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Decolorisation of Textile Dyes using Immobilised PPO from Tomato Peel and Pulp

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ABSTRACT

The textile industry is considered second only to agriculture as the biggest polluter of clean water. Untreated dyes cause chemical and biological changes in our aquatic system, which threaten species of fish and aquatic plants causing water pollution. In this study an effort has been made to use plant polyphenol oxidase from tomato (*Solanum lycopersicum*) peel and pulp, for the treatment of dyes used in textile industries. Polyphenol oxidase (PPO) enzyme was extracted from tomato (*Solanum lycopersicum*) peel and pulp and immobilized by two methods: Alginate method (entrapment) and Celite 545 (diatomaceous earth, 30–80 mesh) (Adsorption type). PPO enzyme stability was increased after immobilization. The immobilized and free enzymes were employed for the decolorization of textile dyes. The dye solutions were prepared in the concentration of 150 µg/mL in distilled water and incubated with free and immobilized tomato peel and pulp PPO for one hour. The percent decolorization was calculated by taking untreated dye solution as control. Immobilized PPO was significantly more effective in decolorizing the dyes as compared to free enzyme.

Key words : *Immobilisation, Textile Industry, Decolorisation, Dye, Enzyme*

Introduction

The textile industry is one of the largest polluters in the world as it produces a large amount of effluents. Millions of liters of such untreated effluents in the form of wastewater are discharged into natural water reservoirs which can cause serious environmental problems. Effluents of textile mills are heavily loaded with textile dyes (Selvam *et al*., 2003). Untreated dyes cause chemical and biological changes in our aquatic system, which threaten species of fish and aquatic plants. Most of these dyes are recalcitrant in nature, especially azo dyes (Joshi *et al*., 2011). If left untreated, these dyes will remain in environment for long period to time (Olukanni *et al*., 2006). Dyes present in the effluent can lead to acute effects on exposed organisms due to their toxicity. Dye rich effluent cause discoloration of water bodies result of which is reduction in photosynthetic activity in aquatic life by reducing light penetration intensity and the absorbance of light that enters the water (Mester and Tien, 2000). Dyes may also significantly be toxic to some aquatic fauna and flora due to the presence of aromatics, metals, chlorides, etc. (Daneshvar *et al*., 2007).

At present, there is no satisfactory method to economically decolourize textile wastewater. Several physiochemical and biological methods have been proposed for either degradation or removal of dyes to achieve decolorization. Few of physiochemical

methods includes; photochemical degradation, electrochemical destruction, membrane filtration, irradiation, electrokinetic coagulation, ozonation, ion-exchange, adsorption. Each of them has their own disadvantages such as high cost, time consuming, sludge generation, formation of by-products, long retention times (André *et al*., 2007). In case of biological approaches several aerobic and anaerobic microbial processes are thought to facilitate degradation of textile dyes (Abadulla *et al*., 2000). Anaerobic processes are useful for selective dyes and often end up with mutagenic byproduct. Recent studies indicate that an enzymatic approach has attracted much interest in the removal of phenolic pollutants from aqueous solutions as an alternative strategy to the conventional chemical, physical as well as microbial treatments, which pose some serious limitations (Bhunia *et al*., 2001). Oxidoreductive enzymes such as peroxidases and polyphenol oxidases are participating in the degradation/removal of aromatic pollutants from various contaminated sites. These enzymes can act on a broad range of substrates and they can also catalyze the degradation or removal of organic pollutants present in very low concentration at the contaminated sites (Husain, 2000). In view of the potential of enzymes in treating the phenolic compounds, several microbial and plant peroxidases and polyphenol oxidases have been considered for the treatment of dyes but none of them has been exploited at the large scale due to low enzymatic activity in biological materials and their high cost of purification (Nagai *et al*., 2002). Plant peroxidases are catalyzing decolorization of wide spectrum of dyes but they require expensive H_2O_2 as a co-substrate (Bhunia *et al*., 2001).

Plant polyphenol oxidases on the other hand are the simple and cheaper alternative for the decolorization of aromatic pollutants due to its utilization of free molecular oxygen as an oxidant (Shi, 2001). Polyphenol oxidase (PPO) is a group of copper proteins that are widely distributed from bacteria to mammals (Prabha and Patwardhan, 1982). PPO from different plants has been used for dye decolorization (Khan and Husain, 2007). Mostly fruit or its peel has been used for extraction of PPO, which puts limitation on amount of enzyme. But large amount of wastewater generated demands for regular supply of considerable amount of enzyme. To satisfy this need an attempt has been done to use systematically partially purified Tomato polyphenol oxidase (PPOs) for the treatment of various textile and other industrially important dyes.

Immobilization of enzymes (or cells) refers to the technique of confining/anchoring the enzymes (or cells) in or on an inert support for their stability and functional reuse. By employing this technique, enzymes are made more efficient and cost-effective for their industrial use. Immobilized enzymes retain their structural conformation necessary for catalysis.

Several advantages of immobilized enzymes are stable and more efficient in function, can be reused again and again, products are enzyme-free, are ideal for multi-enzyme reaction systems, control of enzyme function is easy are suitable for industrial and medical use and minimize effluent disposal problems. The commonly employed techniques for immobilization of enzymes are—adsorption, entrapment, covalent binding and cross-linking.

Materials and Methods

Preparation of PPO Enzyme Extract

Healthy ripe tomatoes used in this study were purchased from local market and were authenticated by Botanical Survey of India, Hyderabad. Tomatoes were washed with distