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Catalyzed Mediator-Based Decolorization of Five Synthetic Dyes by *Pleurotus ostreatus* ARC280 Laccase

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Authors' contributions

All authors participated in the design of the study and drafted the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The aim of the present study was to evaluate qualitatively the decolorization of five dyes by *Pleurotus ostreatus* (*P. ostreatus*) ARC280 using solid medium. The laccase produced by the fungus was used in terms of its concentration and thermal stability for enzymatic decolorization and also in combination with Hydroxybenzotriazole (HBT) as a redox mediator.
Study Design: Qualitative evaluation of decolorization of dyes and determining the best conditions required for decolorization 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 January 2013 and February 2014.
Methodology: *P. ostreatus* ARC280 fungal ability for dyes decolorization was qualitatively evaluated on solid 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

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of distilled water. The efficiency of decolorization was expressed in terms of decolorization percentage as follows:

$$\text{Decolorization (\%)} = 100 \times \frac{\text{Absorbance}_{t0} - \text{Absorbance}_{tf}}{\text{Absorbance}_{t0}}$$

Where Absorbance $_{t0}$ is the absorbance at the optimum wavelength of the reaction mixture before incubation with the enzyme and Absorbance $_{tf}$ is the absorbance at the optimum wavelength after incubation time.

Results: The enzyme was efficient in decolorizing Acid Blue C.I. 220 (100%), Dichlorophenol indophenol sodium salt D 5110 (92.6%) and Brilliant Green C.I. 42040 (78.6%) after 6 h of incubation at 30ºC. In the presence of HBT (1 mM), Lanasol Red 6G was greatly affected by HBT as a laccase mediator system with decolorization percentage of 53.85% instead of 10.90 in case of laccase alone, however the enzyme could not efficiently decolorize Foron Yellow Brown S 2RFLI dye even in presence of HBT. The decolorization efficiency of all dyes was decreased by increasing reaction temperature from 30 to 50ºC. The absorbance reduction at the maximum wavelength was recorded with all the tested dyes.

Conclusion: The results obtained clearly confirmed the role of *P. ostreatus* ARC280 laccase and its mediated system in the decolorization of structurally different dyes.

Keywords: Pleurotus ostreatus laccase; synthetic dyes; decolorization; hydroxybenzotriazole.

1. INTRODUCTION

Synthetic dyes are widely used in different industrial sectors like textile, food, leather, printing, pharmaceutical, plastic and biomedical industries. The insufficiency of dyeing and printing processes resulted to about 10–20% of the unused dyes are being released into the water bodies [1]. Since some dyes are of potential health hazards because they are toxic or mutagenic to many living organisms [2,3], environmental regulatory agencies made severe restriction regulations for the discharge of colored effluents from textile and dyestuff manufacturers [4,5].

Biological decolorization process can be carried out either by microorganisms or enzymes isolated from them. It is an eco-friendly alternative to physical and chemical degradation and can be performed at feasible rates. Several microorganisms, such as fungi, yeast and bacteria have been investigated for their ability to decolorize many classes of dyes [6,7]. Biochemical studies disclosed that lignin peroxidase, Mn peroxidase and laccase from fungi are engaged in the decolorization of dyes [8].

Laccases (EC 1.10.3.2) only need molecular oxygen as a co-substrate and may be more suitable biocatalysts for several bioremediation applications [9]. Laccases catalyze the processes of oxidation of both phenolic and non-phenolic compounds in presence of some redox mediators and thus can act on a broad range of dyes [10]. This unlimited mechanism of laccase makes it versatile biocatalyst suitable for many applications such as bioleaching, biopulping and wastewater treatment [11,12]. White rot fungi are able to produce laccase isoenzymes, which are participated in the degradations of different resistant compounds like dyes [13], industrial wastewater [14] and endocrine-disrupting chemicals [15].

Laccase–mediator system usage is one of the promising possibilities for decolorization of the textile dyes eco-friendly [16]. The oxidized mediator could base on an oxidation mechanism not obtainable to the enzyme, thereby broad the range of substrates accessible to it [17]. Hydroxybenzotriazole (HBT) is widely studied as laccase redox mediator and has proven to be a very effective mediator in the presence of laccase [18].

In our previous works, we optimized the production, characterized, and purified laccase from *P. ostreatus* ARC280 liquid cultures and assessed its antitumor and antimicrobial activity [7,10,12]. In the current study, the initial qualitative evaluation of dyes decolorization by *P. ostreatus* ARC280 using solid medium was conducted and its laccase was used in terms of its concentration and thermal activity for enzymatic decolorization and also in combination with HBT as a redox mediator.

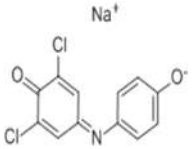
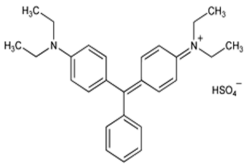
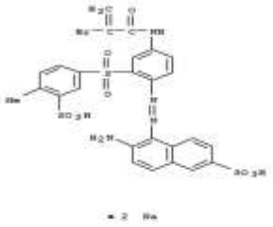
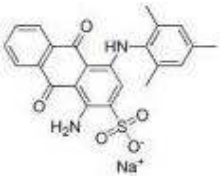
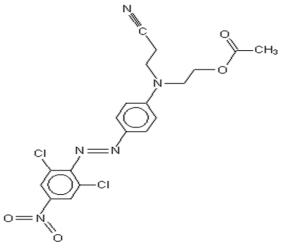
2. MATERIALS AND METHODS

2.1 Microorganism and Media

P. ostreatus ARC280 was maintained on Potato Dextrose Agar (PDA) medium and kept at 4ºC. The medium described by Tlecuitl-Beristain et al. [19] was used for laccase production under static culture conditions at 28ºC for 26 days by *P. ostreatus* ARC280.

