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Decolorization of Industrial Dyes by an Extracellular Peroxidase from *Bacillus* sp. F31

Shamsher S. Kanwar*and Neelam Bansal
Department of Biotechnology, Himachal Pradesh University, Summer Hill, Shimla-171005 India

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Abstract: An extracellular peroxidase purified from a bacterial strain *Bacillus* sp. F31 was evaluated for its ability to decolorize 16 different industrial dyes namely Bromophenol Blue (BPB), Reactive Yellow FN2R (RY), Congo Red (CR), Xylidine (XY), Methyl Orange (MO), Rhodamine B (RB), Erichrome Black Y (EB), Bismark Brown R (BBR), Basic Fuchsin (BF), Bismark Brown Y (BBY), Direct Violet 21 (DV), Direct Black 154 (DB), Methylene Blue (MB), Black RL (BRL), Coomassie Brilliant Blue R-250 (CBBG) and Malachite Green (MG). Each of these dyes was subjected to treatment with purified peroxidase (0.75 U) at 37°C for 30 min in phosphate citrate buffer (pH 5.2). Out of 16 different textile dyes the peroxidase efficiently decolorized five dyes out of which four are triphenyl methane dyes (BF, RB, CBBG and MG) showed decolorization up to (95.5, 70.8, 70 and 40 %) respectively, while a polymeric heterocyclic dye (MB) showed 66.2 % decolorization. These five dyes were studied further to enhance their decolorization by bacterial peroxidase by optimizing different reaction conditions (temperature, time, enzyme concentration, buffer pH, dye concentration and effect of various salt ions).

Key words: *Bacillus* sp. F31, peroxidase, decolorization, industrial dyes, optimization.

Introduction

Industrial dyes in effluents represent one of the most problematic groups of pollutants that are not easily biodegradable [1]. The main consumers of dyes are the textile, plastic, tannery, paper & pulp and electroplating industries [2,3,4]. Dyes are also used as additives in petroleum products, foods, pharmaceutical and cosmetic industries [5]. Based on the chemical structure of the chromophoric group, the dyes are classified as azo, anthraquinone, triarylmethane and phthalocyanine dyes [6]. It is estimated that between 10-20 % of dyestuff being used in the dyeing process could be found in wastewater. Several of these dyes are very stable to light, temperature and microbial attack, making them recalcitrant compounds which act as serious environmental pollutants [7].

The food web/food chain of organisms is also adversely effected with the toxic pollutants/dyes released in the effluents in environment [8,9,10]. Although, the color durability is the most important goal of dying process, however, the textile dyes are highly resistant to both chemical and physical degradation [12,13]. Most of the peroxidases catalyses asymmetric cleavage of bonds present in the dyes [14-18]. The chemical oxidation, reverse osmosis, adsorption, incineration, photocatalysis or ozonation of dyes are highly efficient but they suffer some disadvantages [19]. Due to inherent drawbacks of physical, chemical and photochemical approaches [20], the use of biological methods for the decolorization/ detoxification of textile wastewaters has been encouraged [21-26].

Peroxidases are highly non-specific and are able to transform or mineralize organopollutants as well as have been reported to decolorize various dyes [28,29]. Therefore, the aim of the present investigation was to study the biodegradation of various industrial dyes by an extracellular peroxidase produced from a bacterial isolate *Bacillus* sp. F31

*Corresponding author (Shamsher S. Kanwar)
E-mail: < kanwarss2000@yahoo.com >

which is isolated from crude oil-polluted soil.

Materials and methods

Organism

The bacterial strain *Bacillus* sp. F31 was isolated from the petroleum oil-polluted soil.

Preparation of crude enzyme

The seed culture was raised at 37°C (120 rpm) for 24 h. The seed culture was added (10 %, v/v) to 50 ml of production broth [containing yeast extract (0.2 %), beef extract (0.1 %), glucose (1.4 %), peptone (0.5 %), NaCl (0.5), H_2O_2 (0.06 %; v/v) with final pH of 7.5] and incubated for 48 h under shaking conditions (at 120 rpm at 37°C). Thereafter, the incubated nutrient broth was harvested by centrifugation (10,000 X g for 15 min at 4°C; SIGMA 3K30, Germany) and the cell-free broth was termed as crude peroxidase.

Protein and peroxidase assay

Protein concentration was measured by a standard method [30] using Bovine serum albumin as a standard. The assay of peroxidase in the broth or purified enzyme fraction was done by a colorimetric method using *o*-phenylenediamine (OPD) as a chromogen and H_2O_2 as a substrate [31,32]. The A_{492} values were recorded and activity of the peroxidase was determined. One unit (U) of peroxidase was defined as the amount of enzyme [needed to convert 1.0 µM of chromogenic substrate (OPD) to its product (2, 3 diamino-phenazine)/ min at pH 5.2 and temperature 37°C.

