Available online at www.sciencedirect.com
ScienceDirect
Journal of Environmental Sciences 2011, 23(7) 1135–1142
JOURNAL OF ENVIRONMENTAL SCIENCES
ISSN 1001-0742
CN 11-2629/X
www.jesc.ac.cn

Catalyzed degradation of disperse dyes by calcium alginate-pectin entrapped bitter gourd (*Momordica charantia*) peroxidase

Rukhsana Satar[1], Qayyum Husain[2,*]

1. Department of Biochemistry, Faculty of Life Sciences, Aligarh Muslim University, Aligarh 202002, UP, India
2. Faculty of Applied Medical Sciences, Jazan University, Jazan, Post Box 2092, Kingdom of Saudi Arabia

Received 24 July 2010; revised 28 February 2011; accepted 04 March 2011

Abstract

Calcium-alginate pectin entrapped bitter gourd peroxidase (BGP) has been employed for the treatment of disperse dyes: Disperse Brown 1 (DB 1) and Disperse Red 17 (DR 17). Peroxidase alone was unable to decolorize DR 17 and DB 1. However, the investigated dyes were decolorized maximally by BGP in the presence of 0.2 mmol/L redox mediator, violuric acid (VA). A slow decrease in percent decolorization was observed when VA concentration was higher than 0.2 mmol/L which could likely be due to the high reactivity of its aminoxyl radical (> N–O$^{\bullet}$) intermediate, that might undergo chemical reactions with aromatic amino acid side chains of the enzyme thereby inactivating it. Maximum decolorization of the dyes was observed at pH 3.0 and 40°C within 2 hr of incubation. Immobilized peroxidase decolorized 98% DR 17 and 71% DB 1 using 35 U of BGP in batch process in 90 min. Immobilized enzyme decolorized 85% DR 17 and 51% DB 1 whereas soluble enzyme decolorized DR 17 to 48% and DB 1 to 30% at 60°C. UV-visible spectral analysis was used to evaluate the degradation of these dyes and their toxicity was tested by *Allium cepa* test. The generally observed higher stability of the bioaffinity bound enzymes against various forms of inactivation may be related to the specific and strong binding of enzyme with bioaffinity support which prevents the unfolding/denaturation of enzyme. Thus entrapped peroxidase was found to be effective in the decolorization of the investigated dyes.

Key words: bitter gourd peroxidise (BGP); alginate; pectin; decolorization; disperse dyes

DOI: 10.1016/S1001-0742(10)60525-6

Citation: Satar R, Husain Q, 2011. Catalyzed degradation of disperse dyes by calcium alginate-pectin entrapped bitter gourd (*Momordica charantia*) peroxidise. Journal of Environmental Sciences, 23(7): 1135–1142

Introduction

Azo dyes represent the largest and most versatile group of dyes, which occupy 70% of all dyestuff consumed (McMullan et al., 2001). The impact of dyes discharged into environment is of great concern. They cause water bodies to become colored, which in turn interferes with photosynthesis and aquatic ecosystem (Khenifi et al., 2007). Moreover, a wide range of textile dye toxicity has been reported, which might cause both chronic and acute toxicity. The presence of these dyes in the aqueous ecosystem is thus, the cause of serious environmental and health concerns (Asad et al., 2007).

Enzymes catalyze reactions with high specificity and efficiency. In addition, due to mild operating conditions of enzymatic processes, they can be performed in relatively simple equipment, short time and are easy to control (Fernandez-Sanchez et al., 2002; Husain, 2006). The capability of oxidoreductive enzymes to degrade chromophore substrates such as triarylmethane, indigoid, azo and anthraquinonic dyes suggests their potential application in textile dye bleaching processes (Abadullah et al., 2000; Husain and Husain, 2008). However, these processes have been hindered due to unfavorable kinetics between the enzyme and dye. The use of small molecules capable to act as electron transfer mediators between the enzyme and dye has been regarded as a feasible solution to this drawback (Matto et al., 2009). The feasibility of these molecules as effective electron donors is governed by the redox potential difference between the enzyme and mediator, which strongly depends on the pH at which the reaction takes place (Fernandez-Sanchez et al., 2002). The catalytic activity of the enzyme has also been regarded to be strongly dependent upon the interaction between a specific enzyme and the redox mediator (Soares et al., 2001; Husain and Husain, 2008).

The widespread industrial application of enzymes is often hampered by their lack of long term operational stability and shelf-storage life, their cumbersome recovery and re-use. These drawbacks can generally be overcome by the immobilization of enzymes and a major challenge in industrial biocatalysis is the development of stable,

* Corresponding author. E-mail: qayyumhusain@amu.ac.in; qayyumbiochem@gmail.com

robust and preferably insoluble biocatalysts. The entrapment of pre-immobilized enzymes prevented the leakage of enzymes from polymeric matrices (Matto and Husain, 2006; Satar et al., 2008). Moreover, immobilization inside the pores of the support could reduce any negative interaction between enzyme and the sol-gel matrix (Betancor et al., 2005). Enzyme immobilization may also improve enzyme stability by multipoint or multi-subunit attachment (Mateo et al., 2007).

In this study, calcium alginate-pectin entrapped concanavalin A (Con A)-bitter gourd peroxidase (BGP) was used for the remediation of water polluted with azo disperse dyes. The effects of pH, heat, time, and concentrations of violuric acid (VLA) and enzyme on the decolorization of such dyes have been optimized. The operational stability of soluble and entrapped BGP for treatment of dyes has been compared in batch processes at different temperatures and with varying enzyme concentrations.

