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Functional Suitability of Soluble Peroxidases from Easily Available Plant Sources in Decolorization of Synthetic Dyes

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1. Introduction

With the growing commercial availability of dyes, their range and scope of application is also expanding. Consequently, a large amount of unused dyes are released in the industrial effluents. Ninety percent of textile dyes entering modern activated sludge sewage treatment plants pass through unchanged. There is an intense environmental concern about the fate of these unbound dyes. These discharged dyes form toxic products and their strong color causes turbidity which even at very low concentrations has a huge impact on the aquatic environment.

These synthetic reactive dyes bond covalently with fabric and contain chromophoric groups like anthraquinone, azo, triarylmethane etc. along with reactive groups viz., vinyl sulphone, chlorotriazine, trichloropyrimidine etc. (Sumathi & Manju, 2000; Keharia & Madamvar, 2003). Disperse dyes and acid dyes have low solubility in water. They are mainly used in the dyeing of polyesters and find minor use in dyeing cellulose acetates and polyamides. Azo dyes constitute the largest group of colorants used in industry. These dyes can be precipitated or adsorbed only in small amounts, while under anaerobic conditions they are cleaved by microorganisms forming potentially carcinogenic aromatic amines (Chung & Cerniglia, 1992).

Several studies on decolorization of textile dyes used in industries have been conducted. Some non-textile dyes commonly present in industrial effluents have been studied. Dye removal from wastewaters with traditional physicochemical processes, such as coagulation, adsorption and oxidation with ozone is expensive, can generate large volumes of sludge and usually requires the addition of environmentally hazardous chemical additives (Chen, 2006). On the other hand, most of the synthetic dyes are xenobiotic compounds which are poorly removed by the use of conventional biological aerobic treatments (Marco et al., 2007a, b). Although, biodegradation appears to be a promising technology, unfortunately the analysis of contaminated soil and water has shown persistence of toxic pollutants even in the presence of microorganisms (Robinson et al., 2001; Keharia and Madamvar, 2003).

Decolorization of dye wastewater is an area where innovative treatment technologies need to be investigated. The focus in recent times has shifted towards enzyme based treatment of colored wastewater/industrial textile effluents. The peroxidase and polyphenol oxidases

participate in the degradation of a broad range of substrate even at very low concentration. Further, these peroxidases and polyphenol oxidases have been used for treatment of dyes but large scale exploitation has not been achieved due to their low enzymatic activity in biological materials and high cost of purification (Bhunia et al., 2001; Shaffiqu et al., 2002; Verma & Madamwar, 2002). Bioremediation is a viable tool for restoration of contaminated subsurface environments. It is gaining importance due to its cost effectiveness, environmental friendliness and production of less sludge as compared to chemical and physical decomposition processes. Here too microbial treatment has certain inherent limitations (Husain & Jan, 2000; Duran & Esposito, 2000; Torres et al., 2003).

It has been shown that peroxidases catalyze a variety of oxidation reactions and importantly dyes recalcitrant to peroxidase shows significant decolorization in the presence of Redox mediators (Calcaterra et al., 2008). Redox mediated enzyme catalysis has wide application in degradation of polycyclic aromatic hydrocarbons which includes phenols, biphenyls, pesticides, insecticides etc. (Husain & Husain, 2008; Calcaterra et al., 2008).

2. Peroxidases in dye decolorization with Redox mediators

Enzymatic approach has gained considerable interest in the decolorization/degradation of textile and other industrially important dyes present in wastewater. This strategy is ecofriendly and useful in comparison to conventional chemical, physical and biological treatments, which have inherent serious limitations. Enzymatic treatment is very useful due to the action of enzymes on pollutants even when they are present in very dilute solutions and recalcitrant to the action of various microbes participating in the degradation of dyes. Several enzymes (peroxidases, manganese peroxidases, lignin peroxidases, laccases, microperoxidase-11, polyphenol oxidases and azoreductases) have been evaluated for their potential in decolorization and degradation of dyes. Although, some recalcitrant dyes are not degraded/ decolorized in the presence of such enzymes, the addition of certain Redox mediator enhances the range of substrates and efficiency of degradation of the recalcitrant compounds. However, very few Redox mediators are frequently used which includes 1-hydroxybenzotriazole (HOBt), veratryl alcohol, violuric acid (VA), 2-methoxyphenothiazone. The enzymes in soluble form cannot be exploited on large scale due to limitations of stability and reusability and consequently, the use of immobilized enzymes has significant advantages over soluble enzymes. In the near future, technology based on the enzymatic treatment of dyes present in the industrial effluents/wastewater will play a vital role. Treatment of wastewater on a large scale will also be possible by using reactors containing immobilized enzymes.

2.1 Dye decolorization with turnip (*Brassica rapa*) proteins

The potential of partially purified turnip (*Brassica rapa*) proteins have been studied by Matto and Husain, (2007) on decolorization of certain direct dyes like Direct Red 23, Direct Red 239, Direct Blue 80 and Direct Yellow 4. The turnip proteins showed enhanced decolorization in the presence of Redox mediators. Redox mediators explored for dye decolorization of these dyes were HOBt, alpha naphthol, vanillin, L-histidine, VA, catechol, quinol, bromophenol, 4-nitrophenol and gallic acid. Six out of 10 investigated compounds showed their potential in enhancing the decolorization of direct dyes. The performance of each was evaluated at different concentrations of mediator and enzyme. The decolorization of all tested direct dyes was maximum in the presence of 0.6 mM Redox mediator at pH 5.5

and 30°C. Complex mixtures of dyes decolorized maximally in the presence of 0.6 mM Redox mediator (HOBT/VA). In order to examine the operational stability of the enzyme preparation, the enzyme was exploited for the decolorization of mixtures of dyes for different times in a stirred batch process. There was no change in decolorization of an individual dye or their mixtures after 60 min of incubation; the enzyme caused more than 80% decolorization of all dyes in the presence of 1-hydroxybenzotriazole/violuric acid. However, there was no desirable increase in dye decolorization of the mixtures on overnight incubation. The treatment of such polluted water in the presence of Redox mediators caused the formation of insoluble precipitate, which could be removed by the process of centrifugation. Such catalyzed oxidative coupling reactions may be important for natural transformation pathways for dyes and indicate their potential use as an efficient means for removal of dyes color from waters and wastewaters.

2.2 Dye decolorization with fenugreek (*Trigonella foenum-graecum*) seed proteins

Peroxidase from fenugreek (*Trigonella foenum-graecum*) seeds is highly effective in the decolorization of textile effluent. The role of six Redox mediators has been investigated for effective decolorization by FSP (Fenugreek Seed Proteins). The maximum decolorization of textile effluent was observed in the presence of 1.0 mM 1-hydroxybenzotriazole, 0.7 mM H₂O₂, and 0.4 U/ml of FSP in the buffer of pH 5.0 at 40°C in 2.5 h. The decolorization of textile effluent in a batch process by peroxidase is 85% in 5 h, whereas the complete decolorization of textile effluent by membrane entrapped FSP was observed within 11 h of its operation. The absorption spectra of treated effluent exhibited a marked diminution in the absorbance at different wavelengths compared to untreated effluent. The removal of colored aromatic compounds from wastewater by peroxidases is well-known. These enzymes are currently being employed for the treatment of aromatic compounds (Husain & Husain, 2008; Husain et al., 2009). Most of the studies on dye decolorization have been done in a defined media or synthetic wastewater where a single dye or their mixtures are usually present. However, industrial effluents are more complex due to the presence of other contaminating substances along with colored compounds; under such conditions, the treatment of these pollutants is a difficult problem (Husain et al., 2010). Textile effluents alone are recalcitrant to the action of FSP, but in the presence of Redox mediators, they decolorized significantly. Many other aromatic pollutants such as aromatic amines, dyes and bisphenol A have already been oxidized by peroxidases in the presence of Redox mediators (Karim & Husain, 2009; Matto & Husain, 2008).

The oxidation of effluent in the presence 1.0 mM HOBT is quite low compared to the Redox mediators used in earlier studies to treat various colored pollutants (Rodriguez-Couto et al., 2005; Matto & Husain, 2008). The high concentrations of Redox mediators may not be appropriate for wastewater treatment because of high cost of mediators or possibility of creating negative impacts on effluent toxicity or in the environment upon their disposal into receiving waters (Kurniawati & Nicell, 2007). The maximum decolorization of textile effluent with FSP was at 0.7 mM concentration of H₂O₂. The concentration of H₂O₂ >1.2 mM acted as an inhibitor of peroxidase activity possibly by causing irreversible oxidation of enzyme ferri-heme group which is essential for its activity (Vazquez-Duarte et al., 2001).

The differences in time course of removal of various dyes might be due to the structural barrier and the electron localization among them (Jauregui et al., 2003). The maximum decolorization (68%) of textile effluent was obtained by 0.4 U/ml of FSP. FSP catalyzed the decolorization of effluent over a wide range of pH with an optimum at pH 5.0. The

maximum decolorization of effluent was at a temperature of 40°C. FSP successfully removed more than 85% color of the effluent in 5 h. After 5 h, the increase in the rate of effluent decolorization has not been recorded. Complete removal of color has been studied by using enzyme in a membrane bag. This type of reactor would increase the efficiency of dye decolorization because enzyme can be reused as it did not mix with the rest of the treated solution. Some workers reported that *Pseudomonas putida* CCRC14365 peroxidase was capable of removing phenol in hollow-fiber membrane bioreactor. *P. putida* fully degraded 2,000 mg/L of phenol within 73 h; even at a level of 2,800 mg/L, phenol could be degraded by more than 90% after 95 h of operation (Chung et al., 2004). In another report, a novel tube membrane bioreactor has been used for the treatment of an industrially produced wastewater arising in the manufacture of 3-chloronitrobenzene and nitrobenzene; in 1.7 h, over 99% of each 3-chloronitrobenzene and nitrobenzene from the wastewater was degraded (Livingston, 2004).

For the decolorization and removal of colored compounds from textile effluent, spectral analysis is an important aspect to demonstrate a loss in these compounds after treatment with enzyme. The decrease in absorbance peaks in the UV-visible region is a strong evidence for the decolorization of aromatic pollutants from wastewater. Some earlier spectral analyses of the enzymatically treated aromatic amines and their mixtures have shown a significant decrease in absorbance (Kurniawati & Nicell, 2007). There was 80% decolorization of the chromophoric groups and a significant reduction in the peak associated with the aromatic ring. The UV-visible absorbance spectrum for diluted textile effluent has been taken before and after treatment by FSP in the presence of various Redox mediators. The treatment of effluent by FSP in the presence of Redox mediator, HOBT, produces insoluble aggregates which can successfully be removed by centrifugation. The peroxidase from fenugreek seeds has potential in the remediation of hazardous aromatic pollutant and its use could be extended to the large-scale treatment of textile effluents and other related aromatic compounds by employing more effective and cheaper Redox mediators.

2.3 Dye decolorization with Horse Radish Peroxidase (HRP)

HRP is extracted from horse radish roots, and its performance has been evaluated in soluble and immobilized form by conducting batch experiments in the presence of H₂O₂ (Vasantha et al., 2006). The oxidation of Direct Yellow 12 dye has been tested as a function of HRP at fixed concentration of H₂O₂, and at constant HRP activity (1.8 units/ml). The optimum contact time required for dye removal was 1 h 45 min for vials containing 5 ml of dye solution (10 mg/L), 1.8 units of enzyme 1.5 ml/L of H₂O₂ were added and the reaction mixture (24°C, pH 4) was agitated for 2 h 15 min. After this period, dye removal was negligible up to remaining 1 h 35 min.

The Direct Yellow 12 dye at varying aqueous-phase pH of the reaction mixture between 2 and 10 by keeping the dye concentration at 10 mg/L, enzyme concentration at 1.8 units, H₂O₂ dose at 1.5 ml/L, reaction temperature at 24°C and the contact time (1 h 45 min) constant exhibited 70% of the dye removal at an aqueous-phase pH of 4. The dye removal dropped significantly from pH 5 to 8 and the same trend continued up to an aqueous phase of pH 10. Aqueous phase of pH 4 resulted in higher HRP activity compared to other pH ranges from 3 to 9. Hydrogen peroxide acts as a co-substrate to activate the enzymatic action of peroxide radical. It contributed in the catalytic cycle of peroxidase oxidizing the native enzyme to form an enzymatic intermediate which accepts the aromatic compound to carry

out its oxidation to form a free radical form. Experiments were carried out to find out the optimum H_2O_2 concentration required to bring out the conversion of dye by varying the H_2O_2 dose from 1 to 3 ml/L in the reaction mixture by keeping all the other experimental conditions constant (dye concentration 10 mg/L; temperature 24°C ; enzyme concentration 1.8 units; reaction time 1 h 45 min). Their findings indicate maximum dye removal at H_2O_2 concentration of 2 ml/L and similar degradation with 2.5 and 3 ml/L; therefore, 2 ml/L was taken as the optimum H_2O_2 dose for dye removal (Vasantha et al., 2006).