Purification

The extracellular bacterial peroxidase from *Bacillus* sp. F31 was purified by $(NH_4)_2SO_4$ salting out, dialysis and anion-exchange chromatography on a DEAE-cellulose column. The molecular mass was determined by SDS-PAGE.

Screening of industrial dyes for decolorization by purified peroxidase

A total of 16 different dyes namely, BPB, RY, CR, XY, MO, RB, EB, BBR, BF, BBY, DV, DB, MB, BRL, CBBG and MG were studied for their decolorization by peroxidase of *Bacillus* sp. F31. The decolorization reaction system contained 700 µl (0.1 M) phosphate citrate buffer (pH 5.2), 15 µl H_2O_2, 50 µl of the dye (20 µM) and 0.75 U peroxidase to make the final volume 1 ml. After 30 min incubation at 37°C, the absorbance at the respective wavelength (λmax) of the dye was recorded and decolorization (%) was determined as follows:

$$\text{Decolorization \%} = \frac{\text{Initial absorbance - Observed absorbance}}{\text{Initial absorbance}} \times 100$$

Optimization of reaction conditions for dye decolorization by peroxidase of *Bacillus* sp. F31

Out of 16 dyes tested for decolorization, only five dyes were efficiently decolorized by bacterial peroxidase and thus these five dyes (BF, RB, MB, CBBG and MG) were selected for further studies. The purified peroxidase was used to evaluate the effect of temperature, reaction time, enzyme quantity, buffer system pH, effect of dye concentration, effect of salt-ions on degradation of selected dyes and optimized conditions for dye degradation were ascertained.

Effect of temperature on dye decolorization

In order to determine optimum temperature for selected dyes (BF, RB, MB, CBBG and MG), the degradation reaction facilitated by peroxidase [1 ml reaction mixture containing 700 µl (0.1 M) phosphate citrate buffer (pH 5.2), 15 µl H_2O_2, 0.75 U purified peroxidase and 50 µl (20 µM) of different dyes] was carried out at few selected temperatures (25, 30, 35, 37, 40 and 45°C) for 30 min and decolorization % was determined.

Effect of reaction time on dye decolorization

To determine the optimum reaction time for degradation of selected dyes (BF, RB. MB, CBBG, and MG) the time of dye degradation assay [1 ml reaction mixture containing 700 µl (0.1 M) phosphate citrate buffer (pH 5.2), 15 µl H_2O_2, 0.75 U purified peroxidase and 50 µl (20 µM) of different dyes] was varied from 0 to 40 min.

Effect of biocatalyst concentration on dye decolorization

The enzyme concentration was varied from 0.70 U to 1.1 U of purified peroxidase in 1 ml final

reaction volume, to perform the dye degradation assay [1 ml reaction mixture containing 700 µl (0.1 M) phosphate citrate buffer (pH 5.2), 15 µl H_2O_2 and 50 µl (20 µM) of different dyes]. Then decolorization (%) of different dyes was calculated after incubation at 35°C for BF after 35 min, at 40°C for RB after 40 min, at 30°C for CBBG after 40 min and for MG after 40 min at 40°C, respectively.

Effect of buffer system pH on dye decolorization

The effect of reaction buffer pH on the dye degradation by purified peroxidase of different dyes was studied by using different pH values (3-7) of 0.1 M phosphate citrate buffer in (1 ml) reaction mixture (containing 15 µl H_2O_2, 50 µl (20 µM) of selected dye). The decolorization (%) of the dye(s) was calculated after incubation at 35°C for BF after 35 min, at 40°C for RB after 40 min, at 30°C for CBBG after 40 min and for MG after 40 min at 40°C, respectively.

Effect of dye concentration on its decolorization

The concentration of each dye was varied from 100 to 1000 mg/l (in dye degradation assay mixture containing 700 µl (0.1 M) phosphate citrate buffer, 15 µl H_2O_2, 50 µl of different dyes and optimized concentration purified peroxidase for each dye). The decolorization assay was carried out at respective optimized temperature, pH and time for each of the dyes.

Effect of H_2O_2 concentration on dye decolorization

The concentration of H_2O_2 was varied from 0.25 to 3.5 mM in the mixture [containing 700 µl (0.1 M) of phosphate citrate buffer, 50 µl (20 µM) of selected dye and the optimized concentration of peroxidase]. The decolorization assay was carried out at respective optimized temperature, pH and time for each of the dye and decolorization (%) was determined.

Effect of salts and inhibitors on dye decolorization

The decolorization (%) of BF, RB, MB, CBBG and MG with purified peroxidase was determined in the presence of 1 mM (w/v) of selected salt ions/ inhibitors (Li^{+3}, Zn^{+2}, Mg^{+2}, K^{+2}, Na^{+}, Hg^{+2}, Mn^{+2}, Ca^{+2}, Cu^{+2}, Fe^{+2}, EDTA, SDS, sodium azide and DTT) under optimized conditions. The mixture of enzyme and metal ion/ inhibitors in ratio 1: 1 was pre-incubated for 30 min at 37°C followed by the addition of the selected dye to check its decolorization.

