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Decolorization of Synthetic Dyes by *Ficus carica* Latex Peroxidase Isoenzymes

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Authors' contributions

This work was carried out in collaboration between all authors. Author AME performed the practical work and first draft of the manuscript. Author UMH supervised the practical work and managed the analyses of the study. Authors MGAH, SSAG, WHS and AHS managed the literature searches and supervised the work. Author ASF supervised and managed the analyses of the study and revised the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The current study aims to elucidate the potential of *Ficus carica* latex peroxidase isoenzymes for decolorizing different synthetic dyes in comparison to the commercial horseradish peroxidase.
Study Design: The decolorization of 20 dyes was investigated using the purified *F. carica* latex peroxidase isoenzymes (purified FP1 and partially purified FP2, and FP3), and horseradish peroxidase (HRP) as a control.
Place and Duration of Study: Molecular Biology Department, Genetic Engineering and Biotechnology Research Division, National Research Centre, Egypt, between January 2017 and March 2018.

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Methodology: The purified and partially fractions of peroxidase isolated from latex of *F. carica* were used for the present study. Stock solutions of the dyes were prepared in 0.05 M sodium acetate buffer (pH 5.5) and diluted to the requested concentrations ranged from 12 to 330 μM in order to get maximum absorbance does not exceed 1.5 as initial reading. The efficiency of decolorization was expressed in terms of percentage. All experiments were performed in triplicate.
Results: *F. carica* latex peroxidase isoenzymes and commercial horseradish peroxidase were able to decolorize some of tested dyes and the extent of decolorization achieved with different dyes classes were varied according to different chemical structure of each dye. The decolorization efficiency after 3 h of incubation at 40°C using 6.4 U/ml of peroxidase activity of FP1, FP2, FP3 and HRP, was found to be extremely efficient in decolorizing some dyes and relatively low in other dyes.
Conclusion: The efficiency of *F. carica* latex peroxidase isoenzymes toward different synthetic dyes meet the prerequisites needed for environmental and industrial applications.

Keywords: Ficus carica; latex; peroxidase; dyes decolorization.

1. INTRODUCTION

Synthetic dyes are considered the main pollutants class in various industries wastewater like paper, textile, plastics, food, and cosmetics. Nowadays, more than 10,000 dissimilar dye structures have been produced and over 8×10^5 tons of dyestuffs are formed per year [1–3]. Dyes correspond to a very huge and complicated collection of organic compounds, which diverge in their source, physical and/or chemical properties and the application process related properties. In addition, due to the resistance of the mutagenic or carcinogenic dyes to basic and acid conditions, light degradation, bleaching agents, and others, they cause the more complicated ecological troubles [4–6].

Dyes are largely classified in relation to their purpose for groups as disperse dyes, acid or basic dyes, mordant dyes, direct dyes, vat dyes, reactive dyes, and so forth, or on their chemical structure as anthraquinonic dyes, azo dyes, xanthene dyes, carotenoid dyes, phthalocyanine, triphenylmethane dyes, and so forth. In addition, they are extremely opposed to degradation because of their complex aromatic structures and stay colored for an extended time that makes their removal is obligatory from the industrial effluents previous to their discharge into the surroundings. The enormous expansion in the dyestuff manufacturing and textile dyeing industries has direct to a huge increase in the complexity and wastewater volume that discharged to the environment [5].

A variety of approaches have been utilized for removing dyes from textile wastewater and decrease the fees of the whole process comprising physicochemical techniques (adsorption, coagulation/flocculation and reverse osmosis), chemical oxidation, microbial or electrochemical decolorization, and most newly, the utilize of a variety of enzymes. Traditional physical and chemical methods of decolorization of dyes are old-fashioned because expenses are high and they need high quantity of energy and chemicals, further disadvantages are mud development and accumulation of biomass [7,8]. Nowadays, biological treatment technology has increasingly aroused people attention, which is a lower-cost and environment friendly alternative compared with conventional physic-chemical treatment technologies [9].

Dyes biological degradation included different properties such as water solubility, fused aromatic ring structures, and large molecular weight structures that restrain penetration throughout the biological cell membranes. Additional restrictions of utilizing microbes for pollutants treating were slow dyes decolorization procedure, high production costs of microbial cultures, and metabolic activities inhibition [10,11]. On the other hand, enzymatic systems considered as techniques have the advantages of the two conventional categories of biological and chemical procedures, because they contain chemical reactions based on the biological catalysts action [12]. This was principally due to not like the chemical catalysts; enzymes were frequently favored more than the whole organisms having the enzymes as the isolated enzymes presented numerous advantages such as better standardization, greater specificity, easy handle and store, and independence from the bacterial growth rates [11,13].