The concentration of the substrate present in the aqueous phase significantly influences enzyme-mediated reaction. If the amount of enzyme concentration is kept constant and the substrate concentration is gradually increased the reaction will increase until it reaches maximum. After obtaining the equilibrium state, further addition of the substrate will not change the rate of reaction. Studies carried out at different concentrations of the dye, i.e. 5–40 mg/L, keeping the other parameters constant indicated a dye concentration of 25 mg/L to be the cut-off concentration of the dye for optimum removal at the specified experimental conditions along with retaining the activity of HRP enzyme and to protect the protein from denaturation.

Application of free enzyme in industrial process is not economically viable, while immobilization/ entrapment of enzyme results in repeated application. Two types of polymeric materials—alginate and acryl amide—have been used to study their relative efficiency in dye removal for the entrapment of peroxidase. Normally, enzyme immobilization is expected to provide stabilization effect restricting the protein unfolding process as a result of the introduction of random intra- and intermolecular crosslinks. Zille et al. (2003) have reported less availability of the enzyme for interaction with anionic dyes due to the immobilization in a particular matrix. For the immobilization of HRP enzyme acryl amide gel was more efficient in dye removal compared to alginate matrix. About 78% of dye removal was observed with acryl amide gel-immobilized beads, while with alginate matrix it was only 52%. Gel-immobilized HRP was effective in dye removal compared to free HRP (69%), and alginate-immobilized HRP showed inferior performance.

The effect of aqueous-phase pH on the enzyme-catalyzed degradation with alginate- and acryl amide-immobilized HRP enzyme suggests that after pH 4 there is a decrease in the dye removal capacity for both types of the entrapped matrices. About 78% and 54% of the dye removal was observed at an aqueous phase of pH 4 for acryl amide and alginate-entrapped beads respectively. The relative inferior performance of alginate-immobilized HRP compared to acryl amide may perhaps be due to lesser availability of the peroxidase structure to the dye molecule in the alginate mix compared to acryl amide. The effective performance of acryl amide-entrapped beads may be attributed to the nonionic nature of the beads, which results in minimum modification of the enzyme properties and unaffected nature of the charged substrate as well as product diffusion. The electron-withdrawing nature of the azo linkages obstructs the susceptibility of azo dye molecules to oxidative reactions. Only specialized azo dye-reducing enzymes could degrade azo dyes. In addition, when Direct Yellow 12 dye was reduced using the enzymatic method, the oxidation capacity increased with increasing concentrations of HRP and H_2O_2 at pH 4. Gel/alginate based enzyme immobilization reduced the azo dye. The immobilized enzyme beads could further be used two–three times for the removal of the same dye with lower efficiency. The application of enzyme-based systems in waste treatment is unusual, given that many drawbacks are derived from their use, including low efficiency, high costs and easy deactivation of the enzyme.

2.4 Dye color removal with Bitter Gourd (*Momordica charantia*) Peroxidase

Application of partially purified BGP (Bitter Gourd Peroxidase) in the decolorization of textile and other industrially important dyes has been explored. Simple ammonium sulphate precipitated proteins from bitter gourd were taken for the treatment of a number of dyes present in polluted wastewater (Akhtar et al., 2005a, b). Partially purified preparation of BGP was obtained by adding 20–80% ammonium sulphate and this preparation exhibited a specific activity of 99.0 EU (Enzyme units) of peroxidase/mg protein. Peroxidases from bitter gourd were highly stable against pH, heat, urea, water miscible organic solvents, detergents and proteolysis. The dye solution was stable upon exposure to H₂O₂ or to the enzyme alone. The dye precipitation was due to H₂O₂-dependent enzymatic reaction, possibly involving free-radical formation followed by polymerization and precipitation.

Eight textile reactive dyes were tested with increasing concentration (0.133–0.339 EU of BGP/ml) of reaction volume for 2 h at 37°C. The decolorization of Reactive Red 120, Reactive Blue 4, Reactive Blue 160 and Reactive Blue 171 was continuously enhanced by adding increasing concentration of BGP. Reactive Blue 4 was completely decolorized with 0.399 EU of BGP/ml of reaction mixture in the absence of HOBT. Reactive Orange 4, Reactive Orange 86, Reactive Red 11 and Reactive Yellow 84 were recalcitrant to the BGP action. Four reactive textile dyes were treated by step-wise addition of enzyme, adding 0.133 EU of BGP/ml of reaction volume at each step after every 1 h, to the decolorizing solution. Each enzyme addition exhibited rapid disappearance of color. Reactive Blue 160 was completely decolorized after the third enzyme addition while Reactive Blue 4 was almost decolorized completely after the second enzyme addition. Reactive Blue 171 and Reactive Red 120 were decolorized to 56% and 39%, respectively after the third enzyme addition. However, Reactive Red 11, Reactive Orange 4, Reactive Orange 86 and Reactive Yellow 84 were recalcitrant to decolorization by BGP.

Of the reactive dyes incubated with 0.266 EU of BGP/ml of reaction volume for increasing time period only four dyes decolorized on treatment with BGP for 1h at 37°C. Although more color disappeared on incubation for longer duration, the rate of decolorization was slow. All the four dyes were decolorized with varying percentages (30–90%). The decolorization of dyes with 0.266 EU of BGP/ml of reaction volume on incubation at 37°C for 4 h was 88% for Reactive Blue 4, 71% for Reactive Blue 160, 31% for Reactive Blue 171 and 28% for Reactive Red 120. Rest of the four dyes: Reactive Red 11, Reactive Orange 4, Reactive Orange 86 and Reactive Yellow 84 were fully recalcitrant to decolorization by BGP even after 4 h incubation with similar treatment.

Thirteen different non-textile dyes were treated with 0.167 EU of BGP/ml of reaction volume at 37°C for 1h. Carmine, Methyl Orange, Methylene Blue, Coomassie Brilliant Blue G-250, Rhodamine 6G, Methyl Violet 6B, 1:2 naphthaquinone 4-sulphonic acid and Martius Yellow dyes were recalcitrant to decolorization by the BGP action or were slowly decolorized during the progress of the reaction. Maximum decolorization achieved by partially purified BGP was 53% for Naphthalene Black 12B, 57% for Coomassie Brilliant Blue R 250, 96% for Evans Blue, 51% for Eriochrome Black T and 86% for Celestine Blue. Twenty-one dyes used in this study were treated with BGP in the presence of 1.0 mM HOBT and 0.6 mM H₂O₂ at 37°C. Presence of HOBT drastically enhanced the rate of decolorization of recalcitrant dyes. Reactive Orange 4, Reactive Red 11, Reactive Yellow 84 and Reactive Orange 86 were recalcitrant to decolorization in the absence of HOBT. However, these dyes were decolorized up to 98%, 80%, 70% and 78%, respectively by the action of 0.399 EU/ml of BGP in the presence of 1.0 mM HOBT at 37°C for 2 h. Non-textile

dyes e.g., Carmine, Methyl Orange, Coomassie Brilliant Blue G250, Rhodamine 6G, Methylene Blue and Methyl Violet 6B, which were recalcitrant to decolorization by BGP in absence of HOBT, were almost completely decolorized in presence of 1.0 mM HOBT. However, 1:2-naphthaquinone 4-sulphonic acid was recalcitrant to decolorization even in the presence of HOBT.

Decolorization of textile reactive dyes in the absence of HOBT occurred by the formation of precipitate, which settled down and removed by centrifugation. Several earlier investigators have shown that the treatment of phenols and aromatic amines by peroxidases and tyrosinases resulted in the formation of large insoluble aggregates. However, the decolorization of textile and other dyes by BGP in presence of 1.0 mM HOBT appeared without the formation of any precipitate. It suggested that the decolorization of dyes took place via degradation of aromatic ring of the compounds or by cleaving certain functional groups as reported elsewhere (Christian et al., 2003; Husain et al., 2009). HOBT could have a dual role, first as a mediator by increasing the substrate range of dyes for BGP and second enhancing the rate of oxidation.

Complex mixtures of various reactive textile and other industrially important dyes have been studied by mixing three or four different dyes in equal proportions and incubated with 0.266 EU of BGP/ml in the presence of 1.0 mM HOBT and 0.6 mM H₂O₂ for 1h at 37°C. Wave length maxima for each dye mixture were determined and decolorization of mixtures was monitored after the incubation period. All the mixtures decolorized by more than 80%. The decolorization rate of mixtures of dyes was slower than that of pure dye solution. This supports an earlier observation that the biodegradation of various phenols in the form of mixtures was quite slow compared to the independent phenol (Kahru et al., 2000). Bourbonnais and Paice (1990) described for the first time the use of Redox mediators by allowing laccase to oxidize non-phenolic compounds thereby expanding the range of substrates that can be oxidized by this enzyme. The mechanism of action of laccase mediator system has been extensively studied and it is used in the textile industry in the finishing process for indigo stained materials. Several workers have demonstrated that the use of Redox mediator system enhanced the rate of dye decolorization by several folds but these mediators were required in very high concentrations (5.7 mM violuric acid/laccase system, 11.6 mM of HOBT/laccase system) (Soares et al., 2001a,b; Claus et al., 2002). For the first time, it was shown that the decolorization of dyes by BGP was effective at very low concentration of HOBT (1.0 mM). The rate of non-textile dyes decolorization was also enhanced by 2-100 folds (Akhtar et al., 2005a). Several reports indicate the enhancement of laccase activity by free radical mediators; however the enhancement of peroxidase activity by a Redox mediator has been studied for the first time. Peroxidase/ Redox mediator system will prove a sensitive and an inexpensive procedure for the treatment of dyes present in complex mixtures/industrial effluent.

2.5 Dye color removal with tomato (*Lycopersicon esculentum*) peroxidase

Matto and Husain (2009) investigated the role of concanavalin A (Con A)-cellulose-bound tomato peroxidase for the decolorization of direct dyes. Cellulose was used as an inexpensive material for the preparation of bioaffinity support. Con A-cellulose-bound tomato peroxidase exhibited higher efficiency in terms of dye decolorization as compared to soluble enzyme under various experimental conditions. Both Direct Red 23 and Direct Blue 80 dyes were recalcitrant to the action of enzyme without a Redox mediator. Six compounds were investigated for Redox-mediating property. Immobilized peroxidase decolorized both

dyes to different extent in the presence of all the used Redox mediators. However, 1-hydroxybenzotriazole emerged as a potential Redox mediator for tomato peroxidase catalyzed decolorization of direct dyes. These dyes were maximally decolorized at pH 6.0 and 40°C by soluble and immobilized peroxidase. The absorption spectra of the untreated and treated dyes exhibited a marked difference in the absorption at various wavelengths. Immobilized tomato peroxidase showed a lower Michaelis constant than the free enzyme for both dyes. Soluble and immobilized tomato peroxidase exhibited significantly higher affinity for Direct Red 23 compared to Direct Blue 80.

2.6 Dye decolorization with Pointed Gourd (*Trichosanthes dioica*) Peroxidase

The effects of *Trichosanthes dioica* peroxidase along with Redox mediators on decolorization of water insoluble disperse dyes; Disperse Red 19 (DR19) and Disperse Black 9 (DB9) have been studied by Jamal et al., (2010). Nine different Redox mediators; bromophenol, 2, 4-dichlorophenol, guaiacol, 1-hydroxybenzotriazole, m-cresol, quinol, syringaldehyde, vanillin and violuric acid were evaluated [Figure-1]. Among the chosen mediators, 1-hydroxybenzotriazole was most effective for decolorization with PGP (Pointed Gourd Peroxidase). At a concentration of 0.45U/mL the peroxidase could decolorize Disperse Red 19 to a maximum of 79% with 0.2mM 1-hydroxybenzotriazole whereas Disperse Black 9 decolorized upto 60% with 0.5mM 1-hydroxybenzotriazole [Figure-2]. The time, pH and temperature at which maximum decolorization were recorded was 60 min, 4 and 42°C. It has been observed that the dye solutions were recalcitrant upon exposure to HOBT, H₂O₂ or to the enzyme alone but the enzyme in the presence of Redox mediators was much effective in performing the decolorization of the dyes, implying dye decolorization was a result of Redox mediated H₂O₂-dependent enzymatic reaction. It has already been reported that Redox mediators have the potential to mediate an oxidation reaction between a substrate and an enzyme.