Results

Purification of the extracellular peroxidase of Bacillus sp. F31

The purification of peroxidase from the culture broth of *Bacillus* sp. F31 was done by ammonium sulphate precipitation, dialysis and anion exchange chromatography (DEAE-cellulose), respectively. The SDS-PAGE and the native-PAGE resulted in a single band of ~37 kDa and 95 kDa, respectively. The peroxidase enzyme was purified up to 14.6-fold with a yield of 12.6 %.

Screening of industrial dyes for decolorization by peroxidase

Out of 16 textile dyes, the purified peroxidase efficiently decolorized only five dyes out of which four were triphenyl methane dyes (BF, RB, CBBG and MG) that showed decolorization up to 95.5, 70.8, 70.0 and 40 %, respectively. In contrast, a polymeric heterocyclic dye (MB) showed 66.2% decolorization (Table 1 and Table 2).

Optimization of reaction conditions for decolorization of selected dyes by peroxidase of *Bacillus* sp. F31

Effect of temperature on dye decolorization

The temperature of reaction system was varied from 30 to 45°C for decolorization of BF, RB, MB, CBBG and MG. The reaction mixture (1 ml) contained 0.75 U of purified peroxidase. The optimum temperature for each of these dyes was 30°C for RB (72.1 %), 35°C (82.0 %) for MB; 40°C for BF (92.1 %), CBBG (90.2 %) and MG (65.2 %), respectively (Fig. 1).

Effect of reaction time on dye decolorization by purified peroxidase

The reaction time of dye decolorization for each

Table 1. Screening of dyes for decolourization by *Bacillus* sp. F31 peroxidase

No.	Name of dye (1 mM)	Type of dye	Dye decolourization (%)
1	BPB	Triphenylmethane	3.0
2	RY	Azo	5.0
3	CR	Azo	-
4	XY	*Dimethylaniline*	-
5	MO	Azo	3.0
6	RB	Triphenylmethane	70.8
7	EB	Azo	-
8	BBR	Diazo	5.0
9	BF	Basic dye	95.5
10	BBY	Diazo	7.0
11	DV	Azo	-
12	DB	Azo	8.0
13	MB	Polymeric heterocyclic	66.2
14	BRL	Azo	4.0
15	CBBG	Triphenylmethane	70.0
16	MG	Triphenylmethane	40.0

Table 2. Efficient decolorization of dyes by peroxidase of *Bacillus* sp. F31

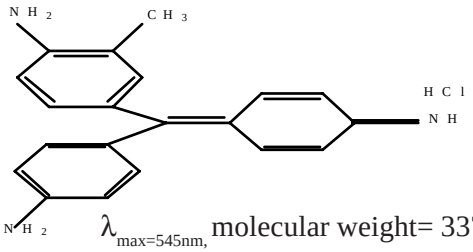
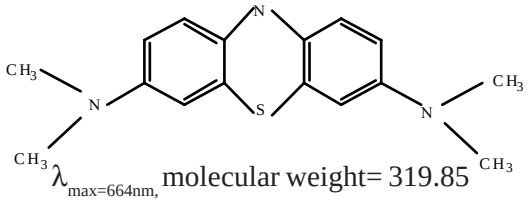
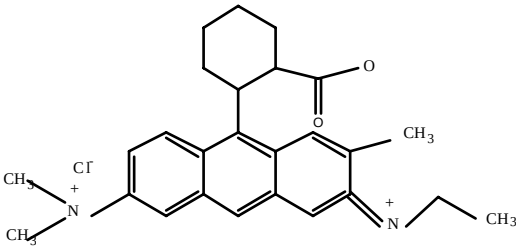
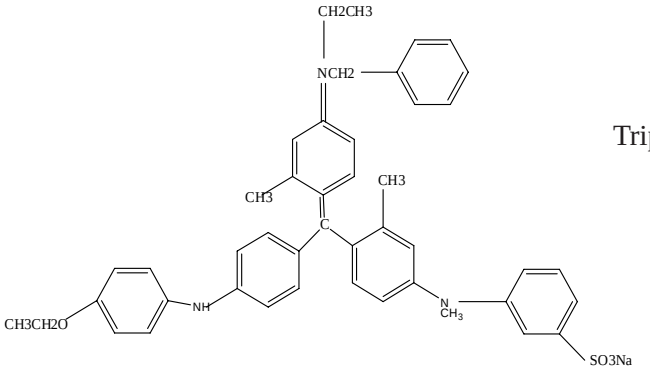
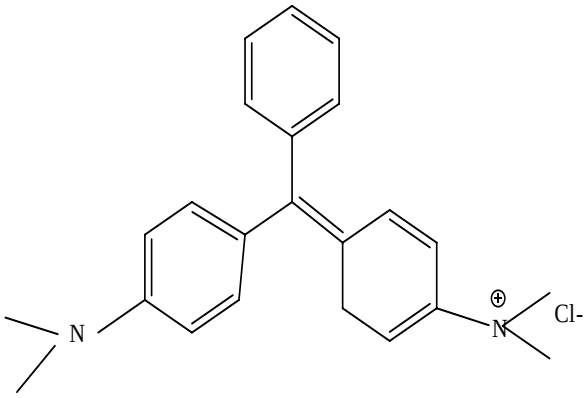
Dye	Structure of dye/ λ_{max}/ Molecular weight (g/mol)	Type of dye	Decolorization (%)
BF	 $\lambda_{max=545nm,}$ molecular weight= 337.85	Triphenyl methane dye	95.5
MB	 $\lambda_{max=664nm,}$ molecular weight= 319.85	Triphenyl methane dye	66.2
RB	 $\lambda_{max=555nm,}$ molecular weight= 479.01	Polymeric/heterocyclic dye	70.8

table 2. (continued).

Dye	Structure of dye/ λ_{max}/ Molecular weight (g/mol)	Type of dye	Decolorization (%)
CBBG	 $\lambda_{max= 610\ nm,}$ molecular weight =854.02	Triphenyl methane dye	70.0
MG	 $\lambda_{max=550\ nm,}$ molecular weight= 364	Triphenyl methane dye	40.0

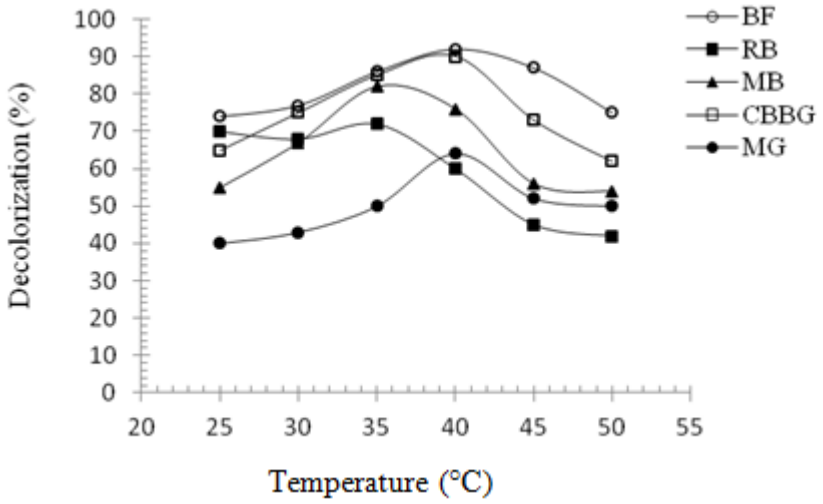


Fig. 1. Effect of temperature on dye decolorization by peroxidase of *Bacillus* sp. F31

of the selected dyes (BF, RB, MB, CBBG and MG) was varied from 0 to 45 min. The maximum decolorization by peroxidase was observed at 30-45 min for MB (82.1 %, 30 min) at 35°C, BF (96.1 %, 35 min) at 40°C, RB (76.2 %, 40 min) at 30°C, CBBG (90.0 %, 40 min) and MG (78.3 %, 40 min) at 40°C, respectively (Fig. 2).

Effect of biocatalyst concentration on dye decolorization

The enzyme concentration used in dye decolorization assay of five selected dyes (BF, RB, MB, CBBG and MG) was varied either from 0.77 to 1.05 U for peroxidase in 1 ml final volume of reaction mixture. The maximum decolorization was observed with 0.94 U of peroxidase for BF (95.1 %) at 40°C in 35 min, 1.05 U for RB (82.2 %) at 35°C in 40 min, MB (85.1 %) at 35°C in 30 min and MG (78.2 %) at 35°C in 40 min and 1.01 U for CBBG (92.1 %) at 40°C in 40 min, respectively (Fig. 3).

Effect of buffer system pH on dye decolorization

In order to determine optimum pH of the decolorization assay system for efficient decolorization of each dye (BF, RB and MB, CBBG and MG), the studies were performed with peroxidase

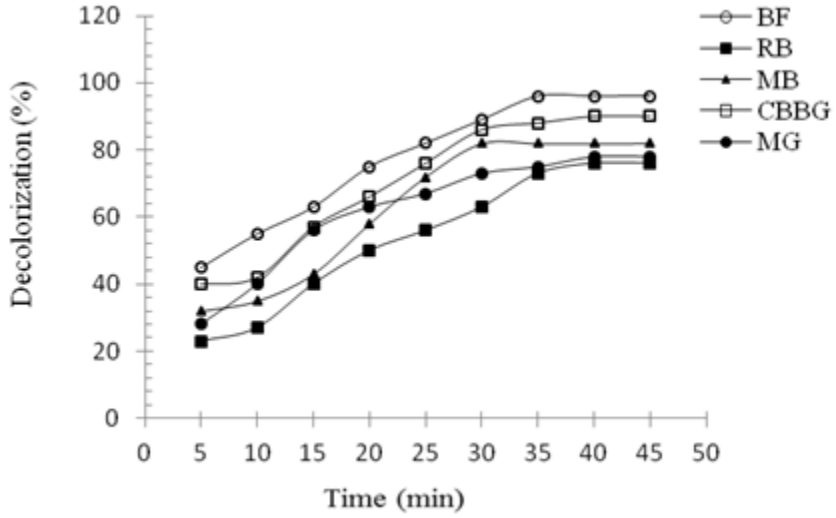


Fig. 2. Effect of reaction time on dye decolorization by peroxidase of *Bacillus* sp. F31