1 Materials and methods

1.1 Materials

Disperse dyes: Disperse Brown 1 (DB 1) and Disperse Red 17 (DR 17) were the gift from Atul Chem. Co. (India). *o*-Dianisidine HCl were obtained from Sigma Chemical Co. (USA). Ammonium sulphate, VLA and Tween 20 were obtained from SRL Chemicals (Mumbai, India). Bitter gourd was purchased from local vegetable market. Other chemicals and reagents employed were of analytical grade and were used without any further purification.

1.2 Ammonium sulphate fractionation of bitter gourd proteins and preparation of Con A-BGP complex via entrapment into calcium alginate-pectin beads

Bitter gourd (250 g) was homogenized in 500 mL of 0.1 mol/L sodium acetate buffer, pH 5.5 (Akhtar et al., 2005).

Con A-BGP complex (1350 U) was prepared using sodium alginate (2.5%) and pectin (2.5%) (Satar et al., 2008; Matto and Husain, 2006). Beads were then washed and stored in 0.1 mol/L sodium acetate buffer, pH 5.5 at 4°C for further use.

1.3 Preparation of synthetic dye solutions and procedure for dye treatment

DR 17 and DB 1 were solubilized using 0.025% (*V*/*V*) Tween 20 prepared in 0.1 mol/L glycine HCl buffer, pH 3.0 (Satar and Husain, 2009a).

DR 17 (25 mg/L, 5.0 mL) and DB 1 (50 mg/L, 5.0 mL) were independently incubated with BGP (0.15 U/mL) in 0.1 mol/L glycine HCl buffer, pH 3.0 in the presence of 0.75 mmol/L H_2O_2 for indicated times at 40°C. Redox mediator, VLA, was added at concentrations specified in each section. Dye decolorization was determined spectrophotometrically as described by Satar and Husain (2009a).

1.4 Effect of varying concentrations of VLA on BGP catalyzed dye decolorization

Each dye (5.0 mL) was incubated independently with soluble and immobilized BGP (0.15 U/mL) in the presence of varying concentrations of VLA (0.1–0.6 mmol/L) and 0.75 mmol/L H_2O_2 in 0.1 mol/L glycine HCl buffer, pH 3.0 for 2 hr at 40°C. The reaction was stopped and the intensity of color was recorded as described earlier.

1.5 Effect of pH and time on BGP catalyzed dye decolorization

Dye solutions were prepared in the buffers of different pH (3.0–10.0). The buffers used were glycine-HCl (pH 2.0 and 3.0), sodium acetate (pH 4.0 and 5.0), sodium phosphate (pH 6.0–8.0) and Tris-HCl (pH 9.0 and 10.0). The molarity of each buffer was 0.1 mol/L. Each dye (5.0 mL) was treated independently with soluble and immobilized BGP (0.15 U/mL) in the buffers of various pH (3.0–10.0) in the presence of 0.75 mmol/L H_2O_2 and 0.2 mmol/L VLA for 2 hr at 40°C. Dye decolorization was monitored at the respective λ_{max} of each dye: 510 nm and 460 nm for DR 17 and DB 1, respectively. The percent decolorization was calculated by taking untreated dye solution in each buffer as control and activity at pH 3.0 as 100%.

Each dye solution (5.0 mL) was treated independently with soluble and immobilized BGP (0.15 U/mL) in the presence of 0.75 mmol/L H_2O_2 and 0.2 mmol/L VLA in 0.1 mol/L glycine HCl buffer, pH 3.0 at 40°C for varying time intervals. The reaction was stopped by keeping in a boiling water bath for 5 min.

1.6 Effect of temperature and enzyme concentration on treatment of dyes in batch process

In order to check the stability of BGP at higher temperatures, decolorization of the dyes by soluble and immobilized BGP was monitored in stirred batch processes at 40, 50 and 60°C for 4 hr. Dye solutions (250 mL each) were treated independently with soluble and immobilized BGP (35 U) in the presence of 0.75 mmol/L H_2O_2 and 0.2 mmol/L VLA for varying times under stirring conditions. Aliquots from the treated dyes were taken at indicated time intervals for the measurement of remaining color.

In subsequent experiments, decolorization of the dyes was monitored in stirred batch processes in the presence of 25 U and 15 U of soluble and immobilized BGP independently at 40°C. Treatment of dye solutions (250 mL each) was carried out as mentioned above.

1.7 Dye decolorization reusability of immobilized BGP and storage stability of soluble and immobilized BGP

Each dye (5.0 mL) was incubated with immobilized BGP (0.15 U/mL) in the presence of 0.75 mmol/L H_2O_2 and 0.2 mmol/L VLA in 0.1 mol/L glycine HCl buffer, pH 3.0 for 2 hr at 40°C. After the reaction, enzyme was separated by centrifugation and stored in assay buffer for over 12 hr at 4°C. The similar dye decolorization experiment was repeated seven times with the same preparation of immobilized BGP and each time with a fresh batch of dye solutions. Dye decolorization was monitored at the respective λ_{max} of each dye. The percent decolorization

was calculated by taking untreated dye solution as control (100%).

Soluble and immobilized BGP were incubated in 0.1 mol/L glycine-HCl buffer, pH 3.0 at 4°C for 30 days. Appropriate aliquots from each BGP preparation were taken out in triplicates at the gap of 5 days and were analyzed for remaining enzyme activity. The enzyme activity measured on first day was considered as control (100%) for the calculation of remaining storage activity.

1.8 *Allium cepa* test for BGP treated dyes

The *Allium cepa* bioassay for the dye samples was carried out according to the method described by Fiskesjo (1985). Boiling tubes were filled with undiluted and 1:1 diluted control and treated dye samples consisting of BGP (0.15 U/mL), 0.75 mmol/L H_2O_2 and 0.2 mmol/L VLA in 0.1 mmol/L glycine HCl buffer, pH 3.0. All the experiments were performed in dark for 7 days and distilled water was used as control in all experiments (Satar and Husain, 2009b). Inhibition in the growth of *Allium cepa* roots was considered as an index for the degree of toxicity.