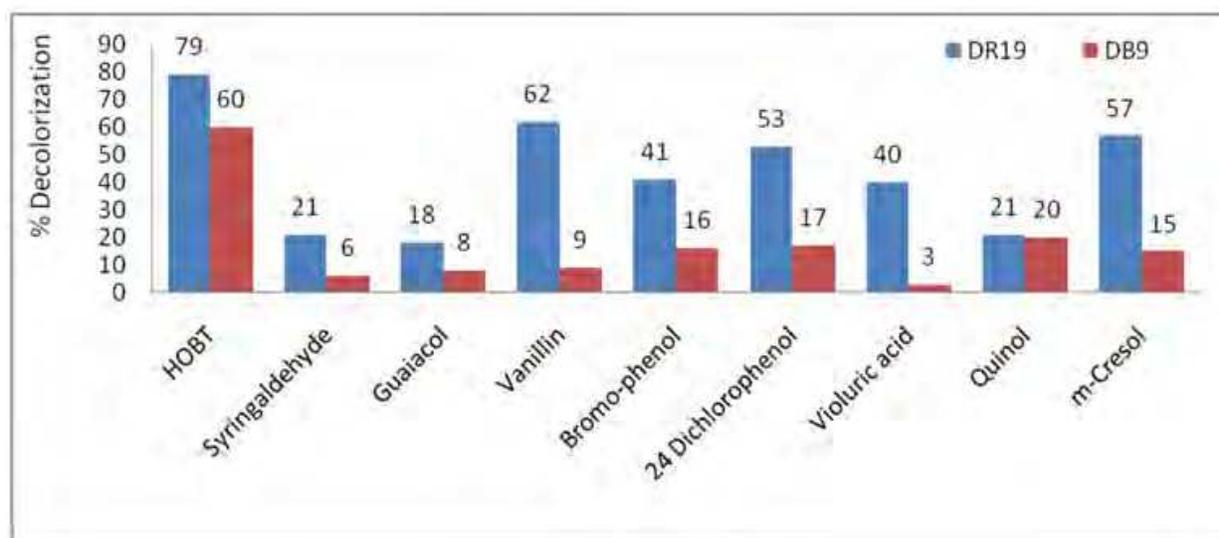


Fig. 1. Percent Dye decolorization as a function of different Redox mediators. The dyes DR19 (25mgL⁻¹,5.0mL) & DB9(50 mgL⁻¹,5.0mL) solutions were incubated independently with PGP (0.45 Uml⁻¹) in the presence of 0.5mM concentration of each Redox mediators; other conditions were 0.8mM H₂O₂, 100mM glycine HCl buffer, pH 4.0 for 60 min at 37°C. (λ_{max} for DR19 and DB9 are 495nm & 461nm respectively) [Jamal et al., 2010]

Different Redox mediators have different mediation efficiency which is governed by Redox potential of the mediator and the oxidation mechanism of the substrate. Oxidation of substrate occurs by free radical formation by the mediator. The free radicals can be formed either by one-electron oxidation of substrate or by abstraction of a proton from the substrate. The pointed gourd peroxidase was effective in decolorizing the dyes at low concentrations of HOBT. Although the extent of decolorization of DR19 and DB9 increased with increasing concentrations of HOBT, the maximum decolorization was observed to be 79% and 60% with 0.2 mM and 0.5 mM for DR19 and DB9, respectively. Further addition of HOBT resulted in a slow decrease in decolorization of both the dyes. This inhibition could likely be due to the high reactivity of HOBT radical, which might undergo chemical reactions with side chains of aromatic amino acid by enzyme thereby; inactivating it. Hence, the dosage of Redox mediator is an important factor for the enzyme-mediated decolorization.

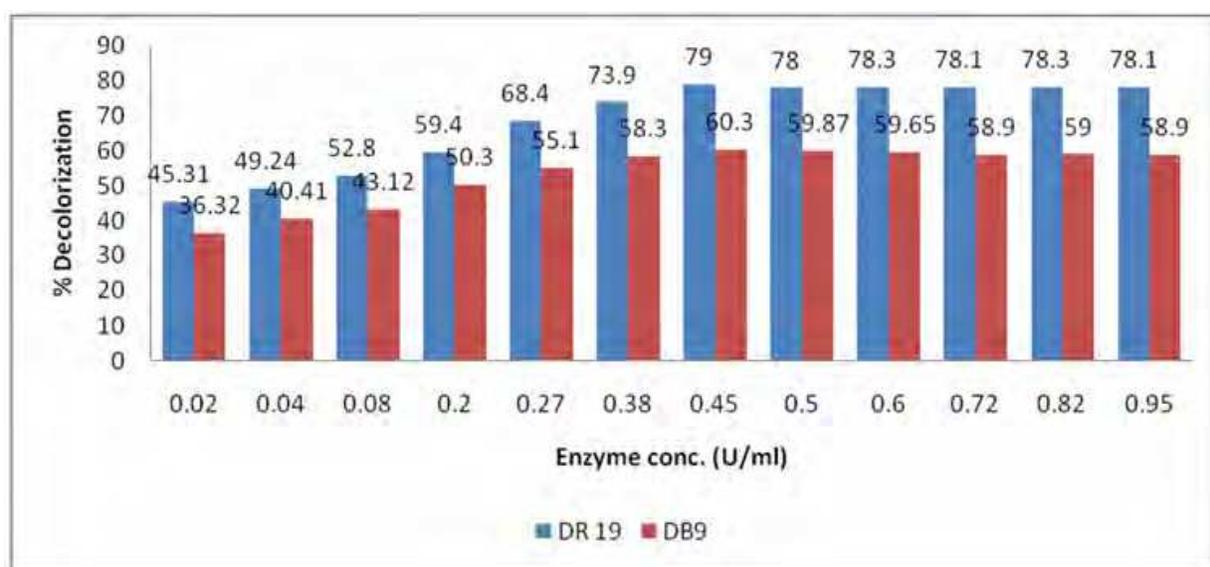


Fig. 2. Percent Dye decolorization as a function of different enzyme (PGP) concentrations. The dyes DR19 (25mgL⁻¹,5.0mL) & DB9(50 mgL⁻¹,5.0mL)solutions were incubated independently with PGP (0.02 to 0.95 Uml⁻¹) in the presence of 0.2mM and 0.5mMconcentration of HOBT for DR19 and DB9 respectively; other conditions were 0.8mM H₂O₂, 100mM glycine HCl buffer, pH 4.0 for 60 min at 37°C. (λ_{max} for DR19 and DB9 are 495nm & 461nm respectively) [Jamal et al., 2010].

The enzyme reacted well to decolorize both the dyes in the presence of 0.8 mM H₂O₂ [Figure-3]. The maximum decolorization was obtained at 0.8mM H₂O₂ which is slightly higher than reported for soybean peroxidase, bitter gourd peroxidase (BGP) and turnip peroxidase. The reaction temperature is an important parameter which effects the decolorization of dyes. The maximum decolorization for both the dyes DR19 and DB9 was at 42°C [Figure-4]. It has been reported that BGP mediated disperse dye decolorization was optimal at 40°C.

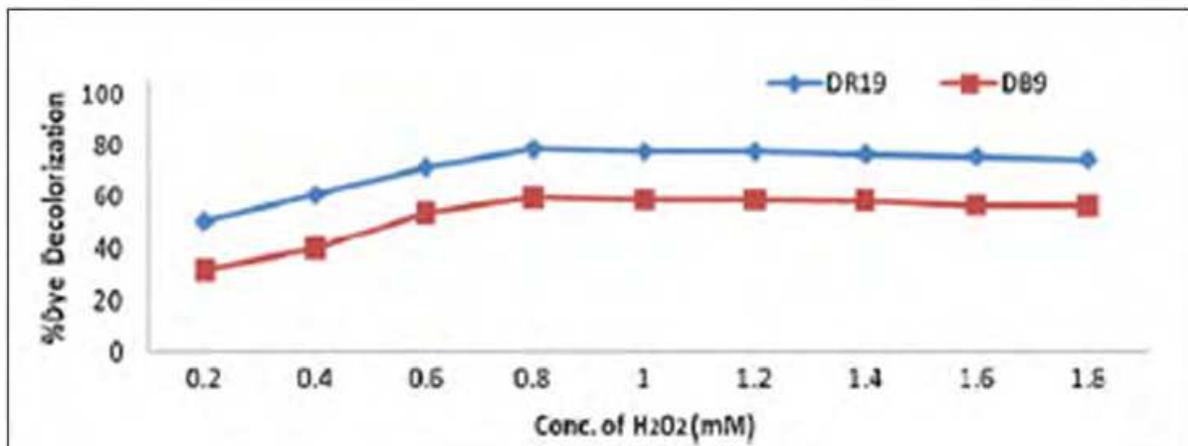


Fig. 3. Percent Dye decolorization at different concentrations of H₂O₂. The dyes DR19 (25mgL⁻¹,5.0mL) & DB9(50 mgL⁻¹,5.0mL) solutions were incubated independently with H₂O₂ concentrations and PGP (0.45 Uml⁻¹) in the presence of varying amounts of H₂O₂ (0.2 to 1.8 mM) for DR19 and DB9 respectively; and other conditions were 100mM glycine HCl buffer, pH 4.0 for 60 min at 37°C. (λ_{max} for DR19 and DB9 are 495nm & 461nm respectively) [Jamal et al., 2010].

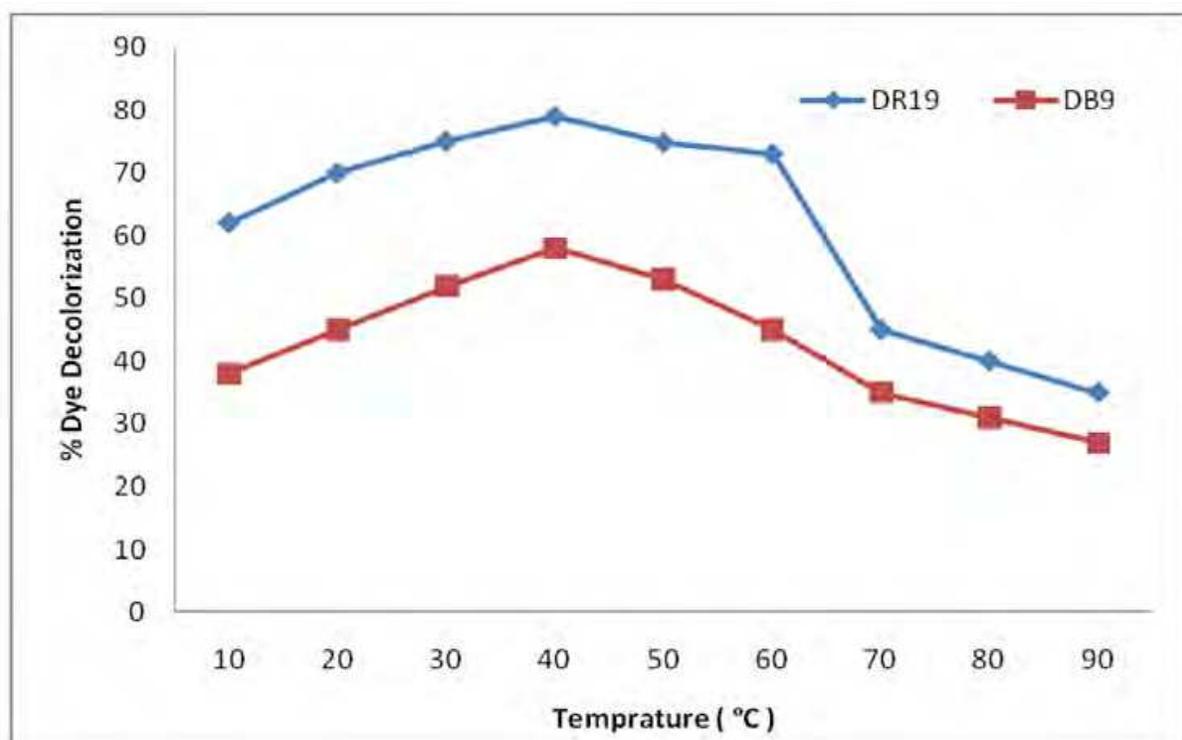


Fig. 4. Percent Dye decolorization as a function of temperature. The dyes DR19 (25mgL⁻¹,5.0mL) & DB9 (50 mgL⁻¹,5.0mL)solutions were incubated independently with PGP (0.45 Uml⁻¹) in the presence of HOBT, 0.8mM H₂O₂, 100mM glycine HCl buffer, pH 4.0 for 60 min at temperatures (20°C to 90°C). (λ_{max} for DR19 and DB9 are 495nm & 461nm respectively) [Jamal et al., 2010].