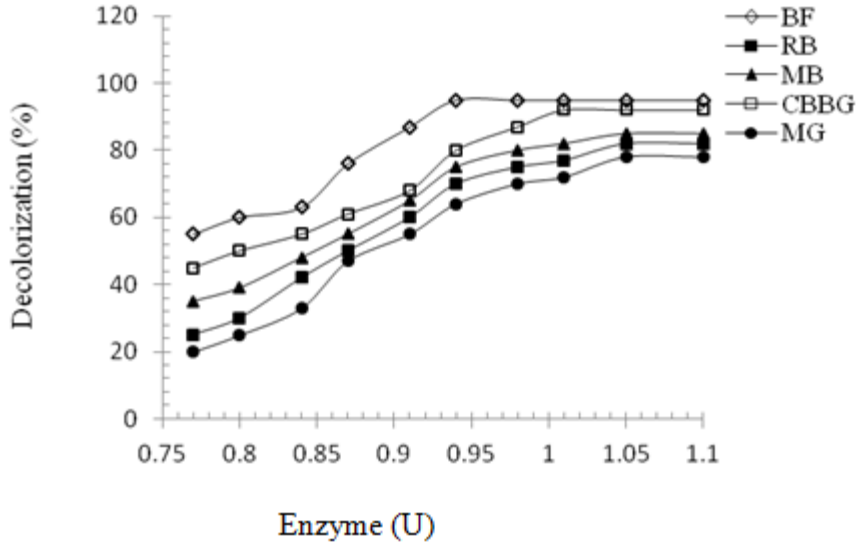


Fig. 3. Effect of biocatalyst concentration on dye decolorization by peroxidase of *Bacillus* sp. F31

at varying buffer pH of phosphate citrate buffer. The optimum buffer pH for decolorization was pH 5.0 for RB (81.2 % at 35°C for 40 min with 1.05 U of peroxidase), pH 5.5 for BF (96.1 % at 40°C for 35 min with 0.94 U peroxidase) and also pH 5.5 for MB (83.5 % at 35°C for 30 min with 1.05 U of peroxidase), and MG (78.3 % at 40°C for 40 min with 1.05 U of peroxidase), pH 6.0 for CBBG (92.2 % at 40°C for 40 min with 1.01 U of peroxidase), respectively (Fig. 4).

Effect of dye concentration on its decolorization

The maximum decolorization of BF was found to be 97.1 % at 800 mg/l at 40°C in 35 min at pH 5.5, for RB (86.2 %) at 600 mg/l at 30°C in 40 min at pH 5.0, MB (84.2 %) at 35°C in 30 min at pH 5.5 and MG (78.1 %) at 400 mg/l at 40°C in 40 min at pH 5.5 and CBBG (92.1 %) at 40°C in 40 min with 200 mg/l at pH 6.0 (Fig. 5).

Effect of H_2O_2 (substrate) concentration on dye decolorization

When the concentration of H_2O_2 was varied from 0.25 to 3.5 mM in dye decolorization assay, the optimized concentration of H_2O_2 for decolorization of the selected dyes (BF, RB, MB, CBBG and MG) using peroxidase was found

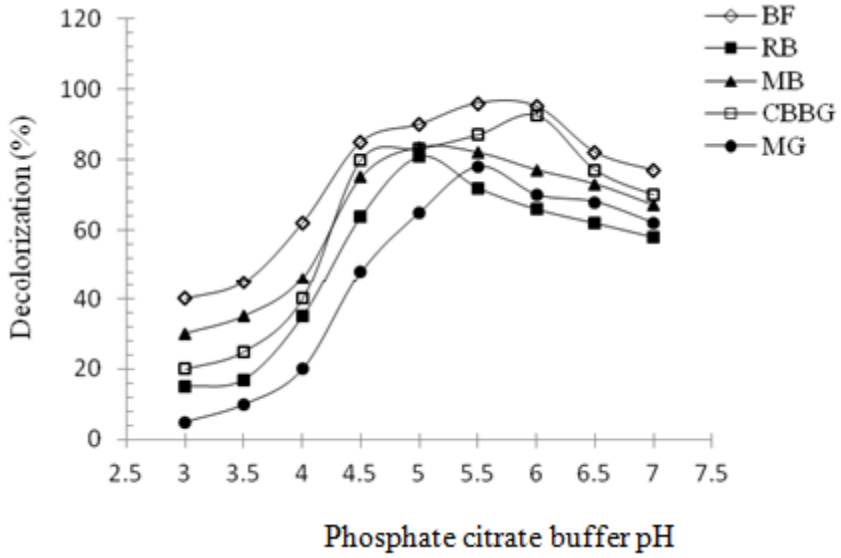


Fig. 4. Effect of buffer pH on dye decolorization by peroxidase of *Bacillus* sp. F31

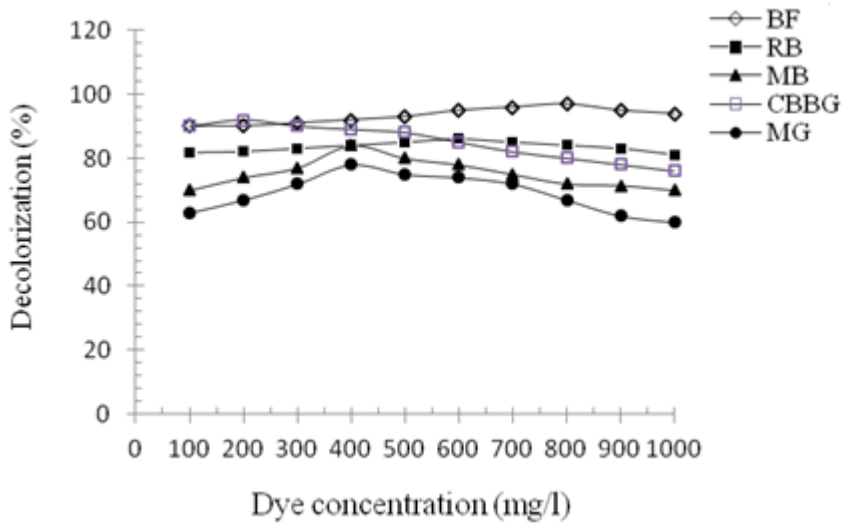


Fig. 5. Effect of dye concentration on its decolorization by peroxidase of *Bacillus* sp. F31

between 1.0 to 1.5 mM with a decolorization of 97.1 % for BF at 40°C in 35 min, 86.2 % for RB at 30°C in 40 min, 84.1 % for MB at 35°C in 30 min, 92.3 % for CBBG at 40°C in 40 min and 78.1 % for MG at 40°C in 40 min, respectively (Fig. 6).

Effect of salts and inhibitors on dye decolorization

The decolorization (%) of BF, RB, MB, CBBG and MG with bacterial peroxidase was determined in the presence of selected salt ions and inhibitors under optimized conditions. The results indicated that the decolorization of all the five dyes by purified peroxidase was inhibited by the presence of Hg^{+2}, EDTA, sodium azide, DTT and SDS. However, the decolorization was found to be slightly stimulated by peroxidase in the presence of Zn^{+2} (BF 100.5 %, RB 101.2 %, MB 101.1 %, CBBG 101.1 % and MG 101.2 %), Mg^{+2} (BF 101.6 %, RB 101.2 %, MB 100.5 %, CBBG 101.6 % and MG 102.5 %) and Mn^{+2} (RB 100.6 % and MG 100.2 %), respectively (Table 3).

Discussion

An extracellular peroxidase purified from a *Bacillus* sp. F31was successfully used in the decolorization of a few selected dyes such as BF, RB, MB, CBBG and MG. Peroxidase from *Bacillus* sp. F31 decolorized four triphenyl methane dyes and one polymeric heterocyclic efficiently so it could be concluded that the peroxidase of *Bacillus* sp. F31 has higher affinity for triphenyl methane dyes than the other ones. In a previous study, the peroxidase from *Hevea brasiliensis* was able to decolorize triphenyl methane dye efficiently as compared to other groups of synthetic dyes [3]. The decolourization of dyes by an enzyme depends upon many factors such as their chemical structure, molecular mass [33], redox potential, complexity of side chains and most important the binding site of enzyme. On the basis of structure and molecular weight the BF having simple structure with small functional groups (NH_2; side chains) and relatively a lower molecular mass (337.8 g/mol) was efficiently decolourized as compare to other dyes bearing more complex side chains accounting for stearic hindrance in binding with enzyme. This can be possibly the reason for greater decolourization of the BF dye by the peroxidase of *Bacillus* sp. F31. In the previous studies, peroxidases purified from diverse sources have been shown to decolorize different industrial dyes [34,35,36]. The manganese peroxidase purified from *Dichomitus squalens* was also able to decolorize selected azo and anthraquinone dyes [37]. The manganese-independent peroxidase sourced from *Auricularia uricular-judae* has been found to be stable in decolorization of the high-redox potential dyes Reactive Blue 5 and Reactive Black 5 [38] and one other bacterial strain *Ganoderma cupreum* AG-

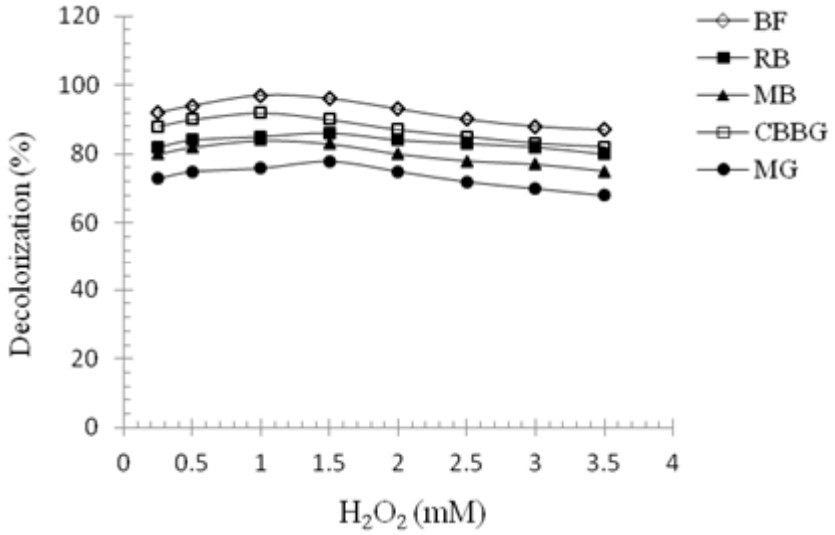


Fig. 6. Effect of H_2O_2 concentration on dye decolorization by peroxidase of *Bacillus* sp. F31

Table 3. Effect of metal ions and inhibitors on decolourization of dyes by peroxidase of *Bacillus* sp. F31

Metal ion/ Inhibitor (1 mM)	Relative decolourization (%) at stated λ_{max}				
	BF(A_{545})	RB (A_{555})	MB (A_{664})	CBBG (A_{610})	MG (A_{550})
None	100.0	100.0	100.0	100.0	100.0
Li^{+3}	97.8	88.4	97.6	98.5	95.0
Zn^{+2}	100.5	101.2	101.1	101.1	101.2
Mg^{+2}	101.6	101.2	100.5	101.6	102.5
K^{+2}	98.9	98.7	98.8	95.5	92.5
Na^{+}	97.8	80.7	84.1	93.3	87.5
Hg^{+2}	63.8	62.8	47.0	38.8	31.2
Ca^{+2}	94.6	98.7	96.4	94.4	88.7
Cu^{+2}	96.8	96.1	97.6	97.7	93.7
Fe^{+2}	95.7	93.5	98.8	96.6	96.2
Mn^{+2}	100.0	100.6	99.4	99.3	100.2
EDTA	88.9	80.9	78.9	74.5	72.0
SDS	62.5	56.5	60.7	58.4	54.2
Sodium azide	45.8	42.3	35.7	50.4	41.5
DTT	58.9	64.5	62.1	60.4	67.0

1 was isolated from the decayed wood was evaluated for its ability to decolorize azo dyes [39].

The optimum temperature for each of these dyes with purified peroxidase of *Bacillus* sp. F31 was 30°C for RB (72.1 %), 35°C (82.0 %) for MB; and 40°C for BF (92.1 %), CBBG (90.2 %) and MG (65.2 %), respectively. The observed data showed that *Bacillus* sp. F31 peroxidase performed efficient decolourization of chosen common textile dyes at 30-40°C. In previous studies for the decolourization of MG, a constant temperature of 25±0.5°C [40] and 30°C by the peroxidase of a fungal strain *Cunninghamella elegans* was required [41]. The peroxidase from *Pleurotus ostreatus* also decolorized triphenyl methane dyes (BPB and MB) at 25°C [42]. BF was 93% decolorized at 30°C by a peroxidase from *Aeromonas hydrophila* [43]. The optimum temperature for decolorization of different dyes by peroxidase from *Trametes versicolor* was found to be 30°C [13]. The peroxidase of *Bacillus* sp. F31 provided optimal decolorization between 30-45 min for MB (82.1 %, 30 min) at 35°C, BF (96.1 %, 35 min) at 40°C, RB (76.2%, 40 min) at 30°C, CBBG (90.0 %, 40 min) and MG (78.3 %, 40 min) at 40°C, respectively. In a previous study, although the decolorization increased with the extension of time yet the increase in decolorization was not significant after 40 min for MB decolourization [40]. In another study, an increase in the Soya bean peroxidase from 10 U/ml to 80 U/ml resulted in a gradual increase in the dye removal (16-64 %) that appeared to be levelling off at 80 U/ml [44].

The intact enzyme may contain both positively and negatively charged groups at any given pH. Such ionizable groups are often part of the active site [45]. Variation in the pH of the medium can result in changes in both the ionic forms of the active site and the activity of enzyme and consequently, the reaction rate [46,47,48]. It was observed that most of the dyes like MG decolorize at strong to moderate acidic (2.0-6.0) pH values [49]. In the initial step, the formation of compound I is favoured by the presence of a network of hydrogen bonds between the Fe-heme/ H_2O_2 adduct and the distal histidine and arginine side chains, whereas, in the subsequent steps, the substrate oxidation may depends on its protonation state [55].