P. ostreatus ARC280 fungal ability for dyes decolorization was qualitatively evaluated on solid 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_2PO_4, 20; $(NH_4)_2SO_4$, 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 [20]. The dyes used are Dichlorophenol indophenol sodium salt D 5110, Brilliant Green C.I. 42040, Lanasol Red 6G, Acid Blue C.I. 220 and Foron Yellow Brown S 2RFLI (Table 1). 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.

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

Name	Class	CAS No	Chemical structure	Molecular formula	Maximum absorbance (nm)
Dichlorophenol indophenols sodium salt D 5110	Redox	620-45-1		$C_{12}H_6Cl_2NNaO_2$	610
Brilliant Green C.I. 42040	Basic	633-03-4		$C_{27}H_{34}N_2O_4S$	620
Lanasol Red 6G	Reactive	85187-33-3		$C_{26}H_{19}BrN_4Na_2O_9S_3$	515
Acid Blue C.I. 220	Acid	72968-73-1		$C_{25}H_{23}N_2NaO_5S$	640
Foron Yellow Brown S 2RFLI	Disperse	5261-31-4		$C_{19}H_{17}Cl_2N_5O_4$	530

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.

2.3 Qualitative Evaluation of Dyes Decolorization Using *P. ostreatus* ARC280 Filamentous Fungus

This experiment was made to evaluate the potential of *P. ostreatus* ARC280 to decolorize Dichlorophenol indophenol sodium salt D 5110, Brilliant Green C.I. 42040, Lanasol Red 6G, Acid Blue C.I. 220 and Foron Yellow Brown S 2RFLI dyes at a concentration of 100 mg/L in a solid medium described by Munari et al [20]. The medium poured in Petri dishes (90 mm diameter) and left to solidify. Mycelia plugs from *P. ostreatus* ARC280 were transferred to the centre of Petri dishes and incubated at 28ºC. Plates were regularly monitored everyday for growth and decolorization activities [20]. Decolorization efficiency was assessed by the disappearance of dyes' color during fungal growth.

2.4 Dyes Decolorization by *P. ostreatus* ARC280 Laccase

The decolorization of dyes namely Dichlorophenol indophenol sodium salt D 5110, Brilliant Green C.I. 42040, Lanasol Red 6G, Acid Blue C.I. 220 and Foron Yellow Brown S 2RFLI was investigated by 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, 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 wavelength 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 (made in 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 610 nm (Dichlorophenol indophenol sodium salt D 5110), 620 nm (Brilliant Green C.I. 42040), 515 nm (Lanasol Red 6G), 640 nm (Acid Blue C.I. 220), and 530 nm (Foron Yellow Brown S 2RFLI) was then observed. The efficiency of decolorization was expressed in terms of decolorization percentage [21] as follows:

$$\text{Decolorization (\%)} = 100 \times \frac{\text{Absorbance}_{t0} - \text{Absorbance}_{tf}}{\text{Absorbance}_{t0}}$$

Where Absorbance_{t0} is the absorbance at the optimum wavelength of the reaction mixture before incubation with the enzyme and Absorbance_{tf} is the absorbance at the optimum wavelength after incubation time [22]. Control samples using denatured enzyme solution were done in parallel under identical conditions. Another control was made for the same reaction mixture without the crude enzyme.

2.5 Optimization of Dyes Decolorization Process by Fungal Laccase

In order to determine dyes decolorization efficiency by enzymatic treatment using *P. ostreatus* ARC280 laccase, batch experiments were conducted by varying process parameters such as dyes concentration, enzyme concentration, reaction temperature and addition of HBT as a mediator. During the incubation period, samples were withdrawn periodically and subjected to residual dye estimation. The strategy adopted for optimization of various process parameters influencing laccase mediated dye decolorization included consecutive evaluation of parameters. Initially one parameter was evaluated and it was then incorporated at its optimized level in the subsequent experiments. The capacity of *P. ostreatus* ARC280 crude laccase in decolorization of dyes was tested at pH 6.0, which is the optimum pH for SGZ oxidation (as a model substrate) by laccase.

2.6 Effect of HBT and Temperature on the Decolorization of Dyes

Influence of HBT-laccase mediator (HBT, 1 mM) over the laccase mediated dyes decolorization

with laccase activity of 1.315 U was used to screen the efficiency of HBT-laccase mediator for dyes decolorization (at pH 6.0, 25 mg/L dye concentration and 30ºC). The effect of temperature was examined by incubating different dyes (25 mg/L) in 100 mM citrate phosphate buffer pH 6.0, 1 mM HBT and in the presence of the laccase of *P. ostreatus* ARC280 (1.315 U) at 30, 40 and 50ºC for 6 h.

2.7 Evaluation of Absorption Spectra Reduction of Dyes by *P. ostreatus* ARC280 Laccase with and without HBT

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

2.8 Statistical Analysis

All the data were statistically evaluated according to the method described by Kenney and Keeping [23]. All data presented are the means of the results of different independent assays with a standard deviation of less than 5%. The SD values have been displayed as Y-error bars in figures.

3. RESULTS AND DISCUSSION

3.1 Qualitative Evaluation of Dyes Decolorization by *P. ostreatus* ARC280

Laccase producing microorganisms especially white rot fungi were extensively applied for dyes decolorization experiments [24]. Decolorization process can be carried out either by enzymes or microorganisms. Given the potential usage of brilliant green dye to color silk and wool in the commercial textile industry, the development and optimization of decolorization of this hazardous recalcitrant dye is essential to reduce pollution. Kumar et al. [25] reported that *Aspergillus sp.* strain CB-TKL-1 isolated from a water sample was found to completely decolorize brilliant green within 72 h when cultured under aerobic conditions at 25ºC. In the present study, the results showed a positive correlation between the fungal growth rate and the decolorization ability of the fungus measured as the diameter of the decolorized zone on the Petri dish. The presence of synthetic dyes in the solid medium reduced *P. ostreatus* ARC280 mycelial growth rate to different extent according to the chemical structure of each dye (Table 2). Results obtained indicated that the mycelial growth covered the agar plate completely on the 9th day of incubation without dyes (control). The highest mycelial growth was obtained in case of Acid Blue C.I. 220 after 12 days of incubation with 100% decolorization zone after 11 days, whereas in case of Dichlorophenol indophenol sodium salt D 5110 the mycelia fungal growth covered the agar plate completely after 20 days of incubation with 100% decolorization zone after 17 days. On the other hand, the lowest mycelial growth was obtained in case of Brilliant Green C.I. 42040, where the mycelial growth covered the agar plate completely after 53 days of incubation with 100% decolorization zone after 55 days (Table 2 and Fig. 1). Lanasol Red 6G and Foron Yellow Brown S 2RFLI were not applied due to the interference of medium components with these two dyes.