1.9 UV-Visible spectral analysis of dyes, measurement of peroxidase activity and statistical analysis

Spectra for treated and control dye samples were recorded on UV-Visible spectrophotometer (UV mini 1240, Shimadzu, Japan). Dye decolorization of the treated and control samples were determined by monitoring the absorbance in UV-Visible range.

Peroxidase activity and statistical analysis was performed as described by Satar and Husain (2009a).

2 Results and discussion

2.1 Effect of varying concentration of VLA on BGP catalyzed dye decolorization

Dyes were decolorized maximally in the presence of 0.2 mmol/L VLA (Fig. 1a). However, the concentrations of VLA above 0.2 mmol/L resulted in gradual decrease in BGP catalyzed dye decolorization. At all the indicated concentrations of VLA, entrapped BGP decolorized dyes more efficiently as compared to its soluble counterpart. A reason for this could be the rigidity provided by the

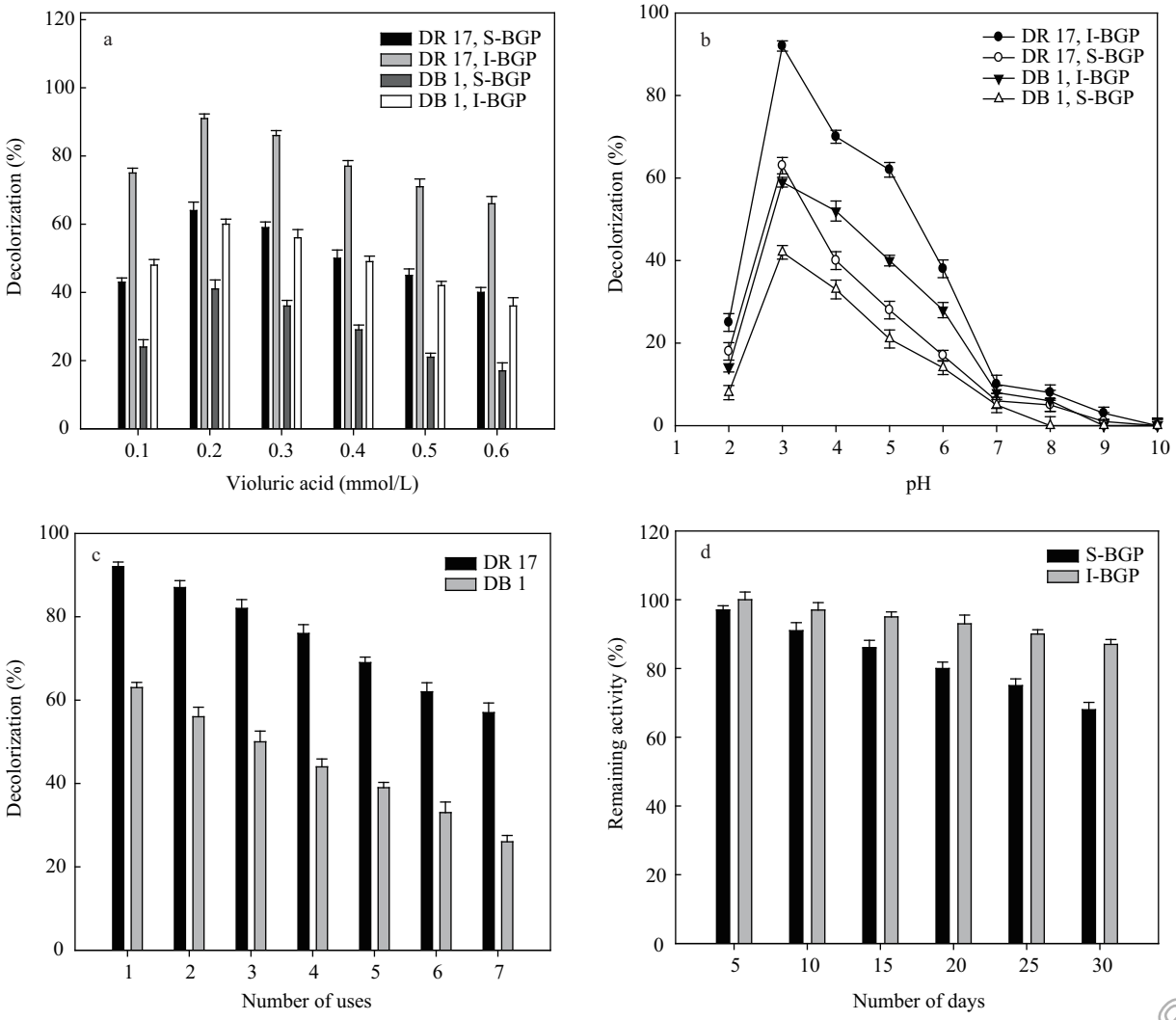

Fig. 1 Effect of VLA concentration (a) and pH (b) on BGP catalyzed decolorization; (c) decolorization reusability of immobilized BGP; and (d) storage stability of soluble BGP (S-BGP) and immobilized BGP (I-BGP).

support via bond formation between the enzyme and the matrix or lower restriction to substrate diffusion in case of immobilized BGP. Several reports are available where the use of redox mediator-enzyme system enhanced dye decolorization to several folds. However, these redox mediators were required in very high concentrations: 5.7 mmol/L VA/laccase system, 11.0 mmol/L of HOBT/laccase system, 2.0 mmol/L HOBT/laccase system, 2.0 mmol/L HOBT/turnip peroxidase system (Soares et al., 2001; Claus et al., 2002; Kulshrestha and Husain, 2007). Such high concentrations may be suitable for some processes, particularly in closed-loop systems where the mediator is retained. However, high concentrations may not be appropriate for applications, such as wastewater treatment processes where important considerations include the potentially prohibitive costs of mediators and the possibility of creating negative impacts on effluent toxicity (Kim and Nicel, 2006). In this study, very low concentration of VLA, 0.2 mmol/L, has been used to enhance the rate of BGP-catalyzed dye decolorization.

