates *Halomonas sp D2, Halomonas sp A3* and *Halomonas sp Gb* were 37%, 46% and 55% after 96 hours respectively under anaerobic condition and a wide range of salinity.

3.6 Effect of Incubation Temperature in the Presence of HBT-laccase System

Studies for obtaining the optimum temperatures for decolorization of chemically different dyestuffs by white rot fungi revealed that it included in the range from 25 to 37ºC [37]. Results obtained indicated that, the optimal temperature for dyes decolorization was 30ºC (Fig.6) and by increasing the temperature above 30ºC, a gradual decrease in dyes decolorization rate was observed depending on the individual dye. This might be attributed to the decrease of enzyme activity at higher temperatures. We previously studied the properties of *P. ostreatus* ARC280 crude laccase [38] and we found that it was stable for 5 h at 30ºC, but it retained about 80.88 and 64.22% of its activity after 5 h of heat exposure at 40 and 50ºC respectively. As indicated from the preceding experiment addition of HBT had potent effect on the decolorization of most dyes but results obtained indicated that addition of HBT to the reaction mixtures containing different dyes had no real change on the behavior of dyes decolorization on subjecting to different temperature degrees ranging between 30-50°C in comparison to reaction mixtures received laccase only. The finding in this experiment indicated that the temperature degrees are the limiting factor. Results showed that a decrease in decolorization percentage occurred by increasing temperature degree from 30°C to 50°C with 22.1, 32.71, 57.99, 46.52% for Congo Red, Acid Green 27, Lanapel Red BM 143-PL and Cibacron D Blue respectively. On the other hand Maxilon Blue and Methylene Blue were not decolorized on subjecting to 50 ^{o}C instead of 30°C (Fig.6).

3.7 Effect of *P. ostreatus* ARC280 Laccase on Dyes' Absorption Spectra

To obtain a relevant decolorization degree of dyes in waste water, no absorbance of dyes in the visible spectrum must be noticed after decolorization process [39]. The aim of the present experiment was to detect the reduction in the absorbance of each dye at its maximum wave length which reflects the performance of the decolorization process. Results in Fig. 7 recorded reduction in absorbance at the maximum wavelength of most dyes and no more peaks of absorbance were appeared in the

visible spectra range (400-800 nm). Results obtained indicated that, high absorbance reduction at the maximum wavelength of Congo Red (505 nm), Acid Green 27 (605 nm) and Acid dye Lanapel Red BM 143-PL (510 nm) was occurred. On the other hand a slight color change for Methylene Blue (665 nm) and Maxilon Blue (610 nm) was observed. In case of

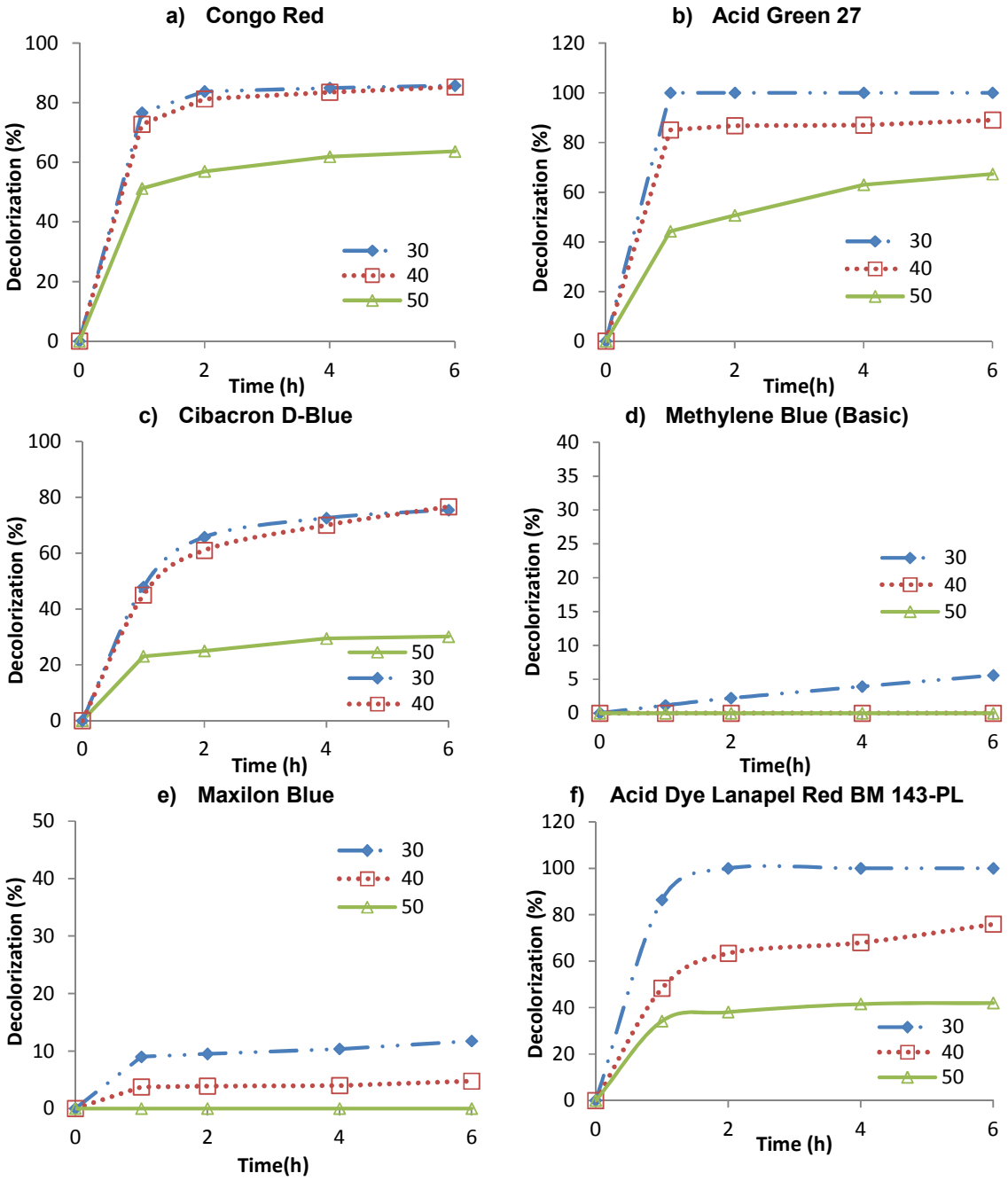


Fig. 6. Effect of incubation temperature on the decolorization of different dyes using HBT-laccase system

Dye concentration: 25 mg/l; dye solution: 20 ml; enzyme: 1.315 U; HBT: 1 mM; pH: 6.0; agitation: 100 rpm; contact time: 6 h and temperature: as indicated

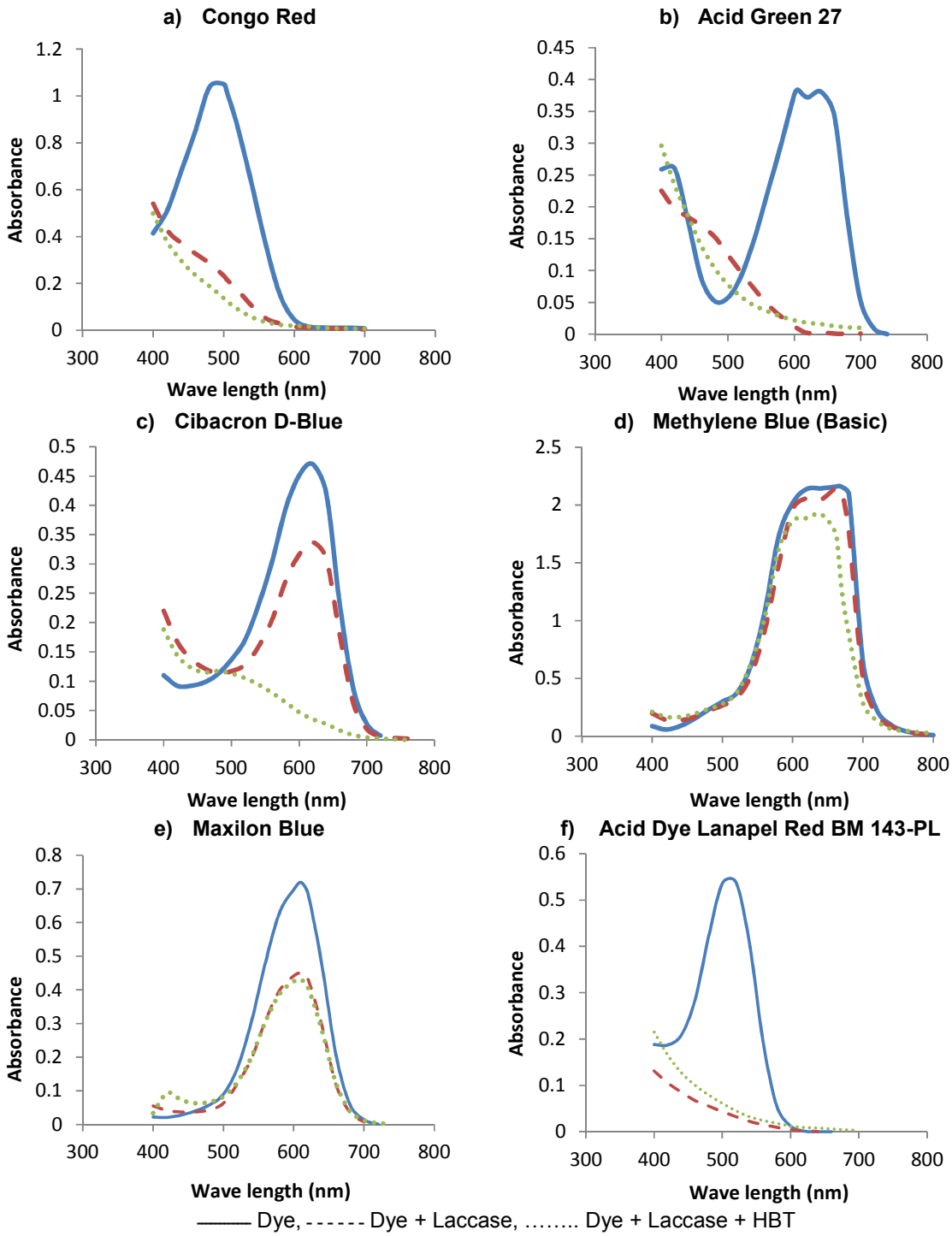


Fig. 7. Changes in the visible absorption spectra of different dyes by the action of the crude laccase produced by *P. ostreatus* ARC280

Dye concentration: 25 mg/l; dye solution: 20 ml; enzyme: 1.315U; HBT: 1 mM; pH: 6.0; agitation: 100 rpm; contact time: 6 hrs and temperature: 30°C.
Dye: Initial spectra of dyes at a concentration of 25 mg/l;
Dye + laccase: Spectra of dyes after decolorization by a laccase concentration of 1.315U;
Dye + laccase + HBT: Spectra of dyes after decolorization by HBT- laccase mediator system

Cibacron D-Blue (620 nm), it was obviously that HBT-laccase mediator system caused higher decolorization percentage (absorbance reduction) than in case of using laccase alone. These observations are in accordance with the results obtained previously with experiments for optimization of parameters for different dyes decolorization using laccase of *P. ostreatus* ARC280 and HBT- laccase mediator system [38].

4. CONCLUSION

The results obtained indicated the effectiveness of *P. ostreatus* ARC280 laccase and its mediated system in the decolorization of six structurally different dyes. Addition of HBT decreased the time required to reach the maximum decolorization percentage of Acid-Green 27 and Acid dye Lanapel Red BM 143-PL to the half. The decolorization percentage of Cibacron D-Blue was greatly affected by HBT-laccase mediator system by about 45% increase. The laccase enzymatic biodecolorization system appears to be a good strategy for dye effluent treatment from different industries due to its high efficiency as an ecofriendly approach.

ACKNOWLEDGEMENT

The Authors would like to express their gratitude to the National Research Centre, Dokki, Giza, EGYPT, for the financial support of this work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Asgher M, Azim N, Bhatti, HN. Decolorization of practical textile industry effluents by white rot fungus Coriolus versicolor IBL-04. Biochem Eng J. 2009;47:61–65.
2. Fahmi MR, Abidin CZA, Rahmat NR. Multi stage ozonation and biological treatment for removal of azo dyes industrial effluent. International Journal of Environmental Science and Development. 2010;1(2):193-198.
3. Hamidzadeh S, Torabbeigi M, Shahtaheri SJ. Removal of crystal violet from water by magnetically modified activated carbon and nanomagnetic iron oxide. J. Environ. Health Sci. Eng. 2015;13(8): 1-7.
4. Devi VM, Inbathamizh L, Ponnu TM, Premalatha S, Divya M. Dye decolorization using fungal laccase. Bull. Environ. Pharmacol. & Life Sci. 2012;1(3):67–71.
5. Elshafei AM, Othman AM, Hassan MM, Haroun BM, Elsayed MA, Farrag AA. Catalyzed Mediator-Based decolorization of Five Synthetic Dyes by *Pleurotus ostreatus* ARC280 Laccase. British Biotechnology Journal. 2015;9(2):1-15.
6. Verma P, Madamwar D. Decolorization of azo dyes using Basidiomycete strain PV 002. World J Microbiol Biotechnol. 2005;21:481–485.
7. Couto SR, Sanroman MA, Gubitz GM. Influence of redox mediators and metal ions on synthetic acid dye decolorization by crude laccase from *Trametes hirsute*. Chemosphere. 2005;58:417–422.
8. Atallah MM, Kheiralla HZ, Hamed ER, Youssry AA, Abd El, Aty AA. Characterization and kinetic properties of the purified *Trematos phaeriamangrovei* laccase enzyme. Saudi J. Biol. Sci. 2013;20:373–381.
9. Rodrı´guez Couto S, Toca Herrera JL. Lacasses in the textile industry. Biotechnol Mol Biol Rev. 2006;1:115–20.
10. Laura L, Melignani E, Ramos AM. Effect of nitrogen sources and vitamins on ligninolytic enzyme production by some white-rot fungi. Dye decolorization by selected culture filtrates. Bioresource Technology. 2010;101:4554–4563.
11. Tlecuitl-Beristain S, Sánchez C, Loera O, Robson-Geoffrey D, Díaz-Godínez G. Laccases of *Pleurotus ostreatus* observed at different phases of its growth in submerged fermentation: Production of a novel laccase isoform. Mycol. Res. 2008;112:1080-1084.
12. Munari FM, Gaio TA, Calloni R, Dillon AJP. Decolorization of textile dyes by enzymatic extract and submerged cultures of Pleurotus sajor-caju. World J. Microbiol. Biotechnol. 2008;24(8):1383-1392.
13. Wang CL, Zhao M, Lu L, Wei XD, Li TL. Characterization of spore laccase from *Bacillus subtilis* WD23 and its use in dye decolorization. Afr. J. Biotechnol. 2011;10(11):2186-2192.
14. Khlifi R, Belbahri L, Woodward S, Ellouz M, Dhouib A, Sayadi S, Mechichi T. Decolorization and detoxification of textile industry wastewater by the laccase

mediator system. J. Hazard. Mater. 2010;175:802–808.
15. Kenney JF, Keeping ES. The standard deviation and calculation of the standard deviation in mathematics of statistics. Pt1, 3rd ed. Princeton, NJ, Van Nostrand. 1962;77-80.
16. Zilly A, Souza CG, Barbosa-Tessmann IP, Peralta RM. Decolorization of industrial dyes by a Brazilian strain of *Pleurotus pulmonarius* producing laccase as the sole phenol-oxidizing enzyme. Folia Microbiol (Praha). 2002;47(3):273-277.
17. Radha KV, Regupathi I, Arunagiri A, Murugesan T. Decolorization studies of synthetic dyes using *Phanerochaete chrysosporium* and their kinetics. Process Biochem. 2005;40:3337-3345.
18. Gomashe A, Suriya SS, Dharmik PG. Decolorization of textile dyes by *Aspergillus ochraceus* (NCIM-1146). Ecoscan. 2011;5:169-171.
19. Aracagök YD, Cihangir N. Decolorization of reactive black 5 by *Yarrowia lipolytica* NBRC 1658. Am. J. Microbiol. Res. 2013;1:16-20.
20. Young L, Yu J. Ligninase-catalized decolorization of synthetic dyes. Water Resour. 1997;31:1187–1193.
21. Chakraborty S, Basak B, Dutta S, Bhunia B, Dey A. Decolorization and biodegradation of congo red dye by a novel white rot fungus *Alternaria alternata* CMERI F6. Bioresource Technology. 2013;147:662–666.
22. Roriz MS, Osma JF, Teixeira Rodríguez JA, Rodríguez Couto S. Application of response surface methodological approach to optimise Reactive Black 5 decolouration by crude laccase from *Trametes pubescens*. J. Hazard. Mater. 2009;169(1-3):691–6.
23. Hadibarata T, Yusoff ARM, Kristanti RA. Decolorization and metabolism of anthraquionone-type dye by laccase of white-rot fungi *Polyporus sp*. S133. Water Air Soil Pollut. 2012;223:933–941.
24. Mogharabi M, Nassiri-Koopaei N, Bozorgi-Koushalshahi M, Nafissi-Varcheh N, Bagherzadeh G, Faramarzi MA. Immobilization of laccase in alginate-gelatin mixed gel and decolorization of synthetic dyes. Bioinorg. Chem. Appl; 2012.
DOI: 10.1155/2012/823830
25. Soares GMB, Amorim MTP, Hrdina R, Costa-Ferreira M. Studies on the biotransformation of novel disazo dyes by laccase. Process Biochem. 2002;37:581-587.
26. Benzina O, Frikha F, Zouari-Mechichi H, Woodward S, Belbahri L, Mnif E, Mechichi T. Enhanced decolourization of the azo dye Sirius rose BB by laccase–HBT system. Biotech. 2012;2:149–157.
27. Lehninger AL, Nelson DL, Cox M. Principles of biochemistry, 4th Edition. Palgrave Macmillan: Basingstoke, UK; 2004.
28. Zouari-Mechichi H, Mechichi T, Dhouib A, Sayadi S, Martinez AT, Martinez MA. Laccase purification and characterization from *Trametes trogii* isolated in Tunisia: Decoloration of textile dyes by the purified enzyme. Enzyme Microb. Technol. 2006;39:141-148.
29. Kunamneni A, Ghazi I, Camarero S, Ballesteros A, Plou FJ, Alcalde M. Decolorization of synthetic dyes by laccase immobilized on epoxy-activated carriers. Process Biochemistry. 2008;43(2):169–178.
30. Dwivedi UN, Singh P, Pandey VP, Kumar A. Structure–function relationship among bacterial, fungal and plant laccases: A review. J. Mol. Catal. B: Enzym. 2011;68:117–128.
31. Mate DM, Alcalde M. Laccase engineering: From rational design to directed evolution. Biotechnol. Adv. 2015;33:25–40.
32. Murugesan K, Dhamija A, Nam IH, Kim YM, Chang YS. Decolorization of reactive black 5 by laccase: Optimization by response surface methodology. Dyes Pigm. 2007;75:176-184.
33. Forootanfar H, Moezzi A, Aghaie-Khozani M, Mahmoudjanlou Y, Ameri A, Niknejad F, Faramarzi MA. Synthetic dye decolorization by three sources of fungal laccase. J. Environ. Health Sci Eng. 2012;9:27-36.
34. Moreira-Neto SL, Mussatto SI, Machado KMG, Milagres AMF. Decolorization of salt-alkaline effluent with industrial reactive dyes by laccase-producing basidiomycetes strains. Letters in Applied Microbiology. 2013;56:283-290.
35. Amoozegar MA, Hajighasemi M, Hamedi J, Asad S, Ventosa A. Azo dye decolorization by halophilic and halotolerant microorganisms. Annals of Microbiology. 2011;61(2):217–230.

36. Asad S, Amoozegar MA, Pourbabaee AA, Sarbolouki MN, Dastgheib SMM. Decolorization of textile azo dyes by newly isolated halophilic and halotolerant bacteria. Bioresour Technol. 2007;98: 2082–2088.
37. Asgher M, Yasmeen Q, Iqbal HMN. Enhanced decolorization of solar brilliant red 80 textile dye by an indigenous white rot fungus *Schizophyllum commune* IBL- 06. Saudi J. Biol. Sci. 2013;20:347–352.
38. Hassan MM, Elshafei AM, Haroun BM, Elsayed MA, Othman AM. Biochemical characterization of an extracellular laccase from *Pleurotus ostreatus* ARC280. J. Appl. Sci. Res. 2012;8(8):4525-4536.
39. Tavares APM, Coelho MAZ, Agapito MSM, Coutinho JAP, Xavier AMRB. Optimization and modeling of laccase production by *Trametes versicolor* in a bioreactor using statistical experimental design. Appl. Biochem. Biotechnol. 2006;134:233-248.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/20411