3.2 Effect of Dyes Concentration

In this connection Young and Yu, [26] reported that high dye concentration will cause slower decolorization rate. Each dye molecule contains a chromophore such as azo and anthraquinone, and its color disappears only after the chromophore structure is destroyed. Destroying the chromophore structure of one dye molecule may require numerous attacks of many enzymes. High dye concentration implies less average attacks of enzyme to each dye molecule, and hence slower color removal rate was noticed [26]. Chen and Yien-Ting [27] reported that the implications of high initial dye concentrations conforms to most observations [28-30], suggesting that dye toxicity may be a factor that limits dye decolorization.

The five tested dyes with final concentrations of 10, 25 and 50 mg/L were used to determine the decolorization capacity of the *P. ostreatus* ARC280 crude laccase (1.315 U). Results obtained indicated that, the crude laccase from *P. ostreatus* ARC280 was found to be extremely efficient in decolorizing Acid Blue C.I. 220 completely with all tested dye concentrations even at the earlier time (1 h) of exposure to enzymatic treatment (Fig. 2d).

Table 2. Qualitative evaluation of dyes decolorization by *P. ostreatus* ARC280

Dye (100 mg/L)	Growth colony diameter (mm)[a]				Growth (days)[b]	Decolorization zone diameter (mm)[c]				Complete decolorization (days)[d]
	Incubation time (days)					Incubation time (days)				
	7	9	15	22		7	9	15	22	
Control	75	90	90	90	9	-	-	-	-	-
Dichlorophenol indophenol sodium salt D 5110	42	49	75	90	20	42	52	79	90	17
Brilliant Green C.I. 42040	24	28	35	51	53	22	27	38	54	55
Lanasol Red	75	90	90	90	9	ND	ND	ND	ND	ND
Acid Blue C.I. 220	70	78	90	90	12	69	83	90	90	11
Foron Yellow Brown S 2RFLI	73	80	90	90	11	ND	ND	ND	ND	ND

ND, not detected; Control, colony diameter of P. ostreatus ARC 280 on the medium without dyes; [a]The number represents the diameter of the mycelia colony in mm; [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. The values are presented as the mean±SD of triplicate plates

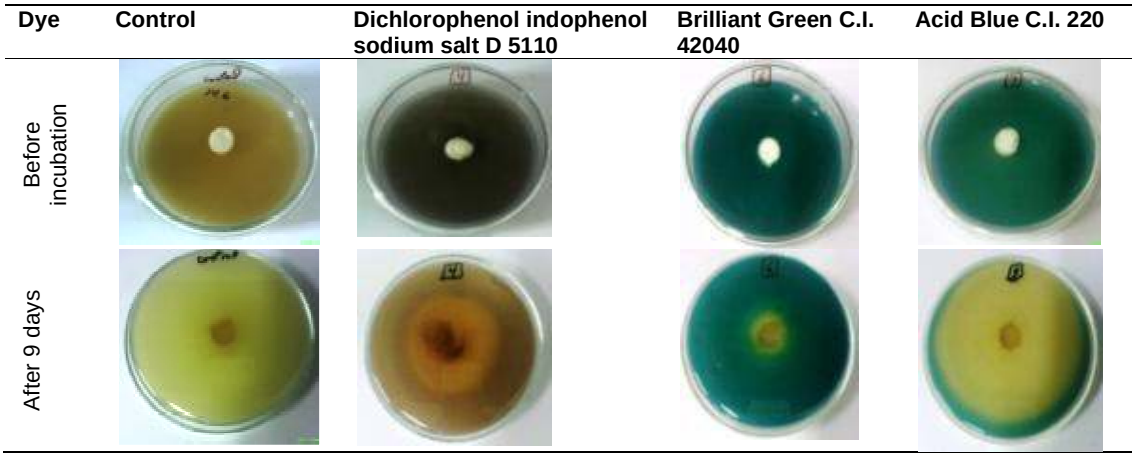


Fig. 1. Qualitative evaluation of dyes decolorization by *P. ostreatus* ARC280
Lanasol Red 6G and Foron Yellow Brown S 2RFLI were not applied due to the interference of medium components with these two dyes

The extent of decolorization achieved with other dyes after 6 h of incubation were varied according to different chemical structure of each dye to give decolorization percentage of 95.70, 92.74, 11.97 and 6.00% for Dichlorophenol indophenol sodium salt D 5110, Brilliant Green C.I. 42040, Lanasol Red 6G and Foron Yellow Brown S 2RFLI respectively, at a concentration of 10 mg/L, whereas the decolorization percentage decreased with the increase of dyes concentration to give decolorization percentage of 88.92, 76.24, 9.95 and 3.45% for the previously mentioned dyes respectively, at a concentration of 50 mg/L (Figs. 2a, b, c, e). The 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. [31] 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

The effect of enzyme concentration on dyes decolorization was studied by increasing laccase activity from 0.657 to 2.629 U. The results obtained indicated that, the decolorization percentage using 0.657U of P. ostreatus

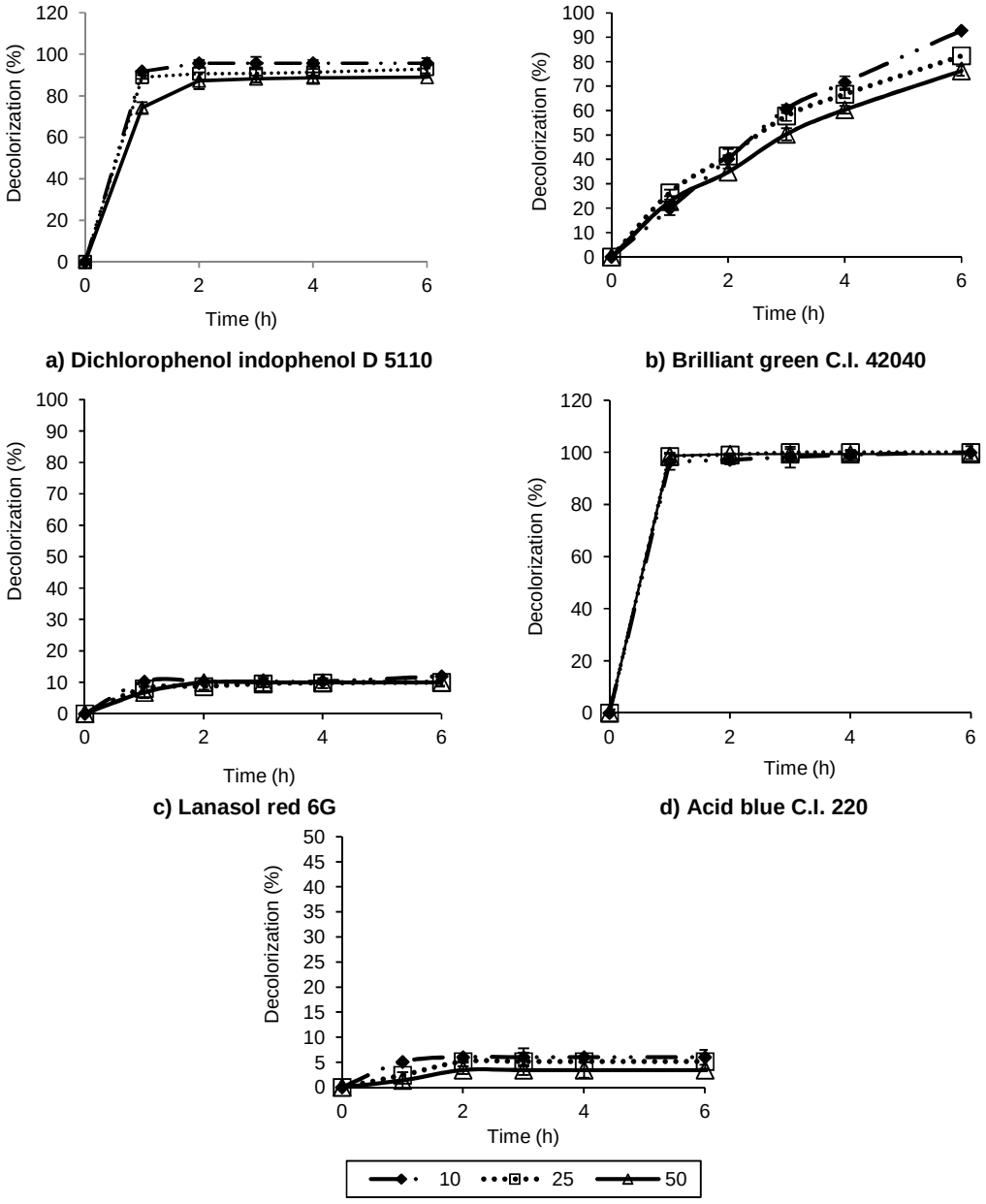


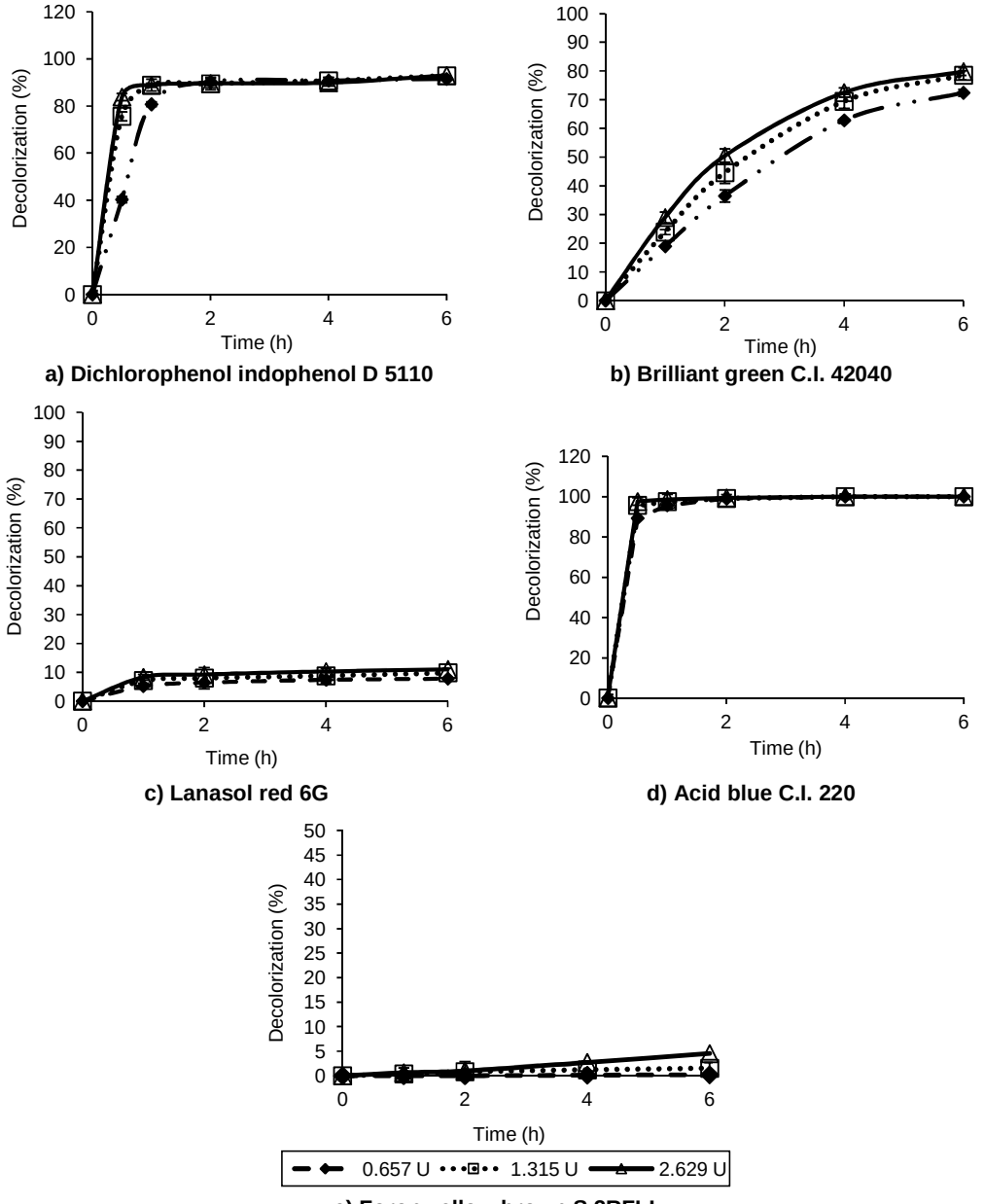
Fig. 2. Effect of dyes concentrations on the decolorization of different dyes by *P. ostreatus* ARC 280 laccase

Dye concentration: 10, 25 and 50 mg/L as indicated; dye solution: 20 mL; enzyme: 1.315 U; pH: 6.0; agitation: 100 r/m; contact time: 6 h and temperature: 30℃. T he values are presented as the mean±SD of duplicate reactions

ARC280 laccase after 6 h of incubation at 30ºC were 91.25, 72.46, 7.82, 100.00 and 0.20% for Dichlorophenol indophenol sodium salt D 5110, Brilliant Green C.I. 42040, Lanasol Red 6G, Acid Blue C.I. 220 and Foron Yellow Brown S 2RFLI, respectively (Fig. 3). However, at 1.315 U enzyme concentration (after 6 h of incubation) the decolorization rates occurs with decolorization percentage of 92.58, 78.60, 9.81, 100.00 and 1.51% for the previously mentioned dyes respectively, which is the same with the behavior of dyes decolorization observed at the

concentration of 2.629 U in most cases and so the concentration of 1.315U was fixed for the next set of experiments to reduce the cost of process.

Hadibarata et al. [32] 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. [33] demonstrated that, the minimum enzyme quantity to obtain maximum decolorization is 2.5 mg/mL. However, Soares et al., [34] reported that increasing the concentration of laccase up to 25 U/mL increased the rate of decolorization of 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 [35].



a) Dichlorophenol indophenol D 5110

b) Brilliant green C.I. 42040

c) Lanasol red 6G

d) Acid blue C.I. 220

e) Foron yellow brown S 2RFLI

Fig. 3. Effect of laccase concentration on dyes decolorization

Dye concentration: 25 mg/L; dye solution: 20 mL; enzyme concentration: as indicated; pH: 6.0; agitation: 100 r/m; contact time: 6 h and temperature: 30℃. The values are presented as the mean±SD of triplicate reactions

3.4 Effect of Temperature

The rates of enzyme catalyzed reactions were reasonable within the temperature range under which the enzyme is stable and retained its activity [36]. In the present study, a considerable decolorization rate was shown at 30ºC (Table 3). With the increase in aqueous phase temperature above 30ºC, a gradual decrease in dyes decolorization rate was observed depending on the individual dye. This might be attributed to the loss of the enzyme activity at higher temperatures. Brilliant green dye C.I.42040 showed higher decolorization rate at 40ºC than 30ºC after the first two hours. In case of Acid blue C.I. 220, the treatment was not affected by changing temperatures from 30 to 50ºC whereas Dichlorophenol indophenol D 5110 was slightly affected with decolorization percentage of 88.32% instead of 90.72% when incubation occurred at 50℃ instead of 30ºC (Table 3).

On the other hand, no decolorization percentage was observed in case of Lanasol Red 6G when incubation temperature occurred at 50ºC in comparison with incubation at 30ºC for 6 h which led to about 10.90% decolorization percentage. The white-rot fungi, *Polyporus sp*. S133 was able to avoid the decrease of decolorization rate after temperature increased to 60ºC [37]. These results are in agreement with those of Zouari-Mechichi et al. [38] who revealed the stability of laccase at high temperature.

3.5 Effect of HBT-laccase Mediator System

In the presence of redox mediators decolorization process of dyes could significantly be efficient [39]. In this study, a concentration of laccase activity of 1.315 U was used to screen the efficiency of HBT-laccase mediator for dyes decolorization. The presence of some redox mediators could expand the catalytic activity of laccase towards many recalcitrant compounds [40,41]. HBT is a frequently used laccase mediator and has been proved to be efficient in promoting the decolorization of different dyes [42]. The decolorization rate depends on the structure and the redox-potential of the enzyme as well as the dye structure [34]. Preliminary results revealed that *P. ostreatus* laccase failed to decolorize some dyes, indicating that the presence of a mediator is required. Similarly, reports from literature show that laccase alone does not decolorize some types of textile dyes [43]. The reason might be that the redox potential of the dye is higher than that of type 1 Cu of the laccase or the dye could not access the type 1 Cu active site because of its steric hindrance. However, such dyes can be oxidized by laccase in the presence of some redox mediators [42,43]. Whereas Dichlorophenol indophenol D 5110 , Brilliant Green C.I. 42040 and Foron Yellow Brown S 2RFLI were slightly affected by HBT-laccase mediator system with a difference in

Table 3. Effect of incubation temperature on decolorization process

Dye	Temperature (ºC)	Decolorization (%)			
		Incubation time (h)			
		1	2	4	6
Dichlorophenol indophenol D 5110	30	89.96±0.21	90.42±2.52	90.57±1.31	90.72±3.22
	40	88.34±1.05	89.64±1.60	90.00±0.53	90.07±1.54
	50	83.2±4.36	85.08±0.01	87.94±2.22	88.32±0.63
Brilliant Green C.I. 42040	30	29.92±0.00	64.61±0.09	89.47±1.32	90.26±0.03
	40	62.52±3.07	85.13±3.84	87.00±0.00	91.87±4.56
	50	62.66±0.91	71.53±1.31	75.28±0.47	75.40±0.71
Lanasol Red 6G	30	7.97±0.43	9.40±0.04	10.90±0.03	10 .90±2.07
	40	2.00±0.53	3.00±0.07	4.00±0.49	5.10±0.04
	50	0.00	0.00	0.00	0.00
Acid Blue C.I. 220	30	96.35±2.78	99.11±0.93	100±0.55	100±4.91
	40	96.58±4.30	100±0.05	100±1.45	100±3.22
	50	98.24±1.00	100±0.63	100±0.68	100±0.99
Foron Yellow Brown S2RFLI	30	3.25±0.54	3.25±0.03	3.25±0.07	3.25±0.08
	40	0.00	0.00	0.00	0.00
	50	0.00	0.00	0.00	0.00

Dye concentration: 25 mg/L; dye solution: 20 mL; enzyme: 1.315 U; pH: 6.0; agitation: 100 r/m; contact time: 6 h and temperature: as indicated. The values are presented as the mean±SD of triplicate reactions

decolorization percentage than those of laccase Lanasol Red 6G dye was greatly affected by HBT-laccase mediator system with an increase in decolorization percentage by about 43% than the corresponding sample with laccase alone (Fig. 4c).

Several authors have pointed out the efficiency of HBT as a laccase mediator. The probable reason is that the dyes containing –NH2 and –OH groups are most vulnerable by laccase attack; however, the steric hindrance may reduce the accessibility of these groups to laccases [43].

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

For mostly white rot fungi the optimum temperatures for decolorization of chemically different dyestuffs have been reported in the range from 25 to 37ºC [44]. Results obtained

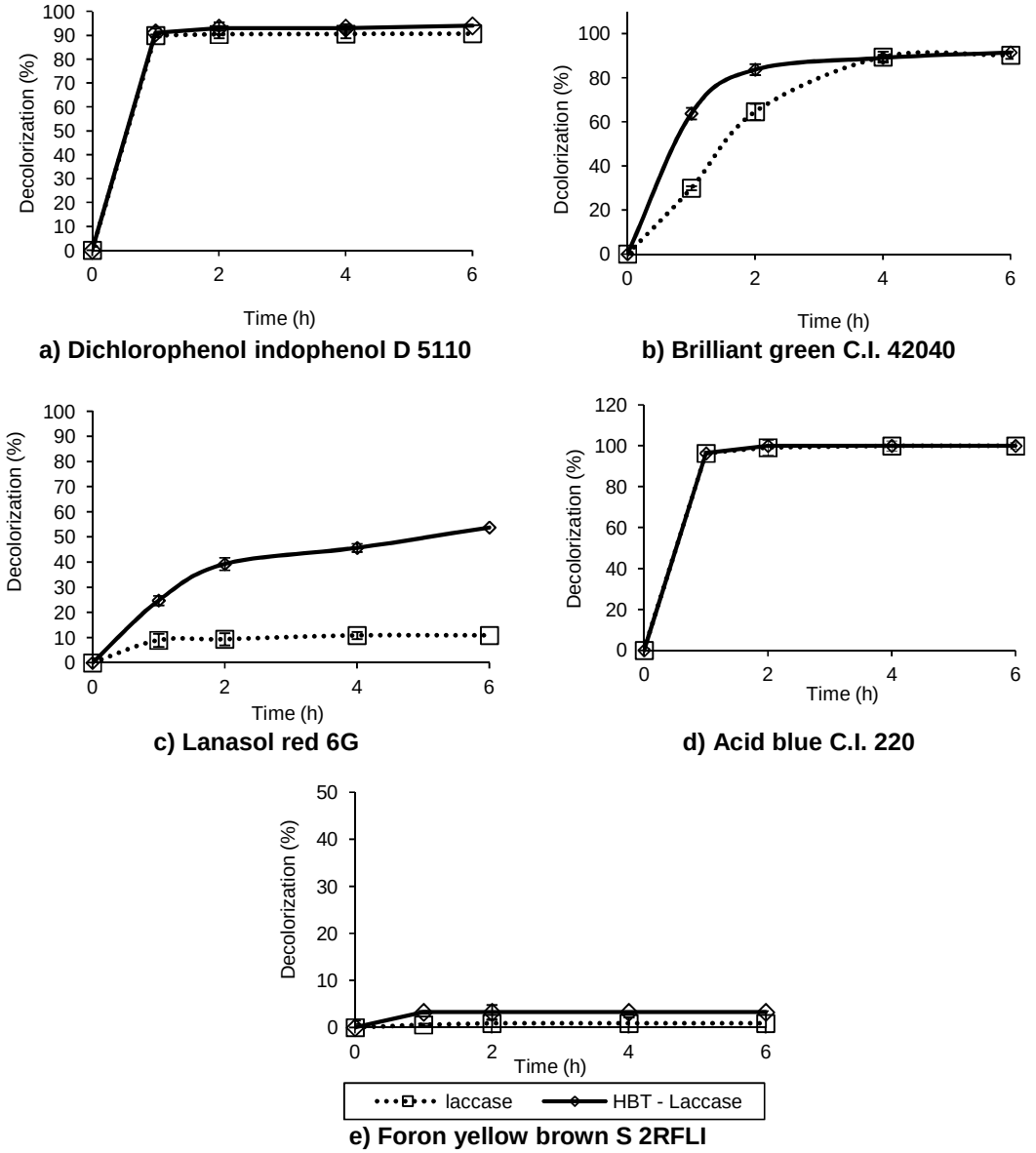
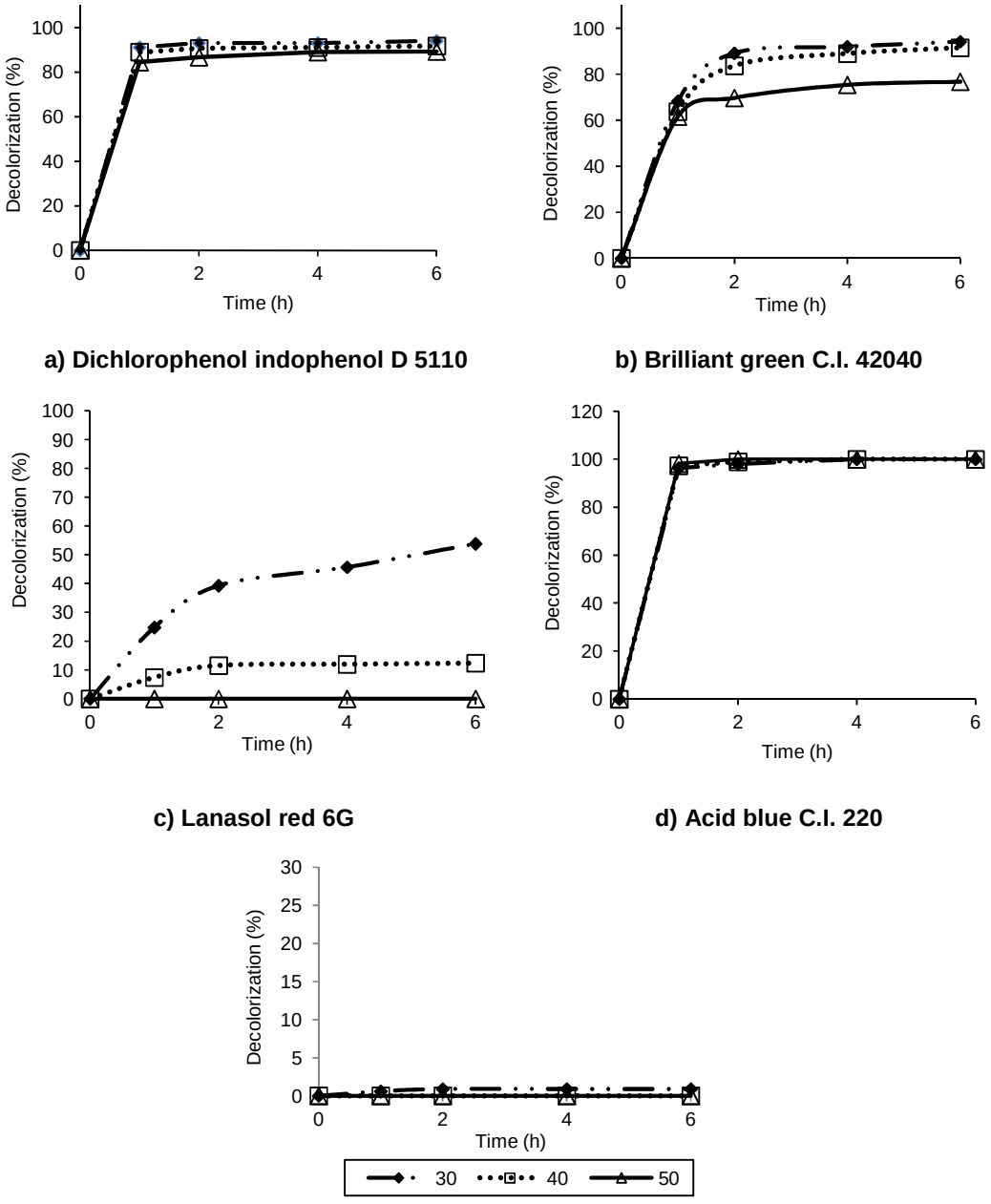


Fig. 4. Effect of HBT-laccase mediator system

Dye concentration: 25 mg/L; dye solution: 20 mL; enzyme: 1.315 U; HBT: 1 mM; pH: 6.0; agitation: 100 r/m; contact time: 6 h and temperature: 30℃. The values are presented as the mean±SD of triplicate reactions

indicated that, the optimal temperature for dyes decolorization was 30ºC (Fig. 5) 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 [10] 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.



a) Dichlorophenol indophenol D 5110

b) Brilliant green C.I. 42040

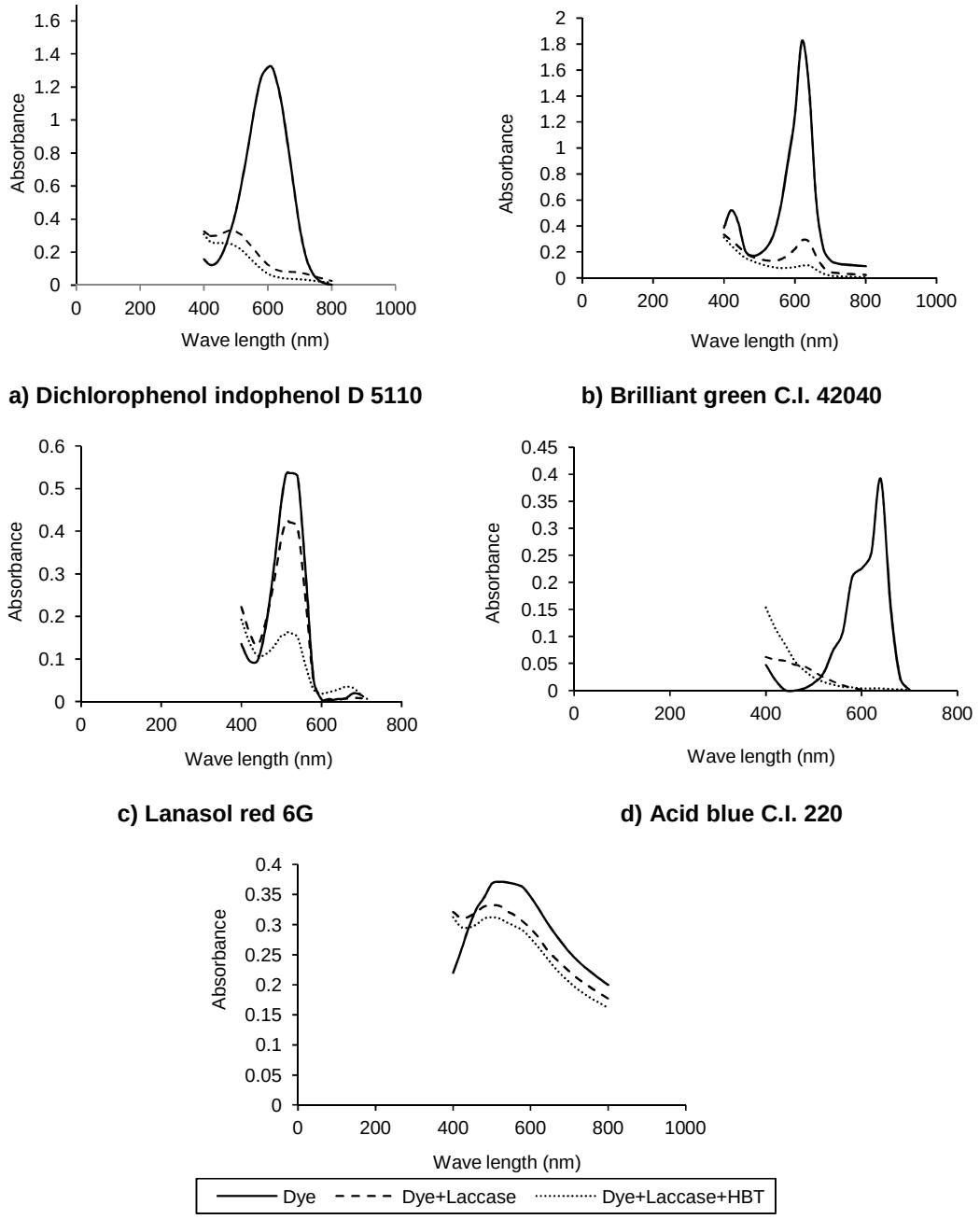
c) Lanasol red 6G

d) Acid blue C.I. 220

e) Foron yellow brown S 2RFLI

Fig. 5. Effect of incubation temperature in the presence of HBT- laccase system

Dye concentration: 25 mg/L; dye solution: 20 mL; enzyme: 1.315 U; HBT: 1 mM; pH: 6.0; agitation: 100 r/m; contact time: 6 h and temperature: as indicated. The values are presented as the mean±SD of triplicate reactions



a) Dichlorophenol indophenol D 5110

b) Brilliant green C.I. 42040

c) Lanasol red 6G

d) Acid blue C.I. 220

e) Foron yellow brown S 2RFLI

Fig. 6. Effect of *P. ostreatus* ARC280 laccase on dyes' absorption spectra

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.
Dye concentration: 25 mg/L; dye solution: 20 mL; enzyme: 1.315U; HBT: 1 mM; pH: 6.0; agitation: 100 r/m; contact time: 6 h and temperature: 30℃.
Data are expressed as the average of three independent assays

Results obtained also indicated that the response of dyes decolorization in the presence of HBT-laccase mediator system is resemble to that obtained with laccase only without mediator with the exception of decolorization rate. In case of Acid blue C.I. 220, the treatment was not affected by changing temperatures from 30 to 50ºC whereas Dichlorophenol indophenol D 5110 was slightly affected with decolorization percentage of 89.27% instead of 94.04% when incubation occurred at 50 and 30ºC, respectively (Figs. 5d, a).

On the other hand, no decolorization percentage was observed in case of Lanasol Red 6G when incubation temperature occurred at 50ºC instead of 53.85% when incubated with HBT-laccase mediator system at 50ºC instead of 30ºC for 6 h (Fig. 5c).

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

According to Tavares et al. [45], for better performance in dye wastewater treatment, no absorbance in the visible spectrum must be detected after decolorization. Full wavelength scans of the reaction products from the spore laccase catalyzed dye decolorization were performed by Zhang et al. [46] From the results obtained in the present study, a higher decolorization based on the absorbance reduction at the maximum wavelength of each dye could be recorded with most of tested dyes and no additional peaks of absorbance were emerged in the entire visible spectrum.

Results cited in Fig. 6, indicated that the higher decolorization based on the absorbance reduction at the maximum wavelength of Dichlorophenol indophenol sodium salt D 5110 (610 nm), Brilliant Green C.I. 42040 (620 nm) and Acid Blue C.I. 221(640 nm), could be recorded and no additional peaks of absorbance were emerged in the entire visible spectrum, whereas in case of Foron Yellow Brown S 2RFLI (530 nm), a slight reduction in absorbance was obtained. The decolorization of Lanasol Red 6G (515 nm) was greatly affected by HBT-laccase mediator system with a decolorization percentage (absorbance reduction) higher than that obtained in case of using *P. ostreatus* ARC280 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. Further studies are needed to identify the decomposed substances.

4. CONCLUSION

The results obtained clearly suggest a role for *P. ostreatus* ARC280 laccase in the decolorization and biotransformation of structurally different dyes. Decolorization capacity was greatly depending on the dye chemical structures. The laccase enzymatic-mediated system appears to be a good strategy for immobilization techniques and use for dye effluent treatment from different industries due to its highly thermal and pH stabilities as reported in our previous studies with this enzyme.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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