2.2 Effect of pH and time on BGP catalyzed dye decolorization

Enzymes have a characteristic pH at which they show maximum activity, the pH optimum being dependent on the nature of substrate used (Fernandez-Sanchez et al., 2002; Ghodake et al., 2009). Results revealed that BGP could decolorize higher level of dyes in acidic pH range 3.0–6.0 (Fig. 1b). However, the dyes were maximally decolorized at pH 3.0. DR 17 was decolorized to an extent of 63% and 92% by soluble and immobilized BGP, while DB 1 was decolorized 42% and 59%, by these BGP preparations, respectively. Thus, the investigated dyes were decolorized to greater extent by immobilized BGP. This implies that immobilized BGP has a higher stability against the pH variation owing to the protection of enzyme by immobilization. However, it has been demonstrated that entrapment of enzymes in gel beads provides a microenvironment of the enzyme, which plays an important role in the state of protonation of the protein molecules (Mattoo and Husain, 2006, Matto et al., 2009). The extent of decolorization was remarkably decreased as pH of treated samples increased above 6.0. This observation was in agreement with earlier studies where peroxidase could decolorize and degrade dyes maximally at acidic pH (Akhtar et al., 2005; Ghodake et al., 2009; Satar and Husain, 2009a). Above and below this pH, decolorization was found to be decreased. Reports are available where decolorization of azo dyes/real wastewaters was maximum at acidic pH and the dye removal dropped significantly from pH 6 to 10 (Maddhinni et al., 2006; Dayaram and Dasgupta, 2008; Matto et al., 2009).

Table 1 shows the effect of time on the decolorization of DR 17 and DB 1. Maximum decolorization of the dyes was observed within 120 min of incubation. DR 17 and DB 1 were decolorized 60% and 41% by soluble BGP while entrapped BGP decolorized DR 17 and DB 1 more efficiently to 90% and 59% within 120 min, respectively. However, there was no effective increase in dye decolorization on further incubation for longer times. This could be due to the end product inhibition which is more in case of soluble enzymes. Sufficient amount of DR 17 was decolorized within 30 min while the decolorization of DB 1 was slow. It has been reported that decolorization rate varies, depending upon the type of dye to be treated (Camarero et al., 2005). Further, it was observed that an incubation period of 60 min was sufficient for maximum decolorization of dyes in batch process by soluble BGP and no further increase in decolorization was observed on incubation for longer periods. This could be due to end product inhibition of soluble BGP. This observation was in accordance with the findings reported earlier, however, immobilized BGP was able to decolorize dyes more efficiently as compared to soluble BGP (Satar and Husain, 2009a).

2.3 Effect of temperature and enzyme concentration on treatment of disperse dyes in batch processes

Treatment of dyes by BGP in stirred batch processes at different temperatures showed a significant loss of color from dye solutions (Table 2). Dyes were decolorized maximally at 40°C in batch process in the presence of 35 U of soluble and immobilized BGP. However, at 50°C and 60°C percent decolorization decreased gradually. The rate of decolorization depends upon enzyme activity which in turn depends upon the reaction conditions. BGP shows its maximum activity at around 40°C, above this temperature activity decreases and this leads to a considerable decrease in dye decolorization. However, at any indicated temperature, decolorization by immobilized BGP was significantly higher as compared the soluble enzyme. This improvement in the thermal stability of calcium alginate-pectin entrapped BGP may be attributed to multipoint attachment of the enzyme with the matrix which provides enhanced

Table 1 Effect of time on BGP catalyzed decolorization

Time (min)	Decolorization (%)			
	DR 17		DB 1	
	S-BGP	I-BGP	S-BGP	I-BGP
15	30.09 ± 2.24	57.14 ± 1.67	16.10 ± 1.34	28.12 ± 1.58
30	39.11 ± 1.35	66.08 ± 1.23	21.11 ± 2.14	36.15 ± 1.62
60	45.13 ± 2.15	76.10 ± 1.76	27.12 ± 1.98	41.08 ± 2.65
90	51.12 ± 2.46	83.07 ± 2.14	35.14 ± 1.64	50.10 ± 1.86
120	60.15 ± 1.68	90.09 ± 1.49	41.14 ± 2.38	59.12 ± 1.34
150	60.14 ± 1.47	90.10 ± 2.25	41.10 ± 1.55	60.09 ± 2.78

Each dye solution (5.0 mL) was treated independently with soluble and immobilized BGP (0.15 U/mL) in the presence of 0.75 mmol/L H_2O_2 and 0.2 mmol/L VLA in 0.1 mol/L glycine HCl buffer, pH 3.0 at 40°C for varying time.

Table 2 Effect of different temperatures on BGP catalyzed decolorization (%) in batch process

Time (min)	40°C				50°C			
	DR 17		DB 1		DR 17		DB 1	
	S-BGP	I-BGP	S-BGP	I-BGP	S-BGP	I-BGP	S-BGP	I-BGP
15	46.11 ± 2.82	78.08 ± 1.57	33.09 ± 2.34	50.11 ± 0.97	40.14 ± 2.32	74.08 ± 1.26	25.11 ± 2.18	41.15 ± 2.89
30	54.09 ± 2.86	87.14 ± 1.34	41.11 ± 1.73	59.08 ± 1.82	46.15 ± 2.78	81.11 ± 1.89	29.13 ± 1.56	50.14 ± 2.14
60	73.08 ± 2.64	95.13 ± 1.85	50.12 ± 2.42	64.15 ± 2.89	58.08 ± 1.57	90.10 ± 1.57	38.10 ± 1.56	56.10 ± 2.16
90	73.12 ± 2.34	98.09 ± 1.43	50.10 ± 1.68	71.13 ± 2.46	58.12 ± 2.41	94.12 ± 2.12	38.12 ± 1.78	60.10 ± 1.8
120	73.11 ± 1.89	98.07 ± 1.36	50.14 ± 2.14	71.10 ± 1.73	59.14 ± 1.26	94.15 ± 2.53	38.12 ± 2.89	60.14 ± 2.14
150	73.15 ± 2.18	98.13 ± 1.82	50.09 ± 1.36	71.08 ± 1.68	59.12 ± 2.78	94.15 ± 2.53	38.15 ± 2.43	60.08 ± 1.89
180	73.07 ± 1.85	98.12 ± 1.89	50.08 ± 2.42	71.12 ± 2.89	59.10 ± 1.89	94.12 ± 2.41	38.08 ± 2.43	60.10 ± 2.16
210	73.09 ± 1.57	98.12 ± 2.86	50.12 ± 2.46	71.12 ± 0.97	59.08 ± 2.67	94.09 ± 2.12	38.11 ± 1.78	60.11 ± 1.84
240	73.12 ± 1.43	98.09 ± 2.64	50.11 ± 2.67	71.09 ± 1.11	59.11 ± 2.65	94.14 ± 2.32	38.11 ± 2.67	60.12 ± 1.84

Time (min)	60°C			
	DR 17		DB 1	
	S-BGP	I-BGP	S-BGP	I-BGP
15	34.10 ± 0.95	70.09 ± 1.48	18.12 ± 1.67	34.12 ± 2.16
30	41.13 ± 2.12	74.5 ± 2.83	23.12 ± 1.68	38.10 ± 2.22
60	48.08 ± 1.45	79.12 ± 2.14	30.15 ± 2.81	45.13 ± 2.16
90	48.10 ± 1.98	85.11 ± 2.12	30.09 ± 1.43	51.12 ± 2.14
120	48.12 ± 1.21	85.14 ± 1.56	30.08 ± 1.12	51.13 ± 2.22
150	48.10 ± 2.12	86.13 ± 1.32	30.10 ± 1.68	51.09 ± 2.12
180	48.08 ± 1.45	85.11 ± 2.14	30.13 ± 2.81	51.10 ± 0.95
210	49.00 ± 1.98	85.14 ± 2.14	30.11 ± 1.43	51.12 ± 1.12
240	48.15 ± 1.21	85.08 ± 1.32	30.09 ± 2.14	51.15 ± 1.56

Dye solutions DR 17 (250 mL) and DB 1 (250 mL) were treated with soluble and immobilized BGP (35 U) in the presence of 0.75 mmol/L H_2O_2 and 0.2 mmol/L VLA in 0.1 mol/L glycine HCl buffer, pH 3.0 for 4 hr at 40°C under stirring conditions. Aliquots of dye solutions were taken at indicated time intervals to monitor decolorization. The percent decolorization was calculated by taking untreated dye solution at each temperature as control (100%).

resistance to the immobilized enzyme (Matto et al., 2009). Decolorization of dyes by soluble BGP at the indicated temperatures increased continuously with time only till 60 min while decolorization of the dyes by entrapped BGP was continuously increased up to 90 min at all the three investigated temperatures. Incubation of dye solutions with immobilized BGP beyond 120 min did not show any further enhancement in decolorization at the indicated temperatures. Lack of improvement in dye decolorization with further increase in incubation time might be attributed to the end product inhibition which was found to be more in case of soluble enzyme. The disappearance of 73% and 50% of color from DR 17 and DB 1 by soluble BGP was observed within 60 min of incubation at 40°C, respectively, while DR 17 and DB 1 were decolorized to 98% and 71% by entrapped BGP in 90 min at 40°C. Decolorization of DR 17 and DB 1 was maximum 58% and 38% in 60 min by soluble BGP at 50°C, whereas maximum decolorization by entrapped BGP was 94% for DR 17 and 60% for DB 1 within 90 min incubation time. However, DR 17 and DB 1 were decolorized to 48% and 30% by soluble BGP in 60 min at 60°C, respectively, whereas decolorization by entrapped BGP was 85% for DR 17 and 51% for DB 1 in 90 min at this temperature.

Table 3 demonstrates the effect of varying concentration of soluble and immobilized BGP on the decolorization of DR 17 and DB 1 at different times of incubation in batch process. Decolorization of the dyes in batch process decreased by decreasing the concentration of enzyme. DR 17 and DB 1 were decolorized to 64% and 40% by soluble BGP in 60 min in the presence of 25 U whereas maximum decolorization by immobilized BGP was 93% for DR 17 and 60% for DB1 in 90 min. In presence of 15 U, soluble BGP could decolorize 50% DR 17 and 29% DB 1 in 90 min. However, DR 17 and DB 1 were decolorized to an extent of 80% and 48%, respectively, by entrapped BGP

Table 3 Effect of enzyme concentration on BGP catalyzed decolorization (%) in batch process

Time (min)	25 U of BGP				15 U of BGP			
	DR 17		DB 1		DR 17		DB 1	
	S-BGP	I-BGP	S-BGP	I-BGP	S-BGP	I-BGP	S-BGP	I-BGP
15	39.12 ± 1.34	70.11 ± 2.82	23.14 ± 2.14	42.09 ± 1.11	31.10 ± 2.67	60.11 ± 1.8	16.12 ± 2.65	34.15 ± 1.89
30	46.09 ± 2.18	79.12 ± 2.67	33.12 ± 1.36	50.15 ± 1.82	36.14 ± 2.43	68.11 ± 2.78	24.13 ± 1.89	41.09 ± 2.34
60	64.11 ± 2.64	88.09 ± 1.85	40.15 ± 2.42	54.08 ± 2.89	50.12 ± 1.56	79.12 ± 1.57	29.11 ± 2.32	46.14 ± 2.16
90	64.11 ± 1.57	93.15 ± 1.43	41.11 ± 1.68	60.10 ± 2.46	52.11 ± 1.78	80.10 ± 2.41	30.10 ± 2.12	48.11 ± 1.84
120	64.13 ± 1.89	93.12 ± 2.86	41.09 ± 0.97	60.11 ± 1.73	52.11 ± 2.89	81.09 ± 1.26	30.11 ± 2.53	49.10 ± 2.14
150	64.12 ± 2.18	93.15 ± 2.67	41.12 ± 1.36	60.12 ± 1.82	52.08 ± 2.43	81.12 ± 2.78	30.14 ± 1.89	49.10 ± 2.34
180	64.15 ± 2.64	93.11 ± 1.85	41.11 ± 2.42	60.12 ± 2.89	52.11 ± 1.56	81.11 ± 1.57	30.14 ± 2.32	49.11 ± 2.16
210	64.11 ± 1.57	93.11 ± 1.43	41.11 ± 1.68	60.14 ± 2.46	52.10 ± 1.78	81.15 ± 2.41	30.13 ± 2.12	49.12 ± 1.84
240	64.10 ± 1.89	93.12 ± 2.86	41.10 ± 0.97	60.12 ± 1.73	52.12 ± 2.89	81.14 ± 1.26	31.11 ± 2.53	49.11 ± 2.14

Dye solutions DR 17 (250 mL) and DB 1 (250 mL) were independently treated with 25 and 15 U of soluble and immobilized BGP in the presence of 0.2 mmol/L VLA and 0.75 mmol/L H_2O_2 in 0.1 mol/L glycine HCl buffer, pH 3.0 for 4 hr at 40°C.

in 90 min. There was no significant enhancement in the decolorization of these dyes after 60 and 90 min by soluble and immobilized BGP, respectively. The optimization of enzyme concentration was carried out to achieve high dye decolorization efficiency. A very low concentration of BGP (0.15 U/mL) was sufficient for maximum decolorization of dyes, indicating high potential of plant peroxidases in the treatment of industrial wastewaters. Increase in enzyme concentration above this value showed no significant increase in decolorization (data not shown). In an earlier investigation, a similar observation has been reported where two-fold increase in concentration of HRP beyond 14.985 U/mL showed no significant increase in decolorization of Remazol Turquoise Blue G 133%. In order to check the efficiency of BGP in batch process, decolorization was carried out in the presence of 25 U and 15 U. However, in batch process, 35 U of BGP resulted in maximum decolorization (data in Table 2 at 40°C), enzyme concentration more than this value did not show any increase in decolorization (data not given). Enzyme concentration less than this showed decreased decolorization (Table 3). On the basis of such findings, it can be concluded that optimization of enzyme concentration is of great importance because higher enzyme concentrations have been reported to show no significant influence on dye decolorization (Ulson de Souza et al., 2007).

2.4 Decolorization reusability of immobilized BGP and storage stability of soluble and immobilized BGP

Reusability is one of the significant indices to evaluate the application of immobilized enzymes in industries. The dye decolorization reusability of soluble BGP was continuously decreased on its repeated use (Fig. 1c). However, immobilized BGP decolorized DR 17 to 57% and DB 1 to 28% even after its 7th repeated use. Reports are available which show that immobilized peroxidase could efficiently be used for the decolorization of dyes (Matto et al., 2009). Our findings have suggested that calcium alginate–starch entrapped BGP has more advantages in removing a significant percentage of color from dye solutions. Thus this preparation could be exploited for the continuous decolorization of dyes for longer durations in a reactor.

The activity of soluble BGP decreased continuously when stored at 4°C for 30 days (Fig. 1d). At the end of the day 30 soluble BGP retained only 68% of its initial activity, while entrapped BGP retained 87% of its initial activity on storage under identical conditions. These observations suggest that entrapped BGP preparation could be used in enzymatic reactors for longer durations without much loss in activity.

2.5 Determination of phytotoxicity of decolorized product

To examine the toxicity of BGP decolorized product of disperse dyes, phytotoxicity experiments were performed using *Allium cepa* test with used dyes and their decolorized product. Table 4 shows the growth of *Allium cepa* roots in terms of length in cm and percent inhibition brought about by treated and untreated dye solutions. *Allium cepa* incubated with undiluted untreated DR 17 solution for 7 days showed 92% inhibition in root length while as 1:1 dilution of the same solution with distilled water exhibited an inhibition of 85% in root length. The average root length was recorded to be 0.35 and 0.62 cm in undiluted and diluted untreated samples of DR 17 as compared to 4.0 cm in control while BGP treated undiluted and diluted DR 17 revealed an inhibition of 77% and 65%, respectively. Minimum inhibition in root length was 70% and 55% for undiluted and 1:1 diluted BGP treated DB 1 product as compared to control. Untreated undiluted and 1:1 diluted DB 1 exhibited 87.5% and 80% inhibition in root length, respectively.

Although toxicity of the treated samples was reduced as compared to the untreated samples, certain extent of toxicity still persisted. Such an extent of detoxification by peroxidase has also been reported earlier (Kulshrestha and Husain, 2007; Satar and Husain, 2009b). The toxicity of several dyes, including azo compounds, was reduced after enzymatic treatment, although there was no strict correlation between decolorization and detoxification (Abadulla et al., 2000; Pereira et al., 2009). In another study, the addition of redox mediator led to 96% decolorization, however, the detoxification was somewhat less effective (Murugesan et al., 2009). Such biological tests are necessary to evaluate the pollution in effluents or water bodies and serve to determine the toxic potential of aromatic pollutants (Palegrini et al., 2007).