In the present study, the maximum decolorization of BF was found to be 97.1 % at concentration

800 mg/l at 40°C in 35 min at pH 5.5, for RB (86.2 %) at 600 mg/l at 30°C in 40 min at pH 5.0, MB (84.2 %) at 35°C in 30 min at pH 5.5 and MG (78.1 %) at 400 mg/l at 40°C in 40 min at pH 5.5 and CBBG (92.1 %) at 40°C in 40 min with 200 mg/l at pH 6.0. It happened possibly due to the reason that if the concentration of enzyme is kept constant and the substrate concentration is gradually increased the reaction will increase until it reaches maximum. The dye concentration in effluent from textile printing house is approximately 200-800 mg/l [50]. The decolourization efficiency often decreased with increasing dye concentration and a marked inhibition effect was exhibited when the dye (Remazol Brilliant Blue R) concentrations were above 100 mg/l [51]. As reported that in study with FTIR spectroscopy, NMR and GC-MS of several dye degradation products from purified peroxidase by *Bacillus cereus*, the results confirmed that decolorization was due to breakdown of dyes into unknown products [52].

The decolourization of BF, RB, MB, CBBG and MG with peroxidase of *Bacillus* sp. F31 was determined in the presence of selected salt ions and inhibitors under optimized conditions. The results indicated that the decolorization of all the five dyes by purified peroxidase was inhibited in the presence of Hg^{+2}, EDTA, sodiun azide, DTT and SDS. Some bivalent metal ions such as Mg^{+2}, Zn^{+2} and Co^{+2} enhanced peroxidase activity so these ions could be used in dye decolorization experiments as additives for efficient decolorization [14,53,54]. In another study, Mg^{+2} and Mn^{+2} (1 mM) ions were observed to significantly enhance the decolorization of MG by peroxidase from *Pseudomonas* sp [4]. H_2O_2 reacts with the peroxidase to oxidize the native enzyme to form an enzyme intermediate, which easily accepts an aromatic compound to carry out its oxidation to a free radical form. In this regard, experiments were done wherein the decolouration of the selected (BF, RB, MB, CBBG and MG) textile dyes was measured as a function of H_2O_2 concentration, while keeping the other reaction parameters constant. When the concentration of H_2O_2 was varied from 0.25 to 3.5 mM in dye-decolorization assay, the optimized concentration of H_2O_2 for decolorization of these dyes using purified peroxidase was found to be between 1 mM to 1.5 mM with a decolorization of 97.1 % for BF at 40°C in 35 min, 86.2% for RB at 30°C in 40 min, 84.1 % for MB at 35°C in 30 min, 92.3 % for CBBG at 40°C in 40 min and 78.1 % for MG at 40°C in 40 min, respectively. H_2O_2 alone or in conjunction with other materials, is used for oxidation and degradation/ decolorization of many harmful organic compounds including dyes. The addition of a small amount of catalyst to a system containing H_2O_2 may lead to the generation of free radicals like •OH with a reasonably high reduction potential (2.3 eV) that facilitates faster degradation of many organic compounds. On the contrary, higher H_2O_2 was detrimental to the process, most likely due to the damage to the enzyme itself. Thus it becomes pertinent to optimize the H_2O_2 concentrations in the enzyme-based dye degradation approaches Zhang *et al.*, [47]. For the decolorization of MB by HRP, 0.15 mM H_2O_2 concentration in the reaction mixture was found to be the optimum [40]. In case of dyes degradation with soya bean peroxidase, H_2O_2 concentration led to increased dye decolorization.

However, after reaching the maximum dye decolourization with 64 mM H_2O_2, further increase in H_2O_2 did not cause any additional effect [13,35,36,55,56]. The MnP purified from *Dichomitus squalens* was also able to decolorize selected azo and anthraquinone dyes [37]. A thermostable peroxidase from *Bacillus stearothermophilus* and peroxidase sourced from *Auricularia auricula-judae* has been found to be stable in decolourization of the high-redox potential dyes such as Reactive Blue 5 and Reactive Black 5 [38,57]. *Ganoderma cupreum* AG-1 recently isolated from the decayed wood was evaluated for its ability to decolorize azo dyes [58]. Thus bacterial peroxidase(s) have emerged as efficient biological tools for the decolourization of most industrial dyes [18,49].

Conclusion

BF, RB and MG are triphenyl methane type of basic dyes used in textile, pharmaceutical and chemical industries; while RB is extensively used in textile industries for dying nylon, wool, silk and cotton. The extracellular peroxidase produced by

Bacillus sp. F31 may be adopted as an effective biological eco-friendly tool to decolorize most of the common textile dyes.

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