The maximum decolorization of DR19 and DB9 was obtained at an acidic of pH 4.0 [Figure-5]. It has earlier been reported that the degradation of industrially important dyes by enzymes such

as horse radish peroxidase, polyphenol oxidase, BGP and laccase was also maximum in the buffers of acidic pH. DR19 and DB9 were maximally decolorized within 20 min of incubation [Figure-6]. There was slow and gradual enhancement of decolorization upto 60 min of incubation. It was also evident from the observation that DR19 was decolorized to a greater extent within 20 min in the presence of only 0.2 mM HOBT. However, DB9 was decolorized maximally in the presence of 0.5 mM HOBT and decolorization rate was slow. This is consistent with reports that decolorization rate varies, depending upon the type of dye to be treated.

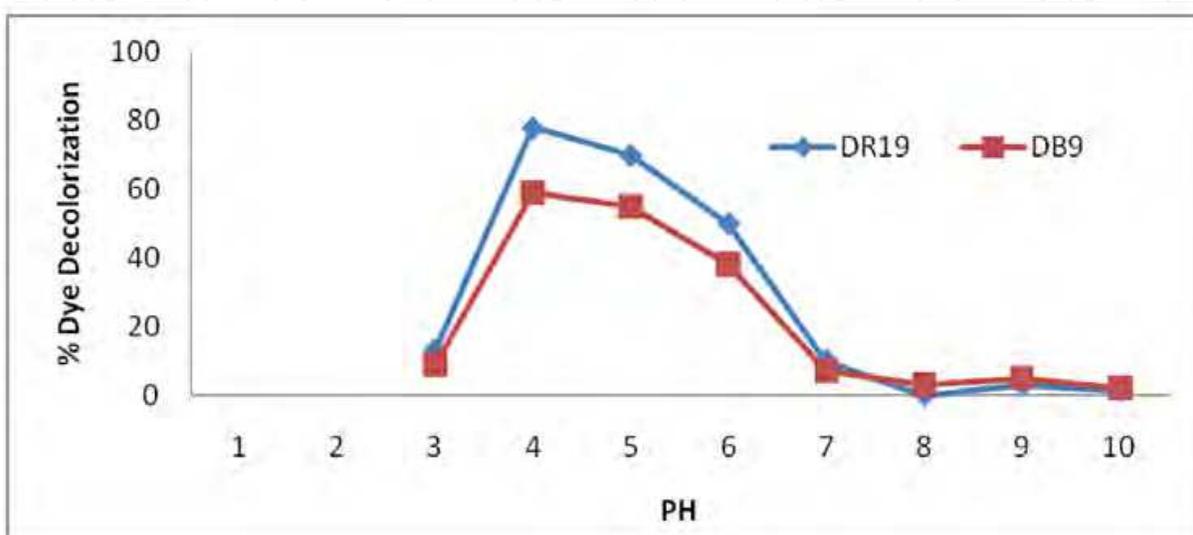


Fig. 5. Percent Dye decolorization as a function of pH. The dyes DR19 (25mgL⁻¹, 5.0mL) & DB9 (50 mgL⁻¹,5.0mL) solutions were incubated independently with PGP (0.45 Uml⁻¹) in the presence of HOBT, 0.8mM H₂O₂, 100mM glycine HCl buffer at different pH (2,3,4,5,6,7,8,9 and 10) for 60 min at 37°C. (λ_{max} for DR19 and DB9 are 495nm & 461nm respectively) [Jamal et al., 2010].

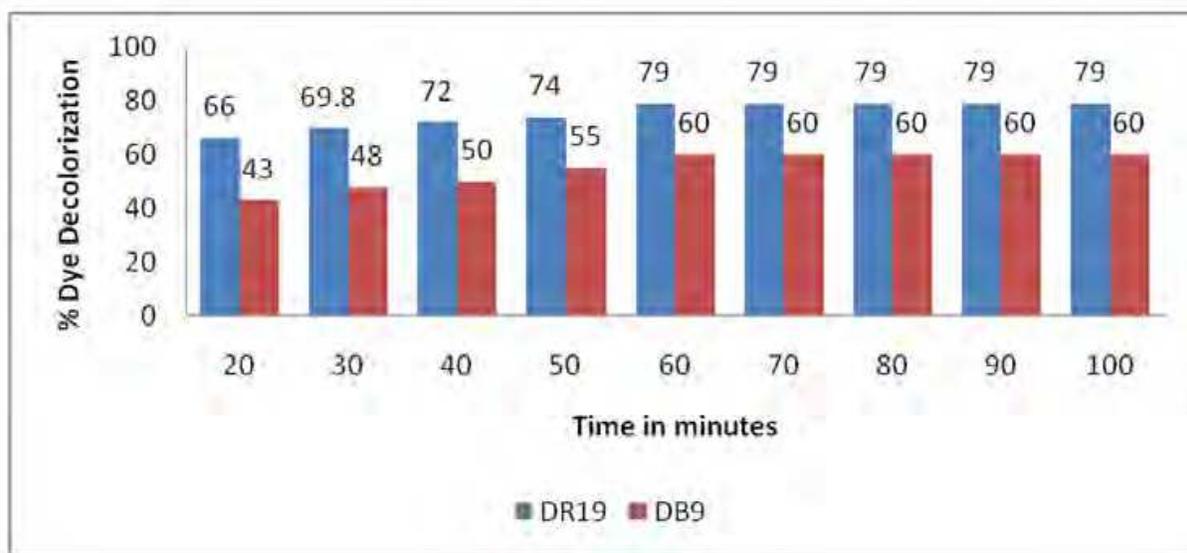


Fig. 6. Percent Dye decolorization as a function of time. The dyes DR19 (25mgL⁻¹, 5.0mL) & DB9(50 mgL⁻¹,5.0mL) solutions were incubated independently with PGP (0.45 Uml⁻¹) in the presence of HOBT, 0.8mM H₂O₂, 100mM glycine HCl buffer, pH 4.0 and for time 20 to 100 min at 37°C. (λ_{max} for DR19 and DB9 are 495nm & 461nm respectively) [Jamal et al., 2010].

2.7 *Trichosanthes dioica* proteins in decolorizing industrially important textile, non-textile dyes and dye mixtures

In one of my recent works, the competency of *Trichosanthes dioica* proteins in decolorizing industrially important textile, non-textile dyes and dye mixtures in the presence of Redox mediators under varying experimental conditions of pH, temperature, time intervals and enzyme concentration on the basis of one-factor-at-a-time (OFAT) method has been studied. All the textile dyes and non-textile dyes, ammonium sulphate, and Tween-20 were procured from Sigma Chemical Co. (St. Louis, MO, USA). Redox mediator's viz., 1-hydroxybenzotriazole (HOBT) and vanillin were obtained from SRL Chemicals (Mumbai, India). All other chemicals were of analytical grade. The pointed gourds were purchased from the local market. The samples were aseptically transferred into sterilized plastic bags.

Briefly, 100 g of pointed gourd was homogenized in 180 ml of 100 mM sodium acetate buffer, pH 5.6. The homogenate was filtered through multi-layers of cheese cloth and then centrifuged at the speed of $10,000 \times g$ on a Remi C-24 cooling centrifuge for 30 min at 4°C. The clear solution thus obtained was used for salt fractionation by adding 10% to 80% (w/v) $(\text{NH}_4)_2\text{SO}_4$. The proteins were precipitated by continuously stirring at 4°C overnight. The precipitate was collected by centrifugation at $10,000 \times g$ on a Remi C-24 cooling centrifuge, dissolved in 100 mM sodium acetate buffer, pH 5.6 and dialyzed against the assay buffer (0.1 M glycine HCl buffer, pH 4.0) (Akhtar et al., 2005a). Protein concentration was estimated by taking BSA as a standard protein and following the procedure of Lowry et al (1951). Peroxidase activity was determined by a change in the optical density ($A_{460 \text{ nm}}$) at 37°C by measuring the initial rate of oxidation of 6.0 mM o-dianisidine HCl in the presence of 18.0 mM H_2O_2 in 0.1 M glycine-HCl buffer, pH 4.0, for 20 min at 37°C. One unit of activity was defined as the amount of enzyme that transformed $1 \mu\text{mol}$ of o-dianisidine HCl as substrate per min.

The dyes (45-210 mg/l) were solubilized in 100 mM glycine HCl buffer, pH 4.0. Each dye was independently incubated with pointed gourd peroxidase (PGP) (0.45 EU/mL) in 100 mM glycine HCl buffer, pH 4.0 in the presence of 0.80 mM H_2O_2 for varying times at 37°C. The reaction was stopped by boiling at 100°C for 7 min. Dye decolorization was monitored by measuring the difference at the maximum absorbance for each dye as compared with control experiments without enzyme on UV-visible spectrophotometer (JASCO V-550, Japan). Untreated dye solution (inclusive of all reagents except the enzymes) was used as control (100%) for the calculation of percent decolorization. The dye decolorization was calculated as the ratio of the difference of absorbance of treated and untreated dye to that of treated dye and converted in terms of percentage.

2.7.1 Effect of Redox mediators on decolorization profile of textile and non-textile dyes

The effect of different Redox mediators on the dye decolorization by PGP is shown in **Table-1**. Out of the two different Redox mediators studied for dye decolorization, HOBT was more effective in decolorizing the dyes under study. The extent of decolorization in the presence of HOBT was in the range of 98.6% to 69.8% for the reactive dyes whereas the disperse dyes studied exhibited decolorization in the range of 79.2% to 61.2%. The effective HOBT concentrations were 1.0 mM and 0.2 mM for reactive and disperse dyes, respectively. The dye decolorization with vanillin was 71.2% to 60.2% for reactive dyes and 55.3% to 34.5% for disperse dyes at 1.0 mM concentration. **Table-1** shows the effect of increasing concentrations of HOBT and vanillin (0.05 to 1.5 mM) on textile and other dyes. With increasing concentration of HOBT or vanillin there was an increase in the extent of decolorization of

both dyes. However, the decolorization of each textile dye was lower at each of the varying concentration of vanillin than HOBT. The non-textile dyes were effectively decolorized at 1.0 mM of HOBT and vanillin independently but here too, influence of HOBT was substantial. There was not much effect in percent decolorization of the dyes above these concentrations of HOBT or vanillin.

Textile Dyes (λ_{max})	Percent Dye Decolorization in the presence of <i>T.dioica</i> peroxidase and Redox Mediators at varying concentrations (mM) incubated for 2hrs									
	HOBT					Vanillin				
	0.05	0.10	0.20	1.0	1.5	0.05	0.10	0.20	1.0	1.5
Reactive Blue 15 (675 nm)	86.5	89.7	97.6	98.6	98.5	46.6	56.7	64.8	71.2	71.1
Reactive Orange 15 (494 nm)	61.3	67.5	71.2	74.6	74.6	35.4	45.2	52.3	60.7	60.5
Reactive Red 4 (517 nm)	61.6	65.4	67.9	68.2	68.1	32.2	43.2	53.1	61.2	61.1
Reactive Yellow 2 (404 nm)	63.1	65.6	67.6	69.8	69.8	37.6	42.3	54.3	67.2	67.1
Disperse Black 9 (464 nm)	54.2	58.7	69.1	68.7	69.1	15.2	24.2	34.5	55.3	55.2
Disperse Orange 25 (457 nm)	56.1	56.6	61.2	61.1	60.9	28.9	37.8	43.7	45.7	45.6
Disperse Red 19 (495 nm)	67.1	69.9	79.2	78.9	79.1	43.6	56.7	61.2	67.9	67.8
Disperse Yellow 7 (385 nm)	58.9	61.3	67.3	67.2	67.3	15.6	24.8	37.9	37.9	37.8
Non Textile Dyes (λ_{max})	0.05	0.10	0.20	1.0	1.5	0.05	0.10	0.20	1.0	1.5
Celestine Blue (642 nm)	46.2	52.3	66.8	79.6	79.3	39.7	46.8	61.3	74.9	74.2
Coomassie Brilliant Blue R250 (553 nm)	56.3	69.4	88.9	98.9	98.1	42.1	61.2	76.2	79.8	79.3
Methylene Blue (664 nm)	60.1	67.8	89.3	98.6	98.5	56.7	68.7	71.2	73.2	73.2
Eriochrome Black T (503 nm)	59.3	68.7	90.2	92.5	92.2	45.3	54.5	68.9	79.8	79.7
Evans Blue (611 nm)	67.5	76.4	87.7	97.1	96.8	43.8	61.3	69.9	78.3	78.2
Martius Yellow (430 nm)	6.4	15.6	35.3	39.1	39.1	5.1	13.4	20.9	33.8	33.7
Methyl Orange (505 nm)	64.2	75.6	87.9	98.4	98.4	55.6	67.3	66.7	78.5	78.4
Naphthol Blue Black (618 nm)	57.6	69.7	78.9	97.5	97.2	51.2	62.9	72.1	84.9	84.3
Rhodamine 6G (524 nm)	62.3	69.8	74.3	98.9	98.6	54.3	58.9	68.9	89.4	89.1

Table1. Percent Dye decolorization of different textile and non-textile dyes in the presence *T. dioica* peroxidase along with two different Redox mediators [HOBT and Vanillin].