In particular, there is a rising attention to the dyes degradation using enzyme due to numerous advantages such as ability to work over a wide range of contaminants concentration [11,14]. In addition, enzymes can specifically react with organic contaminants and eliminate them by converting them into other products. Enzymes can catalyze different reactions at comparatively low temperature and in the entire pH range [15]. Among oxidoreductive enzymes that are implicated in decolorization of dyes, peroxidases such as cytochrome C peroxidase, chloroperoxidase, manganese peroxidase, lignin peroxidase, soybean peroxidase and horseradish peroxidase have been stated as exceptional oxidant agents to corrupt dyes in the hydrogen peroxide occurrence [16–18].

In current study, *F. carica* latex peroxidase isoenzymes were tested to elucidate their potential for decolorizing different synthetic dyes in comparison to the commercial horseradish peroxidase.

2. MATERIALS AND METHODS

2.1 Chemicals and Latex Source

Guaiaciol was purchased from Bio Medical (USA). Hydrogen peroxide was purchased from Rankem (N.D., India). The different dyes classes utilized in the current study were purchased from Sigma Aldrich. All other chemicals and reagents employed were of analytical grade and were used without any further purification. The peroxidase isoenzymes were purified and partially purified from *F. carica* latex that collected from Al-Sharkia governorate, Egypt. Horseradish peroxidase as a standard enzyme was purchased from sigma All buffers utilized through this study were prepared as mentioned in Gomori [19], and pH values were checked by Hanna pH 211 micro processer pH meter.

2.2 Collection of Latex and Crude Extract Preparation

Fresh latex samples were obtained by cutting *F. carica* plant branches and the released latex was collected. Equal volumes of the diluted latex and benzene were mixed and centrifuged under cooling conditions at 5000 rpm for 10 min using BEKMAN 12HS centrifuge to remove any insoluble materials and to separate the mixture components. The peroxidase activity of latex fractions was examined in the three separated layers. The layer contains the peroxidase activity (aqueous layer) was collected and stored at -20°C and designated as crude extract.

2.3 Purification of *F. carica* Latex Peroxidase Isoenzymes

The aqueous layer from benzene fractionation was dialyzed against 20 mM sodium acetate buffer, pH 5.5 containing 10 mM $CaCl_2$. The dialyzed sample was applied directly to a Carboxymethyl (CM) -Sepharose column (12 × 1.5 cm i.d.) pre-equilibrated with the same buffer. The bound proteins were eluted with a stepwise gradient of NaCl range between 0.0 and 0.5 M which prepared in the same buffer at a flow rate of 30 ml/h. The eluted fractions using 0.1 M NaCl were pooled and expressed as FP1 isoenzyme, whereas the negative fractions (0.0 M NaCl) were collected and underwent for further purification step using DEAE-Sepharose column and expressed as FP2 (0.1 M NaCl) and FP3 (0.2 M NaCl) isoenzymes.

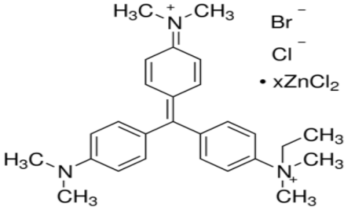
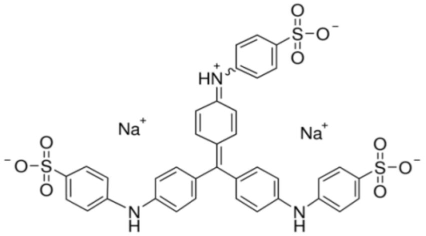
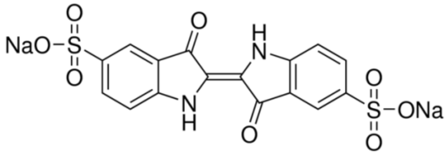
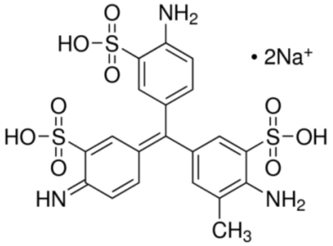
2.4 Peroxidase Assay and Protein Determination

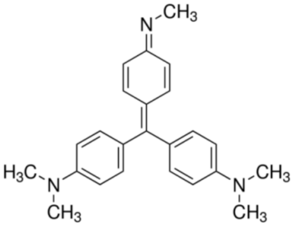
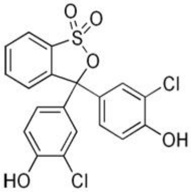
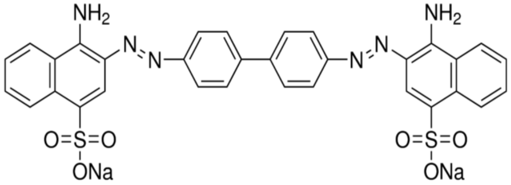
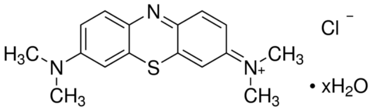
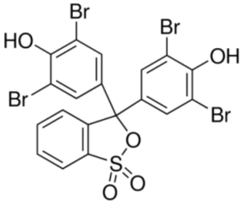
Peroxidase activity was carried out according to Miranda et al. [20]. The reaction mixture containing in 1.0 ml: 8 mM H_2O_2, 40 mM guaiacol, 50 mM sodium acetate buffer (pH 5.5) and least amount of enzyme preparation. Assays were carried out at 30ºC for 1 min. One unit of peroxidase activity is defined as the amount of enzyme which increases the optical density at 470 nm by 1.0 per minute under standard assay conditions using Cary 100 UV-Vis spectrophotometer (Agilent Technologies, Germany). Proteins were determined by the method described by Bradford [21] using bovine serum albumin (BSA) as a protein standard. All experiments were done in triplicates, and the mean value was presented ± standard deviation.

