Chapter V

Decolorization of textile effluent by bitter gourd peroxidase immobilized on concanavalin A layered calcium alginate-starch beads
5.1. INTRODUCTION

Recently bioaffinity based procedures for enzyme immobilization have gained much attention over the other known classical methods, as this method can be exploited for the direct immobilization of enzymes from partially purified preparation or even from crude homogenates (Kulshrestha and Husain, 2006a; Khan and Husain, 2007). Surface immobilized enzymes are far more superior to entrapped enzymes as in the latter case the diffusion of large molecular size products from inside the gel beads is difficult (Le-Tien et al., 2004). In order to prevent the possibility of accumulation of products inside polymeric matrices, immobilization of enzymes on the surface of support will be a preferred choice.

In this study BGP has been immobilized on the surface of Con A layered calcium alginate-starch beads and this preparation was used for the decolorization of colored textile effluent. This study describes the optimization of various experimental conditions for the effective removal of colored compounds from the textile effluent by immobilized BGP (I-BGP). Immobilized BGP has also been used successfully in batch process as well as in continuous reactor for the remediation of colored toxic pollutants from industrial effluent.

5.2. MATERIALS AND METHODS

5.2.1. Materials

Violuric acid was purchased from Fluka Chemicals, Austria. Sodium alginate was the product of Koch-Light, England. Glutaraldehyde and ethanolamine were obtained from Sigma Chemical Co. (St. Louis, MO) USA. Redox mediators; 1-hydroxybenzotriazole, syingaldehyde, vanillin and veratryl alcohol were all procured from SRL Chemicals Pvt. Ltd. Mumbai, India. The untreated textile industrial effluent was obtained from cotton textile industry located in Sector 7, Noida, U.P, India. Jack bean meal was procured from DIFCO, Detroit, USA. Bitter gourd was purchased from local vegetable market. Other chemicals and reagents employed were of analytical grade and were used as supplied.
5.2.2. Ammonium sulphate fractionation of bitter gourd proteins

The salt fractionated bitter gourd proteins were obtained from the procedure described in Chapter III, Section 3.2.2.

5.2.3. Measurement of peroxidase activity

Peroxidase activity was estimated in 100 mM sodium acetate buffer, pH 5.0 at 37 °C (Chapter II, Section 2.2.6).

5.2.4. Immobilization of BGP on the surface of Con A layered calcium alginate-starch beads

Jack bean extract (10%, w/v) was prepared as described in Chapter II, Section 2.2.3. Immobilization of BGP (4900 U) on Con A layered calcium alginate-starch beads was done by the procedure described in Chapter III, Section 3.2.5.

5.2.5. Effluent processing and dilution

The textile effluent was collected from the industrial site situated in Sector 7, Noida, U.P, India. The effluent was centrifuged; and after centrifugation the clear supernatant was diluted by 100 mM sodium acetate buffer, pH 5.0 till the effluent exhibited optical density of 0.550 (approx) at 580 nm (Fig. 18). The $\lambda_{\text{max}}$ of the effluent was determined by using Cintra 10 e UV-visible spectrophotometer.

Textile effluent decolorization was calculated by the same procedure as mentioned in Chapter IV, Section 4.2.5.

5.2.6. Role of redox mediators on the BGP-catalyzed decolorization of textile industrial effluent

The effect of various redox mediators (1.0 mM) on BGP (0.28 U mL$^{-1}$) catalyzed effluent decolorization was investigated in 100 mM sodium acetate buffer, pH 5.0 in the presence of 0.72 mM H$_2$O$_2$ for 1 h at 37 °C. Effluent decolorization by soluble BGP (S-BGP) was stopped by heating reaction mixture in a boiling water bath.
for 5 min and the insoluble product was removed by centrifugation at 3000 g for 15 min. However, in the case of I-BGP treated effluent, the reaction was stopped by removing enzyme by centrifugation. The residual effluent decolorization was monitored at 580 nm. The percent decolorization was calculated by taking untreated effluent as control (100%).

5.2.7. Effect of HOBT on BGP catalyzed effluent decolorization

The decolorization of textile effluent by soluble and immobilized BGP (0.28 U mL⁻¹) in 100 mM sodium acetate buffer, pH 5.0 was monitored in the presence of varying concentrations of HOBT (0.1-1.4 mM) and 0.72 mM H₂O₂ for 1 h at 37 °C. The percent decolorization was calculated by taking untreated effluent as control (100%).

5.2.8. Effect of BGP concentration on the decolorization of textile effluent

Textile industrial effluent was treated with increasing concentrations of soluble and immobilized BGP (0.08-0.32 U mL⁻¹) in 100 mM sodium acetate buffer, pH 5.0 under the other experimental conditions as described in Section 5.2.6. Untreated effluent was used as control (100%) for the calculation of percent decolorization.

5.2.9. Effect of pH and temperature on the decolorization of effluent by BGP

The decolorization of textile effluent by soluble and immobilized BGP (0.28 U mL⁻¹) was investigated in the buffers of varying pH (2.0-10.0) in the presence of 1.0 mM HOBT and 0.72 mM H₂O₂ for 1 h at 37 °C. The molarity of each buffer was 100 mM. Untreated effluent in each buffer was considered as control (100%) for the calculation of percent decolorization.

The effect of temperature on the decolorization of textile industrial effluent by soluble and immobilized BGP (0.28 U mL⁻¹) was investigated at various temperatures (20-80 °C) in the presence of 1.0 mM HOBT and 0.72 mM H₂O₂ for 1 h. Treated effluent exhibiting maximum decolorization at optimal temperature was considered as control (100%) for the calculation of percent decolorization.
5.2.10. Effluent decolorization in batch processes

Industrial effluent (250 mL) was treated with soluble and immobilized BGP (37.5 U) in batch processes for varying times at 37 °C under the experimental conditions mentioned in Section 5.2.6.

5.2.11. Effluent decolorization reusability of immobilized BGP

The textile effluent (5.0 mL) was incubated with immobilized BGP (1.4 U) for 1 h at 37 °C under similar experimental conditions described in Section 5.2.6. After the completion of reaction, enzyme was separated by centrifugation and stored in assay buffer for over 12 h at 4 °C. The similar experiment was repeated 8 times with the same preparation of immobilized BGP and each time with a fresh batch of diluted effluent. Effluent decolorization was monitored at specific wavelength maxima of the industrial effluent, 580 nm. The percent decolorization was calculated by taking untreated effluent as control (100%).

5.2.12. Continuous treatment of effluent by using two-reactor system

A two-reactor system was developed for the continuous removal of colored compounds from the textile effluent. The first column (10.0 x 2.0 cm) was filled with immobilized BGP (1162 U) connected to another column filled with activated silica (10.0 x 2.0 cm). Activated silica was prepared by the procedure described in Chapter IV, Section 4.2.12. The colored effluent (O.D. 0.550) in the presence of 1.0 mM HOBT and 0.72 mM H2O2 at room temperature was passed through the reactor containing immobilized enzyme. Second column received the effluent treated by enzyme in the first reactor; this activated silica column adsorbed most of the oxidized colored compounds. The flow rate of the reactor was maintained at 16 mL h⁻¹. After a gap of 10 d, the samples of treated effluent from the second column were collected, centrifuged and their spectrophotometric analysis was done. The collection and analysis of textile sample from the continuous two-reactor system was continued for over 2 months.
A parallel two-reactor system was operated in which the first column contained calcium alginate-starch beads without enzyme and the second column was filled with activated silica. The effluent was passed through the continuous two-reactor system and samples were collected after a gap of 10 d for over 1 month and finally samples were centrifuged and analyzed.

5.2.13. UV-visible spectra

BGP treated textile effluent samples collected from the two-reactor system were followed by UV-visible spectrophotometric analysis on Cintra 10e UV-visible spectrophotometer. Spectral profiles from 200-700 nm were recorded to measure the progress of effluent decolorization.

5.3. RESULTS

The collected industrial effluent was suitably diluted and its absorption spectrum was recorded. The spectrum of effluent exhibited maximum absorption at 580 nm (Fig. 18). All the effluent decolorization measurements were carried at this wavelength. The effluent was found to be stable upon exposure to alone H₂O₂, enzyme, HOBT, calcium alginate-starch beads and activated silica. It was also recalcitrant to the combined action of BGP and H₂O₂. Thus the effluent decolorization was due to combined action of BGP, H₂O₂ and a redox mediator, HOBT.

5.3.1. Effluent decolorization by BGP in the presence of various redox mediators

Fig. 19 demonstrates the effect of various redox mediators on BGP mediated decolorization of effluent. Among the redox mediators used for the effluent decolorization, HOBT showed highest decolorization 28% and 70% in the presence of soluble and immobilized BGP, respectively. However, syringaldehyde, VA, VLA and VN mediated less decolorization as compared to HOBT.
The textile effluent obtained from the industrial site was centrifuged and clear supernatant was diluted with 100 mM sodium acetate buffer, pH 5.0. The optical density and maximum wavelength of the textile effluent was approximately 0.550 and 580 nm, respectively.

Fig. 18: UV-visible spectra for textile effluent
Fig. 19: Treatment of textile effluent by BGP in the presence of various redox mediators

Decolorization of effluent was catalyzed by BGP (0.28 U mL$^{-1}$) in the presence of various redox mediators (1.0 mM). The diluted effluent (5.0 mL) was incubated in the presence of 0.72 mM $\text{H}_2\text{O}_2$ for 1 h at 37 °C.
5.3.2. Effect of increasing concentration of HOBT

The effluent decolorization by BGP in the presence of varying concentrations of HOBT (0.1-1.4 mM) has been described in Fig. 20. There was an enhancement in the effluent decolorization up to 1.0 mM HOBT. After this concentration, there was a marginal increase in effluent decolorization. Soluble and immobilized BGP could decolorize 28% and 70% color from effluent in the presence of 1.0 mM HOBT, respectively.

5.3.3. Effect of enzyme concentration

The effect of enzyme concentrations was studied to determine the minimum amount of enzyme required for maximum decolorization. The rate of effluent decolorization increased from 0.08-0.28 U mL$^{-1}$ BGP. However, the decolorization of effluent remained almost constant after 0.28 U mL$^{-1}$ of BGP. Effluent was decolorized to 28% and 70% by soluble and immobilized BGP (0.28 U mL$^{-1}$), respectively (Table 18).

5.3.4. Effect of pH

Table 19 shows effluent decolorization by BGP in buffers of various pH (2.0-10.0). About 28% and 70% of the colored effluent was decolorized by soluble and immobilized BGP at pH 5.0, respectively. Subsequently, the effluent removal dropped significantly at pH 6.0 and onwards.

5.3.5. Effect of temperature

The influence of temperature on the decolorization of textile industrial effluent was evaluated by treating effluent at various temperatures by soluble and immobilized BGP (Table 20). The textile effluent was maximally decolorized 28% and 70% in the presence of soluble and immobilized BGP at 40 °C, respectively. However, above optimum temperature, effluent decolorization was further decreased.
Fig. 20: Effect of HOBT concentration on BGP-catalyzed effluent decolorization

The effluent (5.0 mL) was incubated with varying concentrations of HOBT (0.1-1.4 mM) in the presence of BGP (0.28 U mL\(^{-1}\)), 0.72 mM of \(\text{H}_2\text{O}_2\) in 100 mM sodium acetate buffer pH 5.0 for 1 h at 37 °C.
Table 18: Effect of BGP concentration on effluent decolorization

<table>
<thead>
<tr>
<th>Enzyme concentration (U mL(^{-1}))</th>
<th>Decolorization (%)</th>
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<tbody>
<tr>
<td></td>
<td>S-BGP</td>
<td>I-BGP</td>
<td></td>
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<tr>
<td>0.08</td>
<td>11</td>
<td>31</td>
<td></td>
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<tr>
<td>0.12</td>
<td>18</td>
<td>38</td>
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<tr>
<td>0.16</td>
<td>22</td>
<td>46</td>
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<tr>
<td>0.20</td>
<td>24</td>
<td>53</td>
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<tr>
<td>0.24</td>
<td>26</td>
<td>61</td>
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<tr>
<td>0.28</td>
<td>28</td>
<td>70</td>
<td></td>
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<tr>
<td>0.32</td>
<td>28</td>
<td>70</td>
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Textile effluent (5.0 mL) was treated with increasing concentrations of BGP (0.08-0.32 U mL\(^{-1}\)) for 1 h in the presence of 1.0 mM HOBT and 0.72 mM H\(_2\)O\(_2\) in 100 mM sodium acetate buffer, pH 5.0 at 37 °C. Each value represents the mean for three independent experiments performed in duplicates, with average standard deviation, < 5%.
Table 19: Effect of pH on BGP-catalyzed decolorization of textile effluent

<table>
<thead>
<tr>
<th>pH</th>
<th>Decolorization (%)</th>
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<tbody>
<tr>
<td></td>
<td>S-BGP</td>
<td>I-BGP</td>
<td></td>
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<tr>
<td>2</td>
<td>15</td>
<td>46</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>18</td>
<td>52</td>
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<tr>
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<td>22</td>
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<td>17</td>
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<td>12</td>
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<td>9</td>
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<tr>
<td>10</td>
<td>8</td>
<td>29</td>
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Textile effluent (5.0 mL) was treated by BGP (0.28 U mL⁻¹) in the buffers of various pH (2.0-10.0) in the presence of 1.0 mM HOBT and 0.72 mM H₂O₂ for 1 h at 37 °C. Each value represents the mean for three independent experiments performed in duplicates, with average standard deviation, < 5%.
Table 20: Effect of temperature on BGP-catalyzed decolorization of textile effluent

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Decolorization (%)</th>
<th>S-BGP</th>
<th>I-BGP</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td></td>
<td>10</td>
<td>49</td>
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<tr>
<td>30</td>
<td></td>
<td>16</td>
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<tr>
<td>80</td>
<td></td>
<td>5</td>
<td>32</td>
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</table>

Textile effluent was incubated with BGP (0.28 U mL⁻¹) in 100 mM of sodium acetate buffer, pH 5.0 in the presence of 0.72 mM H₂O₂ and 1.0 mM HOBT at 20-80 °C for 1 h. Each value represents the mean for three independent experiments performed in duplicates, with average standard deviation, < 5%.
5.3.6. Decolorization of effluent in batch processes by BGP

Table 21 depicts the effluent decolorization by BGP in batch processes. It was observed that after 150 min of incubation, immobilized BGP decolorized more than 90% textile effluent. However, the decolorization of effluent by soluble BGP was only 48% under similar incubation period. Immobilized BGP showed its superiority over the soluble enzyme in the removal of color from the industrial effluent on long incubation.

5.3.7. Effluent decolorization reusability of immobilized BGP

In order to consider an immobilized BGP for its use in a reactor, it is necessary to investigate the reusability of immobilized enzyme. The effluent decolorization reusability of immobilized BGP was continuously decreased on its repeated use (Fig. 21). Immobilized BGP retained 59% effluent decolorization capacity even after its 8th repeated use.

5.3.8. Continuous treatment of effluent through a two-reactor system

The schematic diagram for the decolorization of textile effluent by immobilized enzyme in a two-reactor system has been shown in Fig. 22. Immobilized enzyme could remove more than 90% colored compounds from the effluent during initial 10 d of operation. However, the effluent decolorization efficiency of the reactor was gradually decreased. Immobilized BGP present in two-reactor system was capable of removing 40% colored compounds from the textile effluent even after 60 d of its operation (Fig. 23).

The textile effluent was passed through a parallel continuous two-reactor system (without immobilized BGP) in order to check the adsorption of colored compounds from effluent on calcium alginate-starch beads as well as on activated silica. There was no change in the optical density of the textile effluent after passing continuously through the two-reactor system.
Table 21: Decolorization of textile effluent by BGP in batch processes

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Decolorization (%)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>S-BGP</td>
</tr>
<tr>
<td>30</td>
<td>22</td>
</tr>
<tr>
<td>60</td>
<td>28</td>
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<tr>
<td>90</td>
<td>33</td>
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<tr>
<td>120</td>
<td>41</td>
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<tr>
<td>150</td>
<td>48</td>
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<tr>
<td>180</td>
<td>48</td>
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<tr>
<td>210</td>
<td>48</td>
</tr>
<tr>
<td>240</td>
<td>48</td>
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</table>

Textile effluent was incubated with BGP (37.5 U) in 250 mL of 100 mM sodium acetate buffer, pH 5.0 in the presence of 0.72 mM H₂O₂ and 1.0 mM HOBT for varying times. Each value represents the mean for three independent experiments performed in duplicates, with average standard deviation, < 5%.
Fig. 21: Textile effluent decolorization reusability of immobilized BGP

Immobilized BGP was incubated with textile effluent for 1 h at 37 °C in triplicates. Decolorization of textile effluent was determined after incubation period. After the completion of the reaction, the immobilized enzyme was collected by centrifugation and stored in assay buffer at 4 °C overnight. Next day, the similar experiment was repeated. This procedure was repeated for 8 successive days.
Fig. 22: Schematic representation of a two-reactor system used for decolorization and removal of colored compounds from textile effluent

A column (10.0 x 2.0 cm) filled with immobilized BGP (1162 U) was connected to a second column containing activated silica. Textile effluent having O.D. approximately 0.550 was continuously passed through the reactor in the presence of 1.0 mM HOBT and 0.72 mM H₂O₂ for a period of 60 d. Effluent removal in two-reactor system was done under the similar experimental conditions as described in Section 5.2.12.
Fig. 23: Evaluation of decolorized textile effluent collected from two-reactor system

Diluted effluent was treated in a continuous two-reactor system as described in the Section 5.2.12. Samples were collected after a gap of 10 d for over 2 month from the second column (containing activated silica) of the two-reactor system. The collected samples were centrifuged and analyzed spectrophotometrically for the presence of remaining color.
5.3.9. Spectral analysis of treated effluent

UV-visible spectrum of textile effluent before and after enzymatic treatment in two-reactor system is shown in Fig. 24. The decrease in absorbance peaks in UV-visible regions with respect to the number of days of operation showed remarkable variation in the absorbance at various wavelengths and thus removal of the colored pollutants from textile effluent treated by immobilized BGP.

5.4. DISCUSSION

BGP has earlier been employed for the decolorization of a number of textile dyes in synthetic solutions (Akhtar et al., 2005a; 2005b). To the best of our knowledge, BGP has not ever been used for the decolorization/removal of aromatic compounds present in industrial effluents. For the first time an effort has been made to treat industrial effluent by BGP immobilized on the surface of Con A layered calcium alginate-starch beads.

It was observed that the diluted effluent was recalcitrant to the action of alone H₂O₂, enzyme, HOBT, calcium alginate-starch beads and activated silica. Thus, the effluent decolorization was a result of redox mediated H₂O₂-dependent enzymatic action, possibly involving free-radical formation followed by the adsorption of the activated compounds on silica. Several earlier workers have also reported the removal of peroxidase treated aromatic pollutants by adsorbing to an adsorbent (Kinsley and Nicell, 2000; Tonegawa et al., 2003).

Some of the chemicals serving as redox mediators facilitate dye degrading activity of enzymes and enhance their specificity to a wide range of dyes (Reyes et al., 1999; Soares et al., 2001b; Husain and Husain, 2008). Among the redox mediators, those presenting the >NOH moiety (HOBT, N-hydroxyphthalimide, VLA) have been proved to be very efficient towards benzylic substrates through a radical H-abstraction route of oxidation involving the aminoxyl radical (>N-O') intermediate (d’Acunzo et al., 2006).

HOBT, a potential redox mediator plays a critical role in enhancing the rate of enzyme mediated dye/effluent degradation, bleaching of pulp and other environmental pollutants (Garcia et al., 2003). Here we have found that, HOBT had an important role
Fig. 24: UV-visible spectra of the textile effluent

Absorption spectrum of industrial effluent treated in a two-reactor system was recorded on UV-visible spectrophotometer Cintra 10e. Absorption spectra of effluent were taken before and after treatment by I-BGP. Spectra in the figure are labeled.
in the decolorization of industrial effluent as compared to other redox mediators (Fig. 19), HOBT satisfied all the three properties of a redox mediator for the decolorization of effluent; (i) it is oxidized by one-electron directly by the action of enzyme to produce free radical, (ii) it produces radical which is stable enough to diffuse and react with the target compound and (iii) it has an appropriate redox potential (Tinoco et al., 2007).

The schematic representation of enzymatic oxidation of recalcitrant substrate (Sub-H) by means of peroxidase and a >NO-H containing redox mediator (HOBT) is given below;

\[
\begin{align*}
\text{H}_2\text{O}_2 & \xrightarrow{\text{Peroxidase}} >\text{NO}^+ \\
\text{H}_2\text{O} & \xrightarrow{\text{Peroxidase}_\text{ox}} >\text{NO-H} \\
\end{align*}
\]

The concentration of HOBT is an important factor for the enzyme catalyzed decolorization/degradation of aromatic compounds (Murugesan et al., 2007). In most of the earlier reports, mediators are used at a very high concentration in the range of 6 to 57 mM (Kurniawati and Nicell, 2007). However, some earlier studies indicated a significant laccase inactivation by 10 mM HOBT/VLA (Li et al., 1999). In the present study, 1.0 mM HOBT was sufficient to mediate BGP catalyzed maximum effluent decolorization, 70\% (Fig. 20).

The optimization of enzyme concentration was carried to aim at high efficiency of effluent decolorization by BGP and 0.28 U mL\(^{-1}\) enzyme was sufficient for maximum decolorization (Table 18). A similar observation has been reported in an earlier study in which HRP (2.985-29.85 U mL\(^{-1}\)) was evaluated for the decolorization of Remazol Turquoise G 133\%. When the concentration of HRP was 14.985 U mL\(^{-1}\), the decolorization of the dye was 58\%. However, when the concentration of HRP was doubled, the decolorization of dye was increased only by 4\%. On the basis of such results it can be concluded that by using higher concentration of enzyme, decolorization was not significantly influenced (Ulson de Souza et al., 2007).

Most enzymes have a characteristic pH at which their activity is maximum. The immobilized BGP showed high effluent decolorization at pH 5.0 (Table 19). This study was in accordance with an earlier report in which the decolorization of reactive
textile dye by soluble and immobilized BGP was maximum in the buffers of acidic pH (Akhtar et al., 2005b). The decolorization of textile effluent by BGP was optimum at 40 °C (Table 20). This is in line with the work demonstrated by earlier investigators that the decolorization of acid/reactive dyes by plant peroxidases was also maximum at 40 °C (Akhtar et al., 2005a; 2005b; Kulshrestha and Husain, 2007).

It has been previously shown that immobilizing bioactive agents (catalase and a bacteriocin) on the surface of calcium alginate beads offer greater stability against various experimental parameters as compared to the free enzyme (Le-Tien et al., 2004). A similar pattern has been observed in this study as the surface immobilized BGP was remarkably more efficient in decolorizing effluent (90%) as compared to 48% color removal by soluble enzyme within 3 h in a stirred batch process (Table 21).

The advantage of immobilized enzymes does not lie only in increasing the stability but also in its reusability. I-BGP retained remarkably very high effluent decolorization activity (59%) even during its 8th repeated use (Fig. 21). The activity loss of the surface immobilized BGP preparation during 8th repeated use in effluent decolorization was appreciably much higher as compared to 50% acid dye removal by polyacrylamide gel entrapped HRP only after its 5th repeated reuse (Mohan et al., 2005). Our observations have suggested that surface immobilized enzyme has more advantages in removing higher color from industrial effluent.

Considering the success of surface immobilized BGP in effluent decolorization, next step was to evaluate its efficiency at large scale by using a continuous two-reactor system (Fig. 22). The two-reactor system used in this study was able to operate continuously and could decolorize textile effluent without any operational problems. An enhancement in decolorization/degredation of effluent by immobilized enzyme was probably due to increased adsorption of oxidized colored compounds/products on adsorbent, activated silica (Fig. 23). Kim and Nicell (2006) have shown that bisphenol A oxidation by laccase was significantly enhanced by the addition of polyethylene glycol because its product got easily adsorbed on polyethylene glycol.

The decolorization and remediation of textile effluent by immobilized BGP in a two-reactor system was further strengthened by UV-visible spectral analysis (Fig. 24). The significant loss of color in UV-visible region with respect to number of days of operation was attributed to the formation of free radical compounds which got adsorbed on the activated silica present in the second column. This indicated the
suitability of the two-reactor system to treat huge volume of effluents from the textile industries. In addition, continuous decolorization of dyes has been scarcely investigated especially dealing with oxidative enzyme (Lopez et al., 2002; Mielgo et al., 2002; Selvam et al., 2003). Thus it pointed out the relevance as well as the novelty of the results obtained in the present work.

Here, we have demonstrated that BGP immobilized on the surface of calcium alginate-starch beads was suitable candidate for wastewater treatment, since it was used efficiently for the decolorization and removal of colored compounds from textile effluent in batch process as well as in a continuous two-reactor system. Indeed the described system was developed with a cheap biocatalyst that was efficient at low concentration. Thus this work may provide a reasonable basis for development of an effective biotechnological process for the removal of colored pollutants from the textile effluents.