2.7.2 Enzyme activity profile of *T. dioica* peroxidase mediated decolorization of textile dyes

Figure-7a shows the extent of decolorization of reactive and disperse dyes with increasing concentration of PGP. The maximal decolorization for these two different classes of dyes was observed at PGP concentration of 0.45 EU/ml after an incubation time of 4 h. Dye decolorization was not significantly exhibited with any further increase of PGP. The presence of 0.2 mM and 1.0 mM HOBT for decolorization of disperse and reactive dyes was effective in disappearance of Reactive Blue 15 and Reactive Orange 15 to the extent of 96.2% and 94.6% respectively whereas the others, Reactive Red 4 and Reactive Yellow 2 disappeared up to 88.2% and 89.8% respectively. Among the disperse dyes only Disperse Black 9 was effectively decolorized up to 79% whereas the others showed disappearance of color below 69.3%.

In the presence of 1.0 mM vanillin, partially purified *T. dioica* peroxidase catalyzed decolorization at 0.45 EU/ml after an incubation period of 4 h in the range of 61.4% to 71.3% for the dyes under study [Figure-7b]. The decolorization profile for all the dyes significantly increased after the first increase in peroxidase concentration in a period of 2 h. There was no significant change on dye decolorization either on increasing peroxidase concentration or the incubation time.

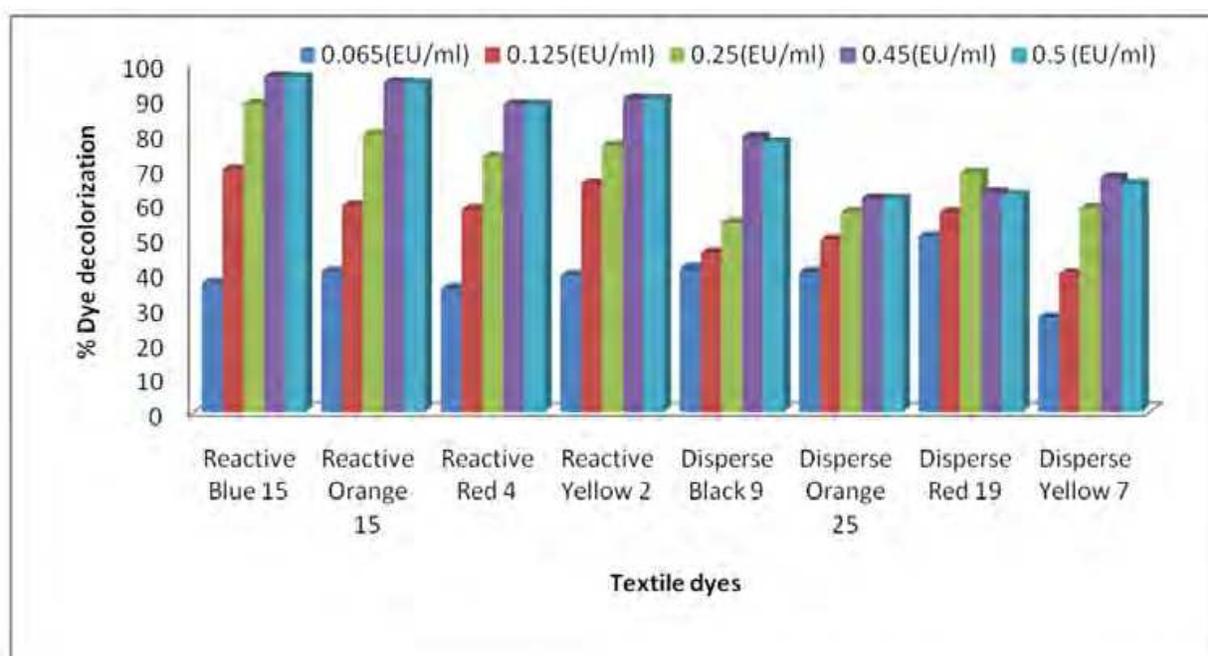


Fig. 7. (a) Percent Dye Decolorization of Textile Dyes in the presence of fixed concentration of HOBT [0.2 mM for Reactive Dyes and 1.0 mM for Disperse Dye] and increasing concentration of *T. dioica* peroxidase enzyme (EU/ml). Please see Table-1 for λ_{\max} of each dye.

2.7.3 H₂O₂ and pH activity profile of decolorization of textile dyes

Figure-8 shows that the percent decolorization improved with the increasing concentration of H₂O₂ and the maximum decolorization was observed at a concentration of 0.8 mM and 1.0 mM of H₂O₂ for disperse and reactive dyes respectively which remained substantially unaffected till 1.2 mM H₂O₂.

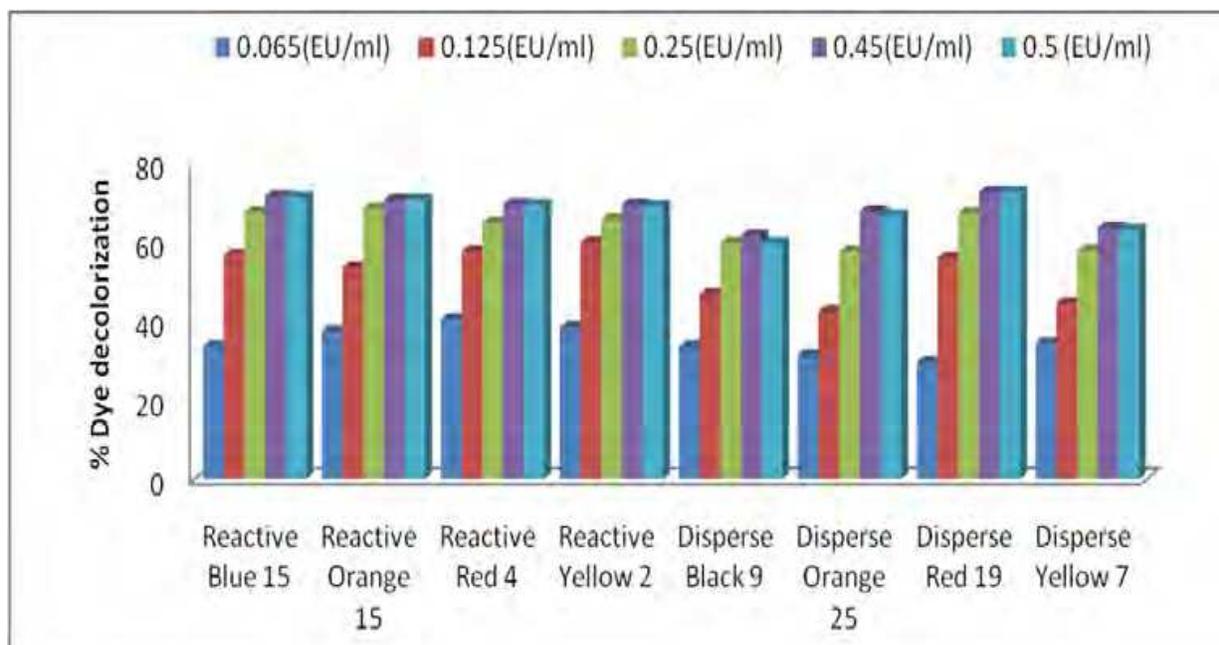


Fig. 7. (b) Percent Dye Decolorization of Textile Dyes in the presence of fixed concentration of vanillin [0.2 mM for Reactive Dyes and 1.0 mM for Disperse Dye] and increasing concentration of *T. dioica* peroxidase enzyme (EU/ml). Please see Table-1 for λ_{max} of each dye.

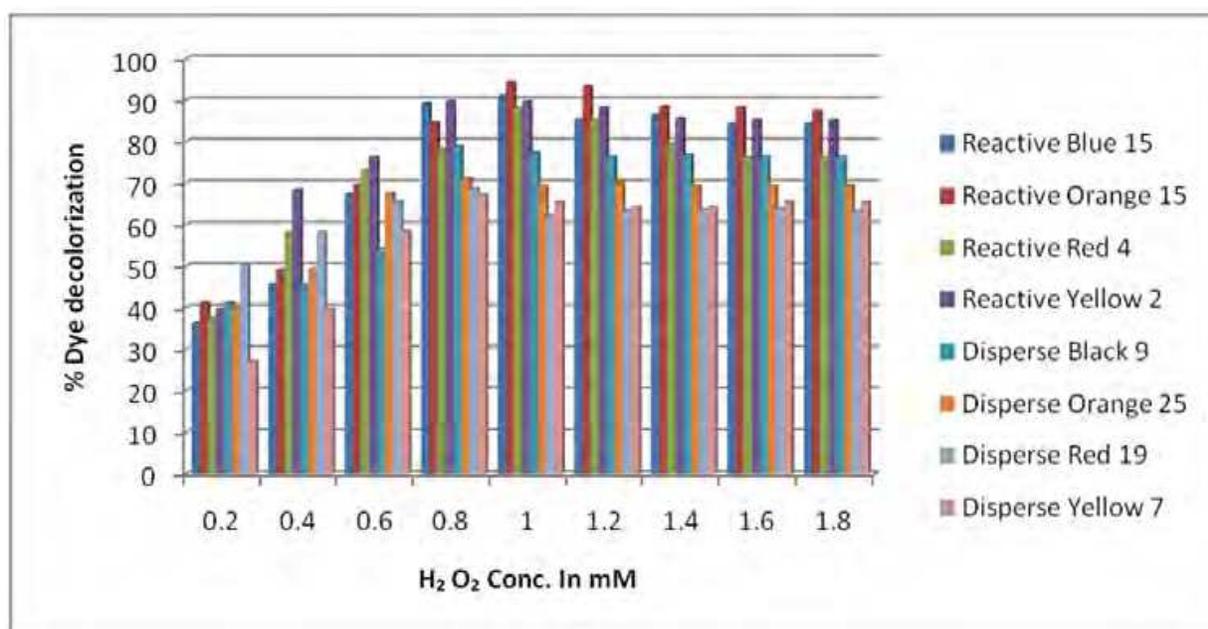


Fig. 8. Percent Dye Decolorization of Textile Dyes in the presence of fixed concentration of HOBT, *T. dioica* peroxidase enzyme (EU/ml) and varying concentration of H₂O₂. Please see Table-1 for λ_{max} of each dye.

To find out the range of pH in which significant decolorization was observed; buffers in the range of pH 2.0 to pH 10.0 were used. The percent decolorization is shown in [Figure-9]. An acidic range of pH (3.0 to 6.0) was better suited for dye decolorization. Maximum

decolorization was observed at pH 4.0 and pH 5.0 at fixed concentration of *T. dioica* peroxidase and HOBT for disperse and reactive dyes respectively. There was significant decrease in the extent of decolorization in an alkaline medium and at pH 10.0 the decolorization action of the enzyme was almost insignificant / lost.