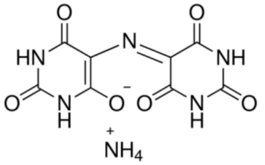
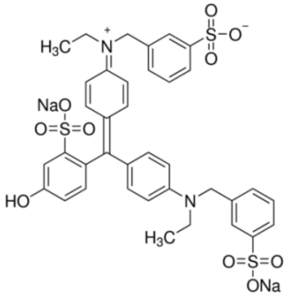
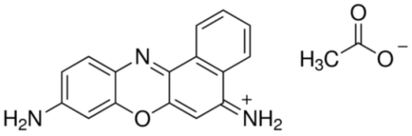
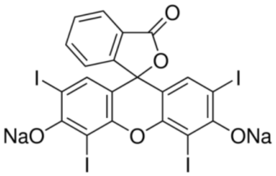
2.5 Dye Decolorization by *F. carica* Latex Peroxidase Isoenzymes

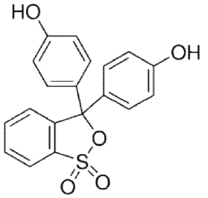
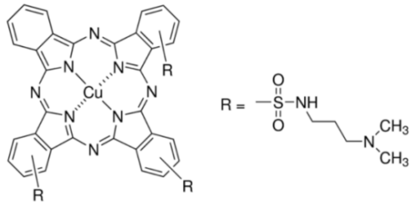
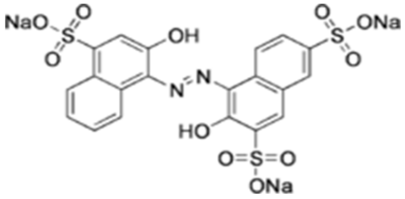
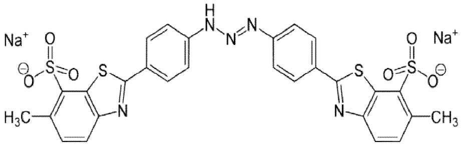
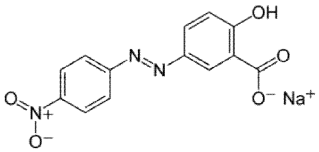
The decolorization of 20 dyes (methyl green, methyl blue, indigo carmine, fuschin, methyl violet, chlorophenol red, congo red, methylene blue,. bromo phenol blue, murexide, fast green, cresyl violet B, erythrosine, phenol red, astra blue, naphthol blue, titan yellow, allizarin yellow, auramine,, and naphthalene black 12B.) was investigated by using the purified three *F. carica* latex peroxidase isoenzymes (purified FP1 and partially purified FP2, and FP3), and horseradish peroxidase (HRP) as a control. Stock solutions of the dyes were prepared in 0.05 M sodium acetate buffer (pH 5.5) and diluted to the

Table 1. Chemical structure, optimum wavelength and concentration of evaluated dyes

	Dye	Dye classification	Chemical structure	Wave-length (nm)	Conc. (μM)
1	Methyl green	Triphenyl methane		630	76
2	Methyl blue	Triphenyl methane		616	63
3	Indigo carmine	Azo dye		608	42
4	Fuschin	Triphenyl methane		546	15

	Dye	Dye classification	Chemical structure	Wave-length (nm)	Conc. (µM)
5	Methyl violet	Triphenyl methane		576	28
6	Chlorophenol red	Triphenyl dye		436	94
7	Congo red	Diazo dye		486	71
8	Methylene blue	Heterocyclic dye		640	63
9	Bromophenol blue	Triphenyl dye		590	30

	Dye	Dye classification	Chemical structure	Wave-length (nm)	Conc. (µM)
10	Murexide	Methine		519	330
11	Fast green	Triaryl methane dye		636	12
12	Cresyl violet B	Heterocyclic dye		557	58
13	Erythrosine	Fluorescein dye		522	22

	Dye	**Dye classification**	**Chemical structure**	**Wave-length (nm)**	**Conc. (µM)**
14	Phenol red	Triphenyl dye		431	56
15	Astra blue	Phthalocyanine		601	110
16	Naphthol blue	Azo dye		616	32
17	Titan yellow	Azo dye		403	72
18	Allizarin yellow	Azo dye		357	129