2.6 UV-Visible absorbance spectra of the dyes treated with immobilized BGP

To confirm the removal of aromatic compounds from the BGP treated dye solutions in the presence of redox mediators, spectral analyses were also performed. The decrease in absorbance at λ_{max} of each treated dye was a clear indication of dye decolorization and loss of chromophoric groups from the solution (Fig. 2). The diminution in the

Table 4 *Allium cepa* test for BGP treated dyes

Dye	Test solution	Root length (cm) (1× dilution)	Inhibition (%)	Root length (cm) (2× dilution)	Inhibition (%)
	Control	4.00 ± 1.24	–	4.00 ± 1.72	–
DR 17	Untreated	0.35 ± 1.54	92.12 ± 2.61	0.62 ± 2.64	85.14 ± 1.64
	Treated	0.91 ± 2.13	77.11 ± 1.86	1.41 ± 2.41	65.11 ± 2.12
DB 1	Untreated	0.52 ± 2.42	87.51 ± 2.17	0.84 ± 1.68	80.15 ± 2.15
	Treated	1.11 ± 2.11	70.15 ± 1.64	1.81 ± 2.16	55.12 ± 1.82

Onion bulbs were placed at the top of each tube containing control and treated samples with root primordial downwards touching the liquid. Distilled water was used as control for all the samples. In order to prevent the gap between onion bulbs and the liquid, respective samples were added to each tube after a gap of 12 hr. The experiments were carried out for 7 days in dark. Inhibition in the growth of *Allium cepa* roots with respect to control was considered as an index for the degree of toxicity.

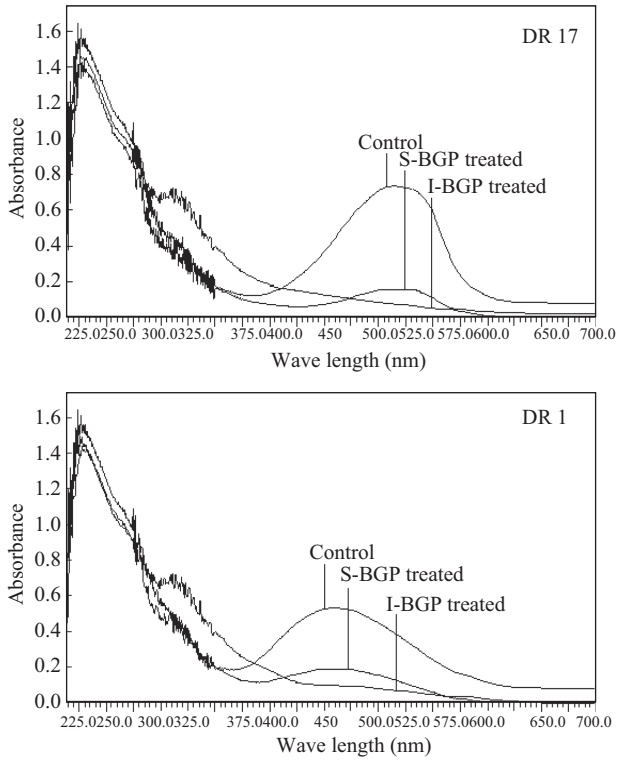

Fig. 2 UV-Visible absorption spectra of DR 17 and DB 1.

peaks at λ_{max} (510 nm for DR 17 and 460 nm for DB 1) in visible region was quite marked in case of immobilized BGP treated solutions of DR 17 and DB 1. The electron-withdrawing nature of the azo linkages obstructs the susceptibility of azo dye molecules to oxidative reactions. It is well documented that only specialized enzymes are found to degrade azo dyes (Husain, 2006; Ghodake et al., 2009; Satar and Husain, 2009b). Moreover, color is usually the first contaminant to be recognized in a wastewater, as very small amounts of synthetic dyes in water (10–15 mg/L) are highly visible, affecting the aesthetic merit, transparency and gas solubility of water bodies (Pereira et al., 2009). Thus the main interest of this study, using dye solutions containing single dye at low concentrations, has been the development of an enzymatic method for the decolorization of water-insoluble disperse dyes. The experimental results indicated that the use of such an efficient immobilized enzymatic treatment process could be a basic and valuable approach for the degradation of disperse dyes from wastewaters. Significant loss of color in visible region indicated the suitability of this enzyme-mediator system in the treatment of colored effluents.

3 Conclusions

On the basis of findings of this work, it can be concluded that immobilized BGP has more potential in the decolorization of dyes even at higher temperatures. Entrapped BGP was better suited for the decolorization/removal of colored compounds from dye solutions in batch processes. These observations may provide a reasonable basis for the application of peroxidase that is easily available, inexpensive and efficient at low concentration in the development of an effective biotechnological process for the removal of colored pollutants from textile effluents at large scale.

Acknowledgments

Aligarh Muslim University, Aligarh is gratefully acknowledged for providing University Grants Commission, New Delhi, sponsored fellowship to one of us (Rukhsana Satar).

References

Abadulla E, Tzanov T, Costa S, Robra K H, Cavaco-Paulo A, Gubitz G M, 2000. Decolorization and detoxification of textile dyes with a laccase from *Trametes hirsuta*. *Applied and Environmental Microbiology*, 66: 3357–3362.

Akhtar S, Khan A A, Husain Q, 2005. Partially purified bitter gourd peroxidase catalyzed decolorization of textile and other industrially important dyes. *Bioresource Technology*, 96: 1804–1811.

Asad S, Amoozegar M A, Pourbabaee A A, Sarbolouki M N, Dastgheib S M M, 2007. Decolorization of textile azo dyes by newly isolated halophilic and halotolerant bacteria. *Bioresource Technology*, 98: 2082–2088.

Betancor L, Lopez-Gallego F, Hidalgo A, Fuentes M, Podrasky O, Kuncova G et al., 2005. Advantages of the preimmobilization of enzymes on porous supports for their entrapment in sol-gels. *Biomacromolecules*, 6: 1027–1030.

Camarero S, Ibarra D, Martinez M J, Martinez A J, 2005. Lignin derived compounds as efficient laccase mediators for decolorization of different types of recalcitrant dyes. *Applied and Environmental Microbiology*, 71: 1775–1784.

Claus H, Faber G, Konig H, 2002. Redox-mediated decolorization of synthetic dyes by fungal laccases. *Applied Microbiology and Biotechnology*, 59: 672–678.

Dayaram P, Dasgupta D, 2008. Decolorisation of synthetic dyes and textile wastewater using *Polyporus rubidus*. *Journal of Environmental Biology*, 29: 831–836.

Fernandez-Sanchez C, Tzanov T, Gubitz G M, Cavaco-Paulo A, 2002. Voltammetric monitoring of laccase-catalyzed mediated reactions. *Bioelectrochemistry*, 58: 149–156.

Fiskesjo G, 1985. The *Allium* test as a standard in environmental monitoring. *Heridates*, 102: 99–112.

Ghodake G S, Kalme S D, Jadhav J P, Govindwar S P, 2009. Purification and partial characterization of lignin peroxidase from *Acinetobacter calcoaceticus* NCIM 2890 and its application in decolorization of textile dyes. *Applied Biochemistry and Biotechnology*, 152: 6–14.

Husain M, Husain Q, 2008. Applications of redox mediators in the treatment of organic pollutants by using oxidoreductive enzymes: A review. *Critical Reviews in Environmental Science and Technology*, 38: 1–41.

Husain Q, 2006. Potential applications of the oxidoreductive enzymes in the decolorization and detoxification of textile and other synthetic dyes from polluted water: A review. *Critical Reviews in Biotechnology*, 60: 201–221.

Khenifi A, Bouberka Z, Sekrane F, Kameche M, Derriche Z, 2007. Adsorption study of an industrial dye by an organic clay. *Adsorption*, 13: 149–158.

Kim Y J, Nicell J A, 2006. Laccase-catalyzed oxidation of bisphenol A with the aid of polymer additives. *Process Biochemistry*, 41: 1029–1037.

Kulshrestha Y, Husain Q, 2007. Decolorization and degradation of acid dyes mediated by salt fractionated turnip (*Brassica*

rapa) peroxidase. *Toxicology and Environmental Chemistry*, 89: 255–267.

Maddhinni V L, Vurimindi H B, Yerramilli A, 2006. Degradation of azo dye with horse radish peroxidase (HRP). *Journal of the Indian Institute of Science*, 86: 507–514.

Mateo C, Palomo J M, Fernandez-Lorente G, Guisan J M, Fernandez-Lafuente R, 2007. Improvement of enzyme activity, stability and selectivity via immobilization techniques. *Enzyme and Microbial Technology*, 40: 1451–1463.

Matto M, Husain Q, 2006. Entrapment of porous and stable concanavalin A-peroxidase complex into hybrid calcium alginate-pectin gel. *Journal of Chemical Technology and Biotechnology*, 81: 1316–1323.

Matto M, Satar R, Husain Q, 2009. Application of calcium alginate-starch entrapped bitter gourd (*Momordica charantia*) peroxidase for the removal of colored compounds from a textile effluent in batch as well as in continuous reactor. *Applied Biochemistry and Biotechnology*, 158: 512–523.

McMullan G, Meehan C, Conneely A, Kirby N, Robinson T, Nigam P et al., 2001. Microbial decolourisation and degradation of textiles dyes. *Applied Microbiology and Biotechnology*, 56: 81–87.

Murugesan K, Yang I H, Kim Y M, Jeon J R, Chang Y S, 2009. Enhanced transformation of Malachite Green by laccase of *Ganoderma lucidum* in the presence of natural phenolic compounds. *Applied Microbiology and Biotechnology*, 82: 341–350.

Palegrini N N B P, Palegrini R T, Paterniani J E S, 2007. Ecotoxicological evaluation of leachate from the Limeira sanitary landfill with a view to identify acute toxicity. *Revista Ambiente and Agua-An Interdisciplinary Journal of Applied Science*, 2: 34–43.

Pereira L, Coelho A V, Viegas C A, dos Santos M M C, Robalo M P, Martins L O, 2009. Enzymatic biotransformation of the azo dye Sudan Orange G with bacterial Cot A-laccase. *Journal of Biotechnology*, 139: 68–77.

Satar R, Husain Q, 2009a. Use of bitter gourd (*Momordica charantia*) peroxidase together with redox mediators to decolorize disperse dyes. *Biotechnology and Bioprocess Engineering*, 14: 213–219.

Satar R, Husain Q, 2009b. Applications of Celite-adsorbed white radish (*Raphanus sativus*) peroxidase in batch process and continuous reactor for the degradation of reactive dyes. *Biochemical Engineering Journal*, 46: 96–104.

Satar R, Matto M, Husain Q, 2008. Studies on calcium alginate-pectin gel entrapped concanavalin A-bitter gourd *(Momordica charantia)* peroxidase complex. *Journal of Scientific and Industrial Research*, 67: 609–615.

Soares G M, de Amorim M T, Costa-Ferreira M, 2001. Use of laccase together with redox mediators to decolorize Remazol Brilliant Blue R. *Journal of Biotechnology*, 89: 123–129.

Ulson de Souza S M A G, Forgiarini E, Ulson de Souza A A, 2007. Toxicity of textile dyes and their degradation by the enzyme horseradish peroxidase (HRP). *Journal of Hazardous Materials*, 147: 1073–1078.