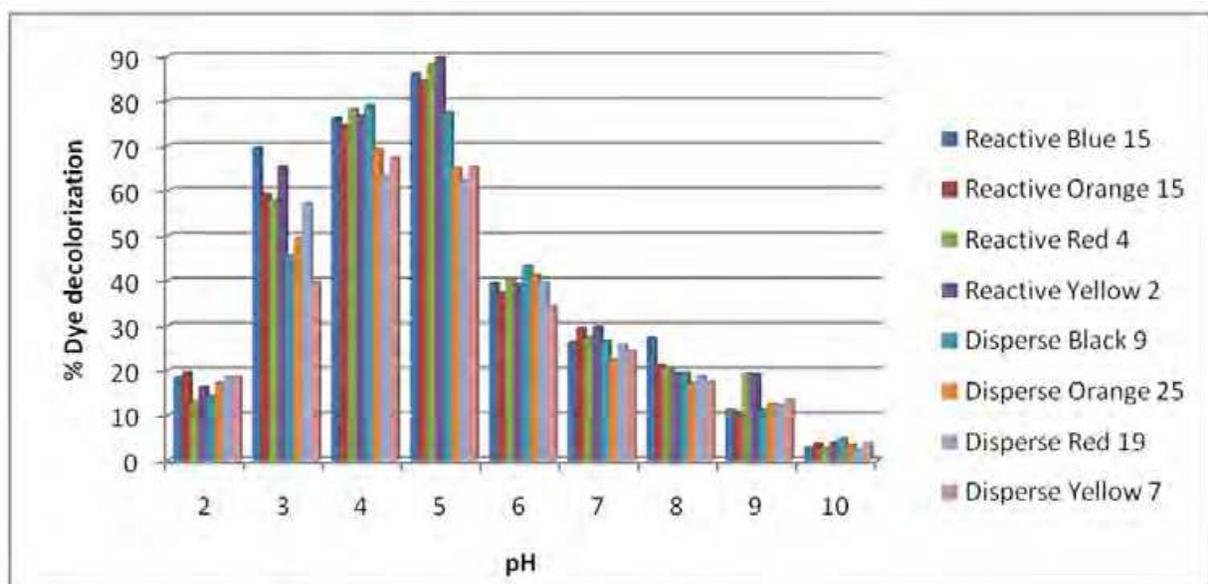


Fig. 9. Percent Dye Decolorization of Textile Dyes in the presence of fixed concentration of HOBT, *T. dioica* peroxidase enzyme (EU/ml) and varying pH. Please see Table-1 for λ_{\max} of each dye.

2.7.4 Temperature activity profile of decolorization of textile dyes

The percent decolorization was plotted as a function of temperature and the results are shown in [Figure-10]. Among the textile dyes the reactive dyes exhibited maximum decolorization at 50°C whereas the disperse dyes showed maximum decolorization at 40°C in the presence of 1.0 mM and 0.2 mM HOBT. Reactive Blue 15 (96.1%), Reactive Orange 15 (94.4%), Reactive Red 4 (85.2%) whereas disperse dyes under study decolorized in the range of 61.2% to 79%.

2.7.5 Time activity profile of decolorization of textile dyes

The extent of decolorization of textile dyes as a function of time is shown in [Figure-11]. Maximum decolorization of reactive and disperse dyes were observed within 1 h of incubation at 50°C and 40°C with 0.45 EU/ml of PGP and 1.0 mM and 0.2 mM HOBT respectively. However, further decolorization of these dyes progressed slowly upto 4 h, although no effective increase was observed even when the dyes were further incubated for longer times. Among the reactive dyes Reactive Blue 15 decolorized almost completely and Reactive Orange 15 up to 78.3% at 4 h incubation, whereas Reactive Red 4 and Reactive Yellow 2 showed decolorization up to 86.3% and 81.2% under similar conditions. The disperse dyes were comparatively resistant to decolorization and only in the presence of HOBT Disperse Red 19, Disperse Yellow 7, Disperse Black 9 decolorized to 79.5%, 72.3% and 71.6% respectively. Disperse Orange 15 was comparatively degraded and decolorized to a lesser extent under similar conditions with a maximum of 61.2%.

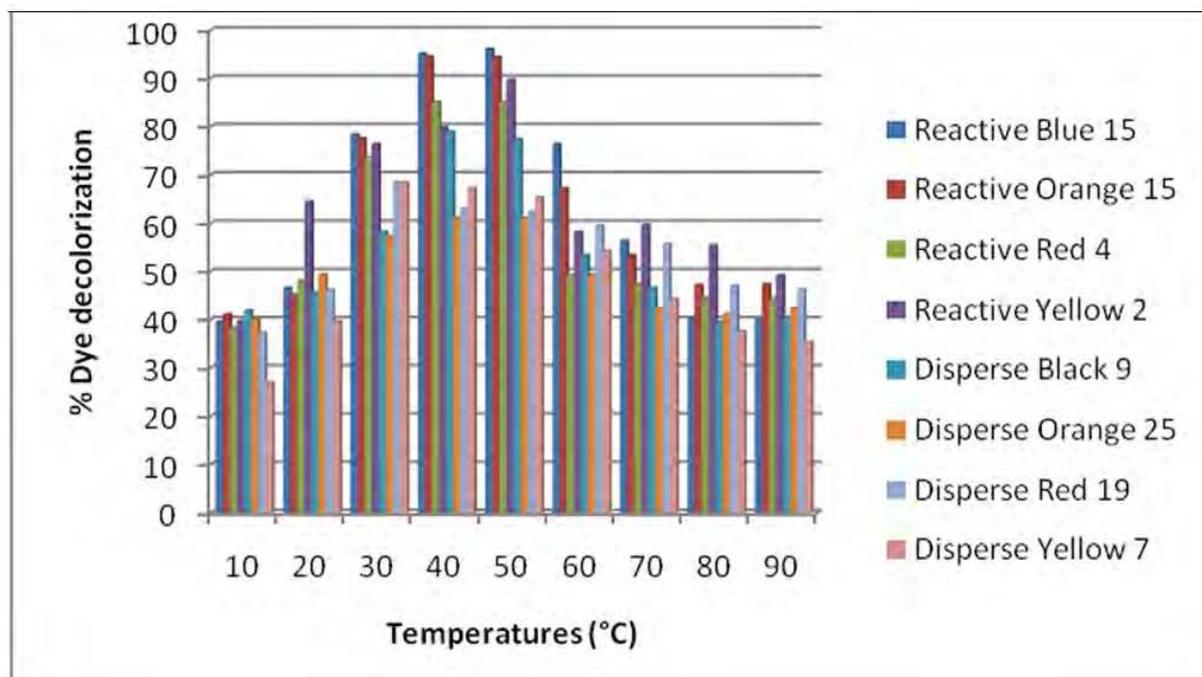


Fig. 10. Percent Dye Decolorization of Textile Dyes in the presence of fixed concentration of HOBT, *T. dioica* peroxidase enzyme (EU/ml) at different temperatures. Please see Table-1 for λ_{max} of each dye.

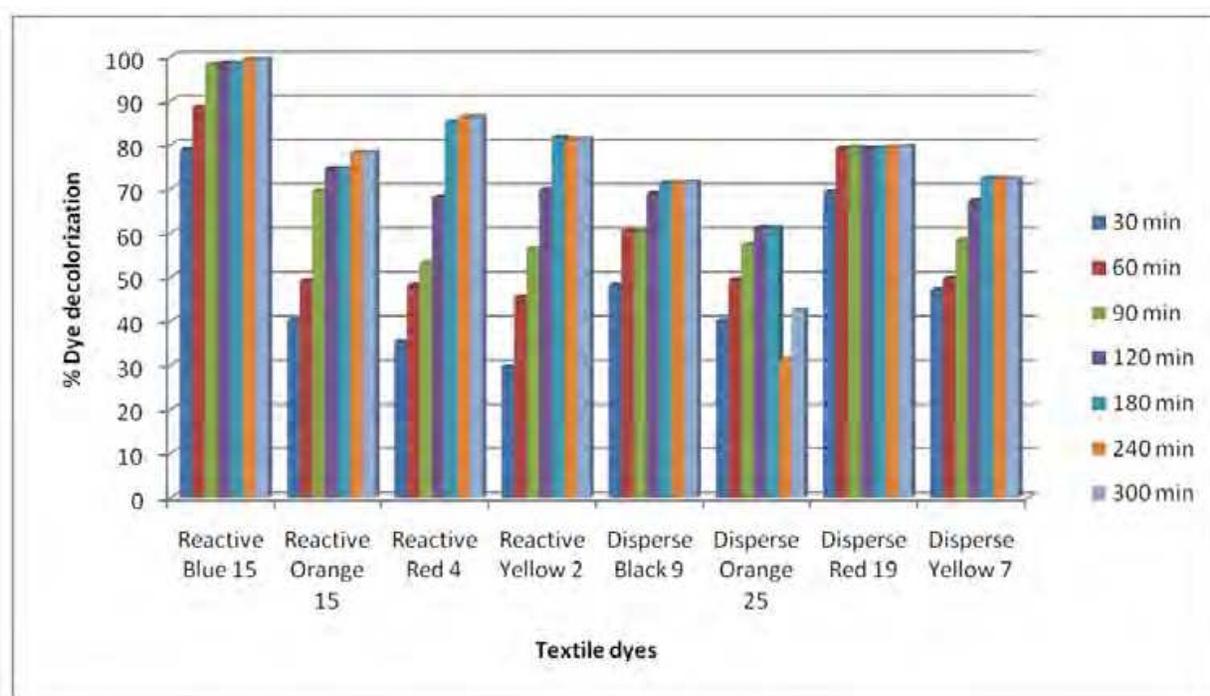


Fig. 11. Percent Dye Decolorization of Textile Dyes in the presence of fixed concentration of HOBT, *T. dioica* peroxidase enzyme (EU/ml) at different time interval. Please see Table-1 for λ_{max} of each dye.

2.7.6 Decolorization profile of non-textile dyes by *T. dioica* peroxidase as a function of enzyme concentration and time

Nine different non-textile dyes were studied. These dyes were treated with different amount of *T. dioica* peroxidase in the range of 0.065 EU/ml to 0.50 EU/ml and incubated for varying time interval with and without the Redox mediator HOBT (1.0 mM) at 37°C as shown in [Table-2]. The results indicated that few non-textile dyes viz., Methylene Blue, Martius Yellow, Methyl Orange, Rhodamine 6G were highly recalcitrant to decolorization by *T. dioica* in the absence of HOBT after 3h of incubation. However, the decolorization progressed slowly with the addition of 1.0 mM HOBT and percent decolorization achieved for Methylene Blue, Martius Yellow and Methyl Orange, was 98.6%, 35.1% and 98.7% respectively; whereas Rhodamine 6G almost decolorized completely. For other dyes, the maximum decolorization exhibited after 3h was 98.7%, 97.3%, 97.1%, 67.8% for Coomassie Brilliant Blue R 250, Naphthol Blue Black, Evans Blue and Eriochrome Black T respectively.

Non Textile Dyes (A _{max}) nm	0.065 EU/ml		0.125 EU/ml		0.250 EU/ml		0.45 EU/ml		0.50 EU/ml	
	-HOBT (60min)	+ HOBT (60min)	-HOBT (120min)	+HOBT (120min)	-HOBT (160min)	+HOBT (160min)	-HOBT (180min)	+HOBT (180min)	-HOBT (240min)	+HOBT (240min)
Celestine Blue (642 nm)	46.6	57.3	49.7	68.7	54.3	69.3	55.6	79.5	58.9	79.6
Coomassie Brilliant Blue R250 (553 nm)	47.6	92.4	48.2	93.2	51.3	98.9	51.2	98.7	51.1	98.7
Methylene Blue (664 nm)	2.5	67.2	3.7	78.5	3.6	98.4	3.5	98.6	3.2	98.9
Eriochrome Black T (503 nm)	48.7	67.7	51.6	77.4	55.4	91.3	55.3	92.0	55.1	92.2
Evans Blue (607nm)	86.4	94.3	87.5	95.4	87.8	96.7	87.2	97.1	86.7	97.2
Martius Yellow (433 nm)	10.2	33.2	12.4	34.5	13.2	35.6	13.5	35.1	12.3	35.2
Methyl Orange (464 nm)	12.3	87.2	13.2	89.2	13.2	98.5	13.1	98.7	12.2	98.6
Naphthol Blue Black (618 nm)	47.2	78.8	49.2	86.2	56.6	97.4	57.2	97.3	57.9	97.1
Rhodamine 6G (525 nm)	11.2	78.4	13.2	86.3	13.2	99.7	14.2	99.6	13.2	99.5

Table 2. Decolorization of Non textile dyes by *T. dioica* peroxidase at different enzyme concentration, incubation period and with fixed concentration of HOBT, H₂O₂.

2.7.7 Decolorization profile of different dye mixtures by *T. dioica* peroxidase

To simulate the decolorization of dyes from industrial effluent, complex mixtures of textile dyes including reactive, disperse and non-textile dyes were prepared by mixing four different dyes in equal proportions and incubated with 0.45 EU/ml of *T. dioica* peroxidase in the presence of 1.0 mM HOBT and 1.0 mM H₂O₂ for 2 h at 40°C. The decolorization was recorded at wave length maxima of each mixture determined spectrophotometrically. The combinations of different dyes showed decolorization by more than 82% [Figure-12]. The rate of decolorization of dye mixture was slower in comparison to that of individual dyes both in the presence and absence of HOBT. However, the HOBT mediated dye decolorization was more effective.