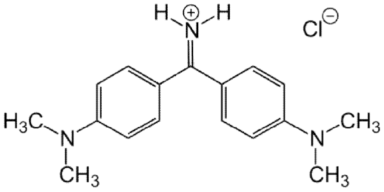
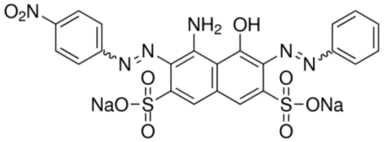
	Dye	**Dye classification**	**Chemical structure**	**Wave-length (nm)**	**Conc. (µM)**
19	Auramine	diarylmethane dye		431	70
20	Naphthalene black12B	Diazo dye		620	32

Table 2. Quantitative evaluation of decolorization of different dyes by *F. carica* latex peroxidase isoenzymes and HRP

	Dye	Wave length (nm)	Conc. (µM)	Decolorization (%)			
				FP1	FP2	FP3	HRP
1	Methyl green	630	76	41.78	41.86	63.79	48.69
2	Methyl blue	616	63	39.21	48.84	51.04	59.3
3	Indigo carmine	608	42	26.42	31.68	44.34	58.48
4	Fuschin	546	15	31.37	35.89	40.45	32.24
5	Methyl violet	576	28	27.35	24.85	18.09	22.49
6	Chlorophenol red	436	94	25.59	24.35	7.30	9.24
7	Congo red	486	71	21.85	19.96	12.18	4.16
8	Methylene blue	640	63	24.56	9.16	5.09	2.682
9	Bromo phenol blue	590	30	16.15	18.09	0	3.10
10	Murexide	519	330	5.78	22.55	16.94	18.04
11	Fast green	636	12	10.84	16.94	17.19	9.49
12	Cresyl violet B	557	58	18.73	1.17	20.85	7.97
13	Erythrosine	522	22	17.74	20.79	5.81	2.71
14	Phenol red	431	56	19.65	20.27	2.77	3.14
15	Astra blue	601	110	9.53	3.86	11.94	15.04
16	Naphthol blue	616	32	4.76	0	12.09	0.944
17	Titan yellow	403	72	8.80	7.60	6.80	6.09
18	Allizarin yellow	357	129	8.85	2.59	2.35	5.63
19	Auramine	431	70	1.29	3.47	8.28	6.14
20	Naphthalene black 12B	620	32	2.77	1.36	10.7	4.55

FP1: Peroxidase isoenzyme of 0.1M CM-Sepharose elution, FP2: peroxidase isoenzyme of 0.1M DEAE Sepharose elution, FP3: peroxidase isoenzyme of 0.2M DEAE Sepharose elution, HRP: Horseradish peroxidase. The reaction mixture consisted of individual dye in a concentration as indicated depending on its absorbance, 6.4 U/ml of peroxidase enzyme, 50 mM sodium acetate buffer, pH 5.5and 8 mM H_2O_2 in a total volume of 1.0 ml at 40°C under static conditions for 3 h.

requested concentrations in order to get maximum absorbance does not exceed 1.5 as initial reading (Table 1). The reaction mixture consisted of individual dye in the range of 12 - 330 µM (depending on its absorbance), 6.4 U/ml of peroxidase enzyme, 50 mM sodium acetate buffer, pH 5.5 and 8 mM H_2O_2 in a total volume of 1.0 ml. The reactions were initiated by the addition of peroxidase preparations and incubated at 40°C under static conditions for 3 h. Decolorization of dyes was followed by measuring the absorbance at different optimum wavelengths. The effect of dyes decolorization was determined by the decrease in absorbance under the maximum absorbance wavelength of each dye (Table 1).

The efficiency of decolorization was expressed in terms of percentage [22]. Controls were done in parallel under identical conditions and contained all components of the reaction mixtures except of the enzyme preparations. All experiments were performed in triplicate. Decolorization was defined as:

$$Decolorization\ (\%) = 100 \times Absorbance_{t0} - Absorbance_{tf} / Absorbance_{t0}$$

Where Absorbance $_{t0}$ is the absorbance at the optimum wavelength of the reaction mixture previous to incubation with the enzyme and Absorbance $_{tf}$ is the absorbance at the optimum wavelength after incubation [23].

2.6 Effect of Dyes Concentration

The influence of dye concentration on enzymatic color removal was investigated by using different dye concentrations (0.1, 1, 10, 100, 1000 µM). All assays were carried out with a minimum of three replicates. The enzyme assay was carried out at the optimum wave length for each dye. The reaction mixture consisted of individual dye in a concentration range depending on its absorbance, 6.4 U/ml of enzyme, 50 mM sodium acetate buffer, pH 5.5 and 8 mM H_2O_2 in a total volume of 1.0 ml. The reactions were initiated by the addition of peroxidase preparations and incubated at 40°C under static conditions for 3 h.

Decolorization of dyes was followed by measuring the absorbance at different optimum wavelengths.

3. RESULTS AND DISCUSSION

3.1 Decolorization of Different Dyes by *F. carica* Latex Peroxidase Isoenzymes and Horseradish Peroxidase

In order to investigate the ability of *F. carica* latex peroxidase purified and partially purified isoenzymes to decolorize different types of hazardous dyes that cause highly negative effects on the environmental ecosystems, twenty dyes species from different classes were studied.

Results obtained are summarized in Table 2 which indicated that, *F. carica* latex peroxidase isoenzymes and commercial horseradish peroxidase enzyme were able to decolorize some of tested dyes and the extent of decolorization achieved with different dyes classes were varied according to different chemical structure of each dye. It can be concluded that the decolorization efficiency after 3 h of incubation at 40°C using 6.4 U/ml of peroxidase activity of FP1, FP2, FP3 and HRP, was found to be extremely efficient in decolorizing some dyes and relatively low in other dyes and in some cases, dyes were rather recalcitrant to degradation by *F. carica* latex peroxidase isoenzymes. The results indicated that the three *F. carica* latex perioxidase isoenzymes (FP1, FP2, and FP3) can decolorize these dyes (methyl green, methyl blue, indigo carmine, fuschin, methyl violet, chlorophenol red) with decolorization persentage higher than that of other dyes

The results obtained in Table 2 indicated that FP1, FP2 and FP3 isoenzymes have the ability to decolorize methyl green, methyl blue, indigo carmine, fuschin and methyl violet efficiently with decolorization percentage ranged from 63 to 18.09%. In this regard, FP1 purified isoenzyme, after 3 h of incubation was found to be efficient in decolorizing methyl green (41.78%), methyl blue (39.21%), indigo carmine (26.42%), fuschin (31.37%), methyl violet (27.35%), chlorophenol red (25.59%), congo red (21.85%), methylene blue (24.56%), phenol red (19.65%), cresyl violet B (18.73%), erythrosine (17.74%), bromo phenol blue (16.15%), and fast green (10.84%). On the other hand, FP2 partially purified isoenzyme was found to be able to decolorize methyl blue (48.86%), methyl green (41.86%), fuschin (35.89%), indigo carmine (31.68%), methyl violet (24.85%), chlorophenol red (24.35%), congo red (19.96%), murexide (22.55%), bromo phenol blue (18.09%), fast green (16.94%), erythrosine (20.79%), and phenol red (20.27%), however, FP3 partially purified isoenzyme was found to be capable to decolorize methyl green (63.79%), methyl blue (51.04%), indigo carmine (44.34%), fuschin (40.45%), cresyl violet B (20.85%), congo red (12.18%), methyl violet (18.09%), murexide (16.94%), fast green (17.19%), Naphthol blue (12.09%), astra blue (11.94%) and Naphthalene black 12B (10.7%) under the same conditions. in (Table 2).

Some peroxidases from different sources can decolorize the synthetic dyes such as from *Hevea brasiliensis* cell suspension that can decolorize triphenyl methane dye group such as aniline blue (83%), brilliant green (68%), bromo cresol purple (52%), crystal violet (60%), fuchsin (55%), malachite green (95%), methyl green (97%), methyl violet (49%) and water blue (88%) within 6 h [24]. However the peroxidase from *Ficus sycomorus* latex can decolorize the synthetic dyes such as bromo phenol blue (73%), methyl green (80%), methylene blue (74%), methyl orange (66%), azo carmine (30%) and titan yellow (72%) within 24 h [8]. Also, peroxidase from *Pleurotus ostreatus* can decolorize triphenyl methane dye such as crystal violet (74%), malachite green (46%) and bromophenol blue (98%); heterocyclic dyes such as methylene blue (10%) and toluidine blue O (10%); and azo dye such as methyl orange (96%) and congo red (32%) within 5 min [25]. Peroxidase from *Azadirachta indica* can decolorize congo red (73%), trypan blue (62%) and methyl orange (59%) within 8 h [26]. Lignine peroxidase from *Streptomyces griseosporeus* SN9 35 had the ability to decolorize remazol brilliant blue (18%), acid blue (35%) and cibacet brilliant blue BG (69%), after 1 h and poly R-478 (5%) within 48 h [27]. Manganese peroxidase from *Bjerkandera adusta* strain CX-9 can decolorize acid blue (91%), Poly R-478 (80%), cibacet brilliant blue BG (77%), remazol brilliant violet 5R (70%), indigocarmine (42%), remazol brilliant blue (38%), and methyl green (12%) within 12 h [28]. In addition, manganese peroxidase from *Cerrena unicolor* can decolorize various types of synthetic dyes including remazol brilliant blue (81.0% in 5 h), congo red (53.9% in 12 h), methyl orange (77.6% in 12 h), bromophenol blue (62.2% in 12 h), and crystal violet (80.9% in 12 h) [18]. Lignin peroxidase from *Lysinibacillus sphaericus* JD1103 was found to be able to decolorize Congo Red and Remazol Brilliant Blue R by 84.38% and 50.00% within 72 h [29].

As a reference peroxidase enzyme, the commercial horseradish peroxidase was found to be capable to decolorize methyl blue (59.3%), methyl green (49%), indigo carmine (58.5%), fuschin (32%), methyl violet (22.5%), murexide (18%), fast green (9.5%), and astra blue (15%) within 3 h. Horseradish peroxidase that compared with *F. sycomorus* latex was able to decolorize the synthetic dyes bromo phenol blue (75%), methyl green (80%), methylene blue (71%), methyl orange (60%), azo carmine (33%) and titan yellow (72%) within 24 hr [8], and the ability of horseradish peroxidase to decolorize two synthetic anthraquinonic dyes such as acid blue 225 (50.63%) and acid violet 109 (85.16%) within 32 min [5].

3.2 Effect of Dyes Concentration on Dyes Decolorization by *F. carica* Latex Peroxidase Isoenzymes

The increase in dye concentration provides an effective increase in color removal [30]. In this connection, the effect of chlorophenol red concentration on FP1 and FP3 peroxidase isoenzymes are indicated in Figs 1a and b. The naphthol blue dye decolorization was increased by increasing the concentration of dye (Figs 2a and b). As another dye candidate, the effect of concentration of methyl blue dye on FP1 and FP3 was illustrated in Figs. 3a and b, where the decolorization of methyl blue dye was increased

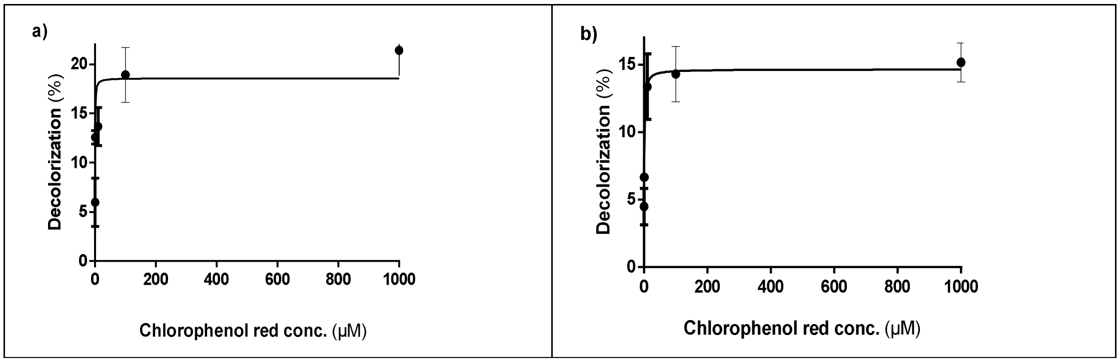


Fig. 1. Effect of chlorophenol red concentrations on its decolorization by *F. carica* latex peroxidase isoenzymes, a) FP1 isoenzyme, and b) FP3 isoenzyme

The reaction mixture consisted of chlorophenol red dye in a concentration range (0 - 1000 µM), 6.4 U/ml of individual peroxidase isoenzyme, 50 mM acetate buffer (pH 5.5) and 8 mM H_2O_2 in a total volume of 1 ml and incubated at 40°C under static conditions for 3 h

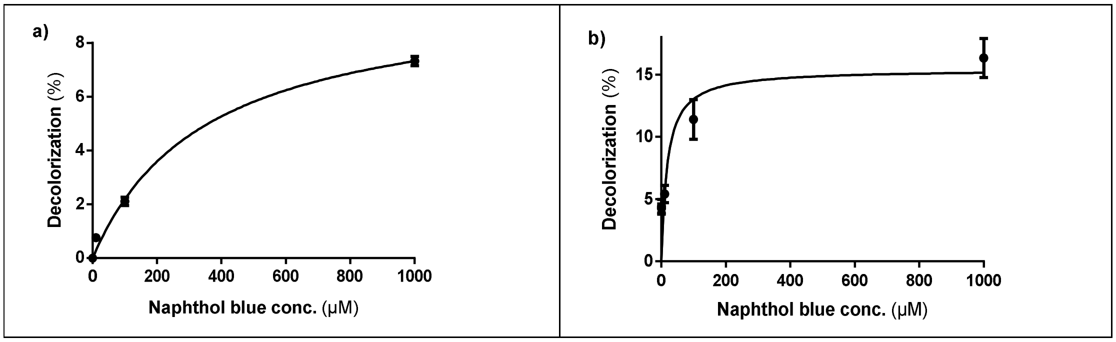


Fig. 2. Effect of naphthol blue concentrations on its decolorization by *F. carica* latex peroxidase isoenzymes, a) FP1 isoenzyme, and b) FP3 isoenzyme

The reaction mixture consisted of naphthol blue dye in a concentration range (0 - 1000 µM), 6.4 U/ml of individual peroxidase isoenzyme, 50 mM acetate buffer (pH 5.5) and 8 mM H_2O_2 in a total volume of 1 ml and incubated at 40°C under static conditions for 3 h

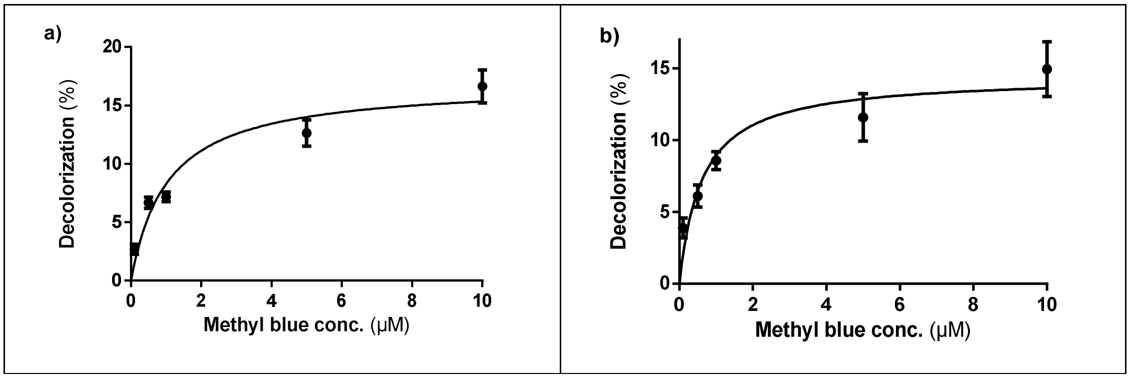


Fig. 3. Effect of methyl blue concentrations on its decolorization by *F. carica* latex peroxidase isoenzymes, a) FP1 isoenzyme, and b) FP3 isoenzyme

The reaction mixture consisted of methyl blue dye in a concentration range (0 - 10 µM), 6.4 U/ml of individual peroxidase isoenzyme, 50 mM acetate buffer (pH 5.5) and 8 mM H_2O_2 in a total volume of 1 ml and incubated at 40°C under static conditions for 3 h

by increasing the concentration of dye until saturation concentration was attained. From the results obtained, it could be conclude that the decolorization rate of different dye species by *F. carica* latex peroxidase isoenzymes (FP1 and FP3) was greatly depended on different concentrations of dyes. Celebi et al., [31] stated that remazol brillant blue R was decolorized by horseradish peroxidase and the decolorization rate was improved by increasing dye concentrations. The increase in Reactive Blue 21 concentration until 40 mg^{L-1} provides an effective increase in color removal. Subsequent increase in dye concentration above 40 mgL^{-1} resulted in negligible dye removal [30]

4. CONCLUSION

F. carica latex peroxidase isoenzymes and commercial horseradish peroxidase were able to decolorize some of tested dyes and the extent of decolorization achieved with different dyes classes were varied according to different chemical structure of each dye. The decolorization efficiency after 3 h of incubation at 40ºC using 6.4 U/ml of peroxidase activity of FP1, FP2, FP3 and HRP, was found to be extremely efficient in decolorizing some dyes and relatively low in other dyes and in some cases, dyes were rather recalcitrant to degradation by *F. carica* peroxidase isoenzymes. It can be concluded that the efficiency of *F. carica* latex peroxidase isoenzymes toward different synthetic dyes meet the prerequisites needed for environmental and industrial applications.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Roriz MS, Osma JF, Teixeira 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.
2. Balan K, Sathishkumar P, Palvannan T. Decolorization of malachite green by laccase: Optimization by response surface methodology. J Taiwan Inst Chem Eng. 2012;43(5):776–82.
3. Othman A, Elshafei A, Elsayed M, Hassan M. Decolorization of Cibacron Blue 3G-A Dye by *Agaricus bisporus* CU13 Laccase - Mediator system: A Statistical study for optimization via response surface methodology. Annu Res Rev Biol. 2018;25(6):1–13.
4. Nyanhongo GS, Gomes J, Gübitz GM, Zvauya R, Read J, Steiner W. Decolorization of textile dyes by laccases from a newly isolated strain of *Trametes modesta.* Water Res. 2002;36(6):1449–56.
5. Šekuljica NŽ, Prlainović NŽ, Stefanović AB, Žuža MG, Čičkarić DZ, Mijin DŽ, et al. Decolorization of anthraquinonic dyes from textile effluent using horseradish peroxidase: Optimization and kinetic study. Sci World J. 2015;2015:1–12.