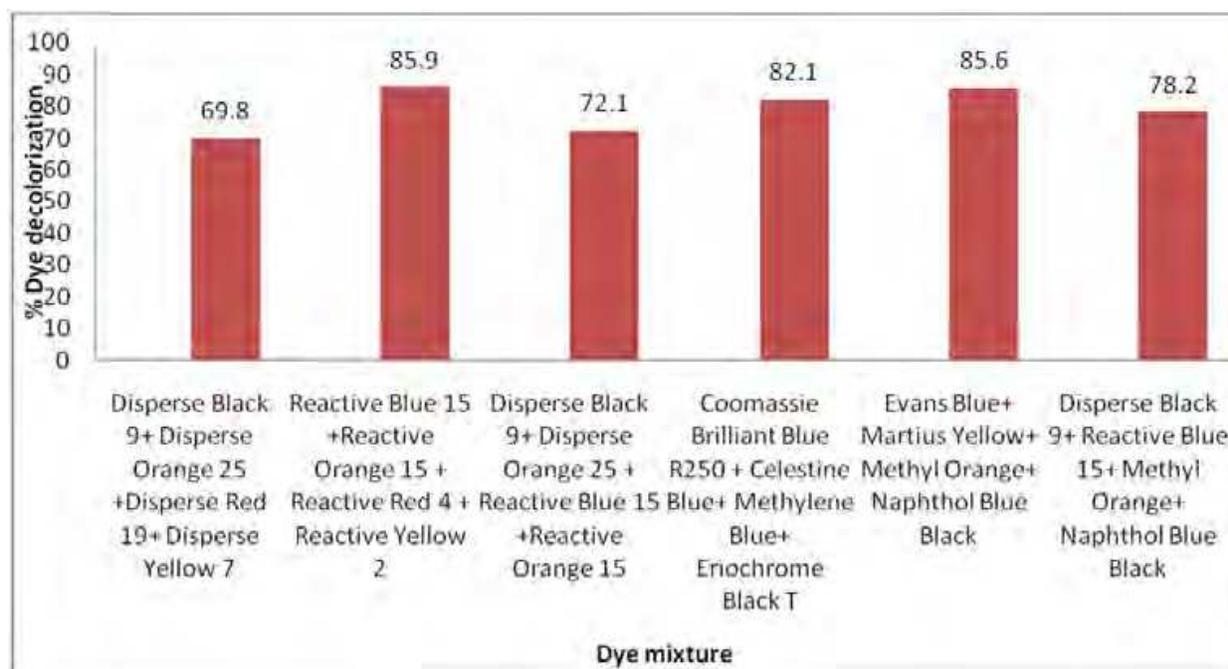


Fig. 12. Degradation/ decolorization of dye mixture by *T. dioica* in the presence of HOBT/H₂O₂ to mimic dyes in industrial effluents. [Disperse Black 9+ Disperse Orange 25 +Disperse Red 19+ Disperse Yellow 7 (λ_{461}); Reactive Blue 15 +Reactive Orange 15 + Reactive Red 4 + Reactive Yellow 2 (λ_{531}); Disperse Black 9+ Disperse Orange 25 + Reactive Blue 15 +Reactive Orange 15 (λ_{521}); Coomassie Brilliant Blue R250 + Celestine Blue+ Methylene Blue+ Eriochrome Black T (λ_{595}); Evans Blue+ Martius Yellow+ Methyl Orange+ Naphthol Blue Black (λ_{541}); Disperse Black 9+ Reactive Blue 15+ Methyl Orange+ Naphthol Blue Black (λ_{571})].

We have earlier reported the potential of a novel *T. dioica* plant proteins in the decolorization of disperse dyes (Jamal et al., 2010). In this paper we intended to widen the spectrum of industrially important textile dyes and included some well known non-textile dyes to study the extent of decolorization in the presence of Redox mediators. The cost of an enzyme depends on its degree of purity hence we opted to use ammonium sulphate precipitated proteins from *T. dioica* to study dye decolorization/degradation. The PGP was partially purified using 10% to 80% ammonium sulphate which retained a specific activity of 96 U/mg of protein. The experiments were performed at different enzyme concentration, pH, temperature, incubation period and Redox mediators namely, HOBT and vanillin. An interesting data profile was obtained for the assessment of this enzyme suitability to treat wastewater contaminated with these dyes.

The reactive dyes underwent decolorization by the formation of precipitate which disappeared in the presence of 1.0 mM HOBT. This finding supports earlier reports that treatment of phenols and aromatic amines by peroxidases resulted in formation of large insoluble aggregates (Wada et al., 1995; Tatsumi et al., 1996; Husain & Jan, 2000; Duran & Esposito, 2000). The other dyes studied showed no formation of precipitate during decolorization in the presence of HOBT. This observation supports earlier view that decolorization of dyes took place via degradation of aromatic ring of the compounds or by cleaving certain functional groups (Akhtar et al, 2005a,b; Satar and Husain, 2009). All the other textile dyes showed insignificant or no decolorization with *T. dioica* peroxidase alone when studied under optimum conditions of dye decolorization in the absence of HOBT (data not shown).

The decolorization profile for all the dyes increased significantly at HOBT concentration of 0.2 mM [Table-1]. Upon increasing the HOBT concentration further to 1.5 mM decolorization increased marginally. The reactive dyes under study exhibited decolorization maximally at 1.0 mM HOBT whereas disperse dyes showed maximum decolorization at lower values of HOBT suggesting that reactive dyes are comparatively more resistant to degradation. Vanillin was able to decolorize both the reactive and disperse dyes at 1.0 mM concentration but the extent of decolorization was sufficiently poor in comparison to HOBT for certain dyes like Reactive blue 15, Reactive orange 15 and most of the disperse dyes studied. Disperse Yellow 7 decolorized poorly (37.9%) with *T. dioica* peroxidase in the presence of relatively higher concentration of vanillin.

The non textile dyes exhibited remarkable decolorization in presence of both the Redox mediators at 1.0 mM concentration. There was no significant change on dye decolorization of non textile dyes at concentrations above 1.0 mM. It has already been reported that Redox mediators have the potential to mediate oxidation reaction between a substrate and an enzyme (d'Acunzo et al., 2006). Different Redox mediators have different mediation efficiency which is governed by Redox potential of the mediator and the oxidation mechanism of the substrate (Baiocco et al., 2003). Oxidation of substrate occurs by free radical formation by the mediator. The free radicals can be formed either by one-electron oxidation of substrate or by abstraction of a proton from the substrate (Xu et al., 2001; Fabbrini et al., 2002). In this study, Redox-mediating property of two different compounds as peroxidase mediators was evaluated and extensive study on textile, non-textile and dye mixtures has been performed with HOBT.

Laccase have been used with Redox mediators to oxidize non-phenolic compounds (Bourbonnais & Paice, 1990). The mechanism of action of laccase mediator system has been extensively studied and it is used in the textile industry in the finishing process for indigo stained materials. Several workers have demonstrated that the use of Redox mediator system enhanced the rate of dye decolorization by several folds but these mediators were required in very high concentrations (Soares et al., 2001a,b; Claus et al., 2002). In the present study we have shown the decolorization of both textile and non-textile dyes as well as dye mixtures mediated by *T. dioica* peroxidase under the influence of low concentration of Redox mediators. The peroxidase reacted well to decolorize at concentration of 0.8 mM and 1.0 mM of H₂O₂ for disperse and reactive dyes respectively and remained substantially unaffected till 1.2 mM [Figure-8]. Although the concentration of H₂O₂ greater than 0.75 mM acted as an inhibitor of peroxidase activity by irreversibly oxidizing the enzyme ferri-heme group essential for peroxidase activity (Satar and Husain, 2009), in this study we observed a relatively higher working concentration of H₂O₂. The enzyme in the presence of Redox mediators was much effective in performing the decolorization of the dyes, implying dye decolorization was a result of Redox mediated H₂O₂-dependent enzymatic reaction. Our results are consistent and very near to values reported earlier for maximum functional concentration of H₂O₂ (Camarero et al., 2005). The enzyme could function better in an acidic medium of pH range 3-6, whereas its decolorizing/ degrading activity was adversely affected in alkaline medium [Figure-9]. This finding supports the earlier view of disperse dye decolorization in acidic medium by *T. dioica* and reports further that acid medium favours catalysis of reactive dyes too by this peroxidase. It has earlier been reported that the degradation of industrially important dyes by enzymes such as horse radish peroxidase, polyphenol oxidase, BGP and laccase was also maximum in the buffers of acidic pH (Galindo et al., 2000).

The reaction temperature is an important parameter which effects the decolorization of dyes. The maximum decolorization for both the reactive and disperse dyes were in the temperature range of 40°C to 50°C in the presence of fixed concentration of HOBT [Figure-10]. The extent of decolorization was remarkable for Reactive Blue 15, Reactive Orange 15, Reactive Red 4 at 50°C whereas disperse dyes could be decolorized in the range of 61.2% to 79% at 40°C. The decolorization varies with the nature of the dyes but Redox mediated decolorization with *T. dioica* peroxidase was a better solution for effective decolorization of recalcitrant compounds. The rate of decolorization varied with time and maximum decolorization in the presence of HOBT was observed within one hour of incubation for Reactive Blue15 and Disperse Red 19 [Figure-11]. However, for other dyes the extent of decolorization progressed slowly and reached a plateau after 4 h of incubation. This data is consistent with reports that decolorization rate varies, depending upon the type of dye to be treated (Camarero et al., 2005).

The non-textile dyes studied for decolorization with *T. dioica* peroxidase exhibited enhanced decolorization in the presence of 1.0 mM HOBT, whereas in the absence of Redox mediator decolorization was much slower. Coomassie Brilliant Blue R250, Methylene Blue, Eriochrome Black T, Martius Yellow, Methyl Orange, Naphthol Blue Black and Rhodamine were extensively decolorized by *T. dioica* peroxidase under the influence HOBT [Table-2]. The decolorization of Evans blue was not significantly affected by the presence of Redox mediator, although in the presence of HOBT decolorization achieved was higher. The performance of this system was maximal during 160 min of incubation. Celestine blue was decolorized to 79.5% in the presence of HOBT at higher concentration of enzyme as well as longer incubation time. The data in [Table-2] is suggestive of *T. dioica* peroxidase in conjunction with low concentration of HOBT to be wonderful decolorization/degradation system for non-textile dyes as well. Further, dye mixtures simulating industrial effluents exhibited more than 82% decolorization with 1-hydroxybenzotriazole [Figure-12]. The rate of decolorization of dye mixture was slower in comparison to that of individual dyes both in the presence and absence of HOBT. The application of inexpensive peroxidase from easily available source can overcome the limitations in current wastewater treatment strategies. The use of peroxidases can be extended to large-scale treatment of wide spectrum of structural dyes by using immobilized PGP along with relatively cheaper Redox mediators. Thus, the study demonstrated that the peroxidase enzyme isolated from *T. dioica* can be coupled with Redox mediator into a system that can serve as an effective biocatalyst for the treatment of effluents containing recalcitrant dyes from textile, dyeing and printing industries.

3. Future perspective

Dye wastewater discharged from textile and dyestuff industries needs to be treated due to their impact on water bodies and to address growing public concern over their toxicity and carcinogenicity. Many different and complicated molecular structures of dyes make dye wastewater treatment difficult by conventional biological and physico-chemical processes. Therefore innovative treatment technologies need to be investigated. The studies performed using peroxidases from different sources indicates that novel enzyme systems can be created to decolorize wide spectrum of textile, non-textile dyes and dye mixtures under varying set of conditions. The efficacy of decolorization drastically improves with Redox mediators and dyes/dye mixtures recalcitrant to peroxidase exhibited remarkable decolorization. The application of inexpensive peroxidases from easily available sources can

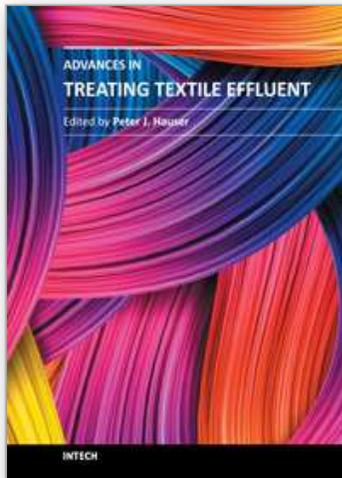
overcome the limitations in current wastewater treatment strategies. The use of peroxidases can be extended to large-scale treatment of wide spectrum of structural dyes by using immobilized peroxidases along with relatively cheaper Redox mediators.