6. Singh RL, Singh PK, Singh RP. Enzymatic decolorization and degradation of azo dyes – A review. Int Biodeterior Biodegrad. 2015;104:21–31.
7. Boucherit N, Abouseoud M, Adour L. Degradation of disperse dye from textile effluent by free and immobilized *Cucurbita pepo* peroxidase. EPJ Web Conf. 2012; 29:00008.
8. Abdel-Aty AM, Hamed MB, Fahmy AS, Mohamed SA. Comparison of the potential of *Ficus sycomorus* latex and horseradish peroxidases in the decolorization of synthetic and natural dyes. J Genet Eng Biotechnol. 2013;11(2):95–102.
9. Pan H, Xu X, Wen Z, Kang Y, Wang X, Ren Y, et al. Decolorization pathways of anthraquinone dye Disperse Blue 2BLN by *Aspergillus* sp. XJ-2 CGMCC12963. Bioengineered. 2017;8(5):630–41.
10. Akhtar S, Husain Q. Potential applications of immobilized bitter gourd (*Momordica charantia*) peroxidase in the removal of phenols from polluted water. Chemosphere. 2006;65(7):1228–35.
11. Husain Q. Peroxidase mediated decolorization and remediation of wastewater containing industrial dyes: A review. Rev Environ Sci Biotechnol. 2010; 9(2):117–40.
12. Saratale RG, Saratale GD, Chang JS, Govindwar SP. Bacterial decolorization and degradation of azo dyes: A review. J Taiwan Inst Chem Eng. 2011;42(1):138–57.
13. Husain Q, Husain M. Peroxidases as a potential tool for the decolorization and removal of synthetic dyes from polluted water. In: Malik A, Grohmann E, editors. Environmental Protection Strategies for Sustainable Development [Internet]. Dordrecht: Springer Netherlands. 2012; 453–98.
    Available:http://link.springer.com/10.1007/978-94-007-1591-2_15
    [Cited 2018 May 7]
14. Arabaci G, Usluoglu A. The enzymatic decolorization of textile dyes by the immobilized polyphenol oxidase from quince leaves. Scientific World Journal. 2014;685975.
15. Calza P, Zacchigna D, Laurenti E. Degradation of orange dyes and carbamazepine by soybean peroxidase immobilized on silica monoliths and titanium dioxide. Environ Sci Pollut Res. 2016;23(23):23742–9.
16. Liu JZ, Wang TL, Ji LN. Enhanced dye decolorization efficiency by citraconic anhydride-modified horseradish peroxidase. J Mol Catal B Enzym. 2006;41(3–4):81–6.
17. Ollikka P, Alhonmaki K, Leppanen VM, Glumoff T, Raijola T. Decolorization of azo, triphenyl methane, heterocyclic and polymeric dyes by lignin peroxidase isoenzymes from *Phanerochaete chrysosporium*. Appl Env Microbiol. 1993; 59(11):4010–6.
18. Zhang H, Zhang J, Zhang X, Geng A. Purification and characterization of a novel manganese peroxidase from white-rot fungus *Cerrena unicolor* BBP6 and its application in dye decolorization and denim bleaching. Process Biochem. 2018;66: 222–9.
19. Gomori G. Preparation of buffers for use in enzyme studies. Methods Enzymol. 1955;1:138–46.
20. Miranda MV, Lahore HMF, Cascone O. Horseradish peroxidase extraction and purification by aqueous two-phase partition. Appl Biochem Biotechnol. 1995; 53(2):147–154.
21. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976;72(1):248–54.
22. Wang C, Zhao M, Lu L, Wei X, Li T. Characterization of spore laccase from *Bacillus subtilis* WD23 and its use in dye decolorization. Afr J Biotechnol. 2011; 10(11):2186–92.
23. Khlifi R, Belbahri L, Woodward S, Ellouz M, Dhouib A, Sayadi S, et al. Decolourization and detoxification of textile industry wastewater by the laccase-mediator system. J Hazard Mater. 2010; 175(1):802–8.
24. Chanwun T, Muhamad N, Chirapongsatonkul N, Churngchow N. *Hevea brasiliensis* cell suspension peroxidase: purification, characterization and application for dye decolorization. AMB Express. 2013;3(1):14.
25. Shin KS, Oh IK, Kim CJ. Production and purification of remazol brilliant blue R decolorizing peroxidase from the culture filtrate of *Pleurotus ostreatus*. Appl Env Microbiol. 1997;63(5):1744–1748.
26. Pandey VP, Rani J, Jaiswal N, Singh S, Awasthi M, Shasany AK, et al. Chitosan

immobilized novel peroxidase from *Azadirachta indica*: Characterization and application. Int J Biol Macromol. 2017;104:1713–20.

27. Rekik H, Nadia ZJ, Bejar W, Kourdali S, Belhoul M, Hmidi M, et al. Characterization of a purified decolorizing detergent-stable peroxidase from *Streptomyces griseosporeus* SN9. Int J Biol Macromol. 2015;73:253–63.
28. Bouacem K, Rekik H, Jaouadi NZ, Zenati B, Kourdali S, El Hattab M, et al. Purification and characterization of two novel peroxidases from the dye-decolorizing fungus *Bjerkandera adusta* strain CX-9. Int J Biol Macromol. 2018;106:636–46.
29. Chantarasiri A, Boontanom P. decolorization of synthetic dyes by ligninolytic *Lysinibacillus sphaericus* JD1103 isolated from Thai wetland ecosystems. AACL Bioflux. 2017;10(4):6.
30. Silva MC, Corrêa AD, Amorim MTSP, Parpot P, Torres JA, Chagas PMB. Decolorization of the phthalocyanine dye reactive blue 21 by turnip peroxidase and assessment of its oxidation products. J Mol Catal B Enzym. 2012;77:9–14.
31. Celebi M, Altikatoglu M, Mustafaeva Akdeste Z, Yildirim H. Determination of decolorization properties of reactive blue 19 dye using horseradish peroxidase enzyme. Turk J Biochem. 2013;38(2):200–6.

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