4. References

- Akhtar, S., Khan, A.A. & Husain, Q. (2005a). Partially purified bitter gourd (*Momordica charantia*) peroxidase catalyzed decolorization of textile and other industrially important dyes. *Bioresour. Technol*, 96, 1804–1811, ISSN: 0960-8524.
- Akhtar, S., Khan, A.A. & Husain, Q. (2005b). Potential of immobilized bitter gourd (*Momordica charantia*) peroxidases in the decolorization and removal of textile dyes from polluted wastewater and dyeing effluent. *Chemosphere*, 60, 291–301, ISSN: 0045-6535.
- Baiocco, P., Barreca, A.M., Fabbrini, M., Galli, C. & Gentili, P. (2003). Promoting laccase activity towards non-phenolic substrates: a mechanistic investigation with some laccase-mediator systems. *Org. Biomol. Chem* 1, 191–197, ISSN: 1477-0520.
- Bhunja, A., Durani, S. & Wangikar, P.P. (2001). Horseradish peroxidase catalyzed degradation of industrially important dyes. *Biotechnol. Bioeng*, 72, 562–567, ISSN: 0006-3592.
- Bourbonnais, R. & Paice, M.G. (1990). Oxidation of non-phenolic substrates. An expanded role for laccase in lignin biodegradation. *FEBS Lett*, 267, 99–102, ISSN: 0014-5793.
- Calcaterra, A., Galli, C. & Gentili, P. (2008). Phenolic compounds as likely natural mediators of laccase : A mechanistic assessment. *J. Mol. Catal. B: Enzym*, 51, 118-120, ISSN: 0039-7911.
- Camarero, S., Ibarra, D., Marti´nez, M.J. & Marti´nez, A.J. (2005). Lignin derived compounds as efficient laccase mediators for decolorization of different types of recalcitrant dyes. *Appl. Environ. Microbiol*, 71, 1775–1784, ISSN: 1432-0614.
- Chen, H. (2006). Recent advances in azo dye degrading enzyme research. *Curr. Protein Pept. Sci*, 7, 101–111, ISSN: 1389-2037.
- Christian, V.V., Shrivastava, R., Novotny, C. & Vyas, B.R. (2003). Decolorization of sulfonaphthalein dyes by manganese peroxidase activity of the white-rot fungus *Phanerochaete chrysosporium*. *Folia Microbiol*, 48, 771–774, ISSN: 1874-9356.
- Chung, K.-T. & Cerniglia, C.E. (1992). Mutagenicity of azo dyes: structure activity relationships. *Mutat. Res*, 277, 201–220, ISSN: 0027-5107.
- Chung, T. P., Wu, P. C. & Juang, R. S. (2004). Process development for degradation of phenol by *Pseudomonas putida* in hollow-fiber membrane bioreactors. *Biotechnol. Bioeng*, 87, 219–227, ISSN: 0006-3592.
- Claus, H., Faber, G. & Konig, H. (2002). Redox-mediated decolorization of synthetic dyes by fungal laccases. *Appl Microbiol Biotechnol*, 59, 672–678, ISSN: 0175-7598.
- d’Acunzo, F., Galli, C., Gentili, P. & Sergi, F. (2006). Mechanistic and steric issues in the oxidation of phenolic and non-phenolic compounds by laccase or laccase mediator systems. The case of bifunctional substrates. *New J. Chem*, 30, 583-591, ISSN: 1144-0546.
- Duran, N. & Esposito, E. (2000). Potential applications of oxidative enzymes and phenoloxidase-like compounds in wastewater and soil treatment: a review. *Appl. Catal. B: Environ*, 28, 83–99, ISSN: 0926-3373.
- Fabbrini, M., Galli, C. & Gentili, P. (2002). Comparing the catalytic efficiency of some mediators of laccase. *J. Mol. Catal. B: Enzy*, 16, 231-240, ISSN: 0039-7911.
- Galindo, C., Jaques, P. & Kalt, A. (2000). Photodegradation of the aminobenzene acid orange 52 by three AOPs: UV/H₂O₂, UV/TiO₂ and VIS/TiO₂. Comparative mechanistic and kinetic investigations. *J. Photochem. Photobiol. A Chem*, 130, 35–47, ISSN: 1010-6030.

- Husain, M. & Husain, Q. (2008). Applications of Redox mediators in the treatment of organic pollutants by using oxidoreductive enzymes. *Critical Reviews in Environmental Science and Technology*, 38, 1–42, ISSN: 1064-3389.
- Husain, Q. & Jan, U. (2000). Detoxification of phenols and aromatic amines from polluted wastewater by using polyphenol oxidases. *J. Sci. Ind. Res*, 59, 286–293, ISSN: 0022-4456.
- Husain, Q., Husain, M. & Kulshrestha, Y. (2009). Remediation and treatment of organopollutants mediated by peroxidases. *Critical Reviews in Biotechnology*, 29, 94–119, ISSN: 0738-8551.
- Husain, Q., Karim, Z. & Banday, Z.Z. (2010). Decolorization of textile effluent by soluble fenugreek (*Trigonella foenum graecum* L) seed peroxidase. *Water Air Soil Pollut*, 212, 319–328, ISSN: 1567-7230.
- Jamal, F., Pandey, P.K. & Qidwai, T. (2010). Potential of Peroxidase Enzyme from *Trichosanthes dioica* to mediate Disperse Dye Decolorization in conjunction with Redox Mediators. *J. Mol. Cat. B: Enzym*, 66, 177–181, ISSN: 0039-7911.
- Jauregui, J., Valderrama, B., Albores, A. & Vazquez-Duhalt, R. (2003). Microsomal transformation of organophosphorus pesticides by white rot fungi. *Biodegradation*, 14, 397–406, ISSN: 0923-9820.
- Kahru, A., Pollumaa, L., Reiman, R., Ratsep, A., Liiders, M. & Maloveryan, A. (2000). The toxicity and biodegradability of eight main phenolic compounds characteristic to the oil-shale industry wastewater: a test battery approach. *Environ. Toxicol*, 15, 431–442, ISSN: 1520-4081.
- Karim, Z. & Husain, Q. (2009). Guaiacol-mediated oxidative degradation and polymerization of bisphenol A catalyzed by bitter melon (*Momordica charantia*) peroxidase. *J. Mol. Cat. B: Enzym*, 59, 185–189, ISSN: 0039-7911.
- Keharia, H. & Madamvar, D. (2003). Bioremediation concept for treatment of dye containing wastewater: a review. *I. J. Exp. Biol*, 41, 1068–1075, ISSN: 0019-5189.
- Kurniawati, S. & Nicell, J. A. (2007). Efficacy of mediators for enhancing the laccase-catalyzed oxidation of aqueous phenol. *Enzym. Microbial. Technol*, 41, 353–361, ISSN: 0141-0229.
- Livingston, A. G. (2004). A novel membrane bioreactor for detoxifying industrial wastewater: II. Biodegradation of 3-chloronitrobenzene in an industrially produced wastewater. *Biotechnol. Bioeng*, 41, 427–936, ISSN: 0006-3592.
- Lowry, O.H., Rosebrough, N.J., Farr, A.L. & Randall, R.J. (1951). Protein measurement with Folin-phenol reagent. *J. Biol. Chem.* 193, 265–275, ISSN: 0021-9258.
- Marco, S.L. & José, A.P. (2007a). Degradation of Reactive Black 5 by Fenton/UV-C and ferrioxalate/H₂O₂/solar light processes. *Dyes and Pigments*, 74, 622–629.
- Marco, S.L., Albino, A., Dias Ana Sampaio, Carla, A. & José, A.P. (2007b). Degradation of a textile reactive Azo dye by a combined chemical-biological process: Fenton's reagent-yeast. *Water Res*, 41, 1103–1109, ISSN: 0143-7208.
- Matto, M. & Husain, Q. (2009). Decolorization of direct dyes by immobilized turnip peroxidase in batch and continuous processes. *Ecotoxicol. Environ. Saf*, 72, 965–971, ISSN: 0147-6513.
- Matto, M. & Husain, Q. (2008). Decolorization of textile effluent by bitter melon peroxidase immobilized on concanavalin A layered calcium alginate-starch beads. *Journal of Hazardous Materials*, 164, 1540–1546, ISSN: 0304-3894.
- Matto, M., & Hussain, Q. (2007). Decolorization of direct dyes by salt fractionated turnip proteins enhanced in the presence of hydrogen peroxide and Redox mediators. *Chemosphere*, 69, 388–345, ISSN: 0045-6535.

- Robinson, T., McMullan, G., Marchant, R. & Nigam, P. (2001). Remediation of dyes in textile effluents: a critical review on current treatment technologies with a proposed alternative. *Bioresour. Technol*, 77, 247–255, ISSN: 0960-8524.
- Rodriguez-Couto, S., Sanroman, M. & Guebitz, G.M. (2005). Influence of Redox mediators and metal ions on synthetic acid dye decolorization by crude laccase from *T. hirsuta*. *Chemosphere*, 58, 417–422, ISSN: 0045-6535.
- Satar, R. & Husain, Q. (2009). Use of Bitter Gourd (*Momordica charantia*) peroxidase together with Redox mediators to decolorize disperse dyes. *Biotechnol. Bioprocess Eng*, 14, 213–219, ISSN: 1226-8372.
- Shaffiqu, T.S., Roy, J.J., Nair, R.A. & Abraham, T.E. (2002). Degradation of textile dyes mediated by plant peroxidases. *Appl. Biochem. Biotechnol*, 102/103, 315–326, ISSN: 0273-2289.
- Soares, G.M., Costa-Ferreira, M. & Pessoa de Amorim, M.T. (2001a). Decolorization of an anthraquinone-type dye using a laccase formulation. *Bioresour. Technol*, 79, 171–177, ISSN: 0960-8524.
- Soares, G.M., Pessoa de Amorim, M.T. & Costa-Ferreira, M. (2001b). Use of laccase together with Redox mediators to decolorize Remazol Brilliant Blue R. *J. Biotechnol*, 89, 123–129, ISSN: 0168-1656.
- Sumathi, S. & Manju, B.S. (2000). Uptake of reactive textile dyes by *Aspergillus foetidus*. *Enzym. Microbial. Technol*, 27, 347–355, ISSN: 0141-0229.
- Tatsumi, K., Wada, S. & Ichikawa, H. (1996). Removal of chlorophenols from wastewater by immobilized horseradish peroxidase. *Biotechnol. Bioeng*, 51, 126–130, ISSN: 0006-3592.
- Torres, E., Bustos-Jaimes, I. & Le Bogne, S. (2003). Potential use of oxidative enzymes for the detoxification of organic pollutants. *Appl. Cat. B: Environ*, 46, 1–15, ISSN: 0926-3373.
- Vasanth, L. M. Vuringi, H.B. & Yerramilli, A. (2006). Degradation of azo dye with horse radish peroxidase (HRP). *J. Indian Inst. Sci*, 86, 507–514, ISSN: 0970-4140.
- Vazquez-Duarte, H., Garcia-Almendarez, B., Regalado, C. & Whitaker, J. R. (2001). Purification and properties of a neutral peroxidase from turnip (*Brassica napus L var purple top white globe*) roots. *Journal of Agricultural and Food Chemistry*, 49, 4450–4456, ISSN: 0021-8561.
- Verma, P. & Madamwar, D. (2002). Decolorization of synthetic textile dyes by lignin peroxidase of *Phanerochaete chrysosporium*. *Folia Microbiol*, 47, 283–286, ISSN: 0015-5632.
- Wada, S., Ichikawa, H. & Tatsumi, K. (1995). Removal of phenols and aromatic amines from wastewater by a combination treatment with tyrosinase and a coagulant. *Biotechnol. Bioeng*, 45, 304–309, ISSN: 0006-3592.
- Xu, F., Deussen, H.J.W., Lopez, B., Lam, L. & Li, K. (2001). Enzymatic and electrochemical oxidation of N-hydroxy compounds: Redox potential, electrontransfer kinetics, and radical stability. *Eur. J. Biochem*, 268, 4169–4176, ISSN: 0014-2956.
- Zille, A., Tzanov, T., Gubit, G. M. & Cavaco-Paulo, A. (2003). Immobilized laccase for decolorization of reactive black 5 dyeing effluent. *Biotechnol. Lett*, 25, 1473–1477, ISSN: 0141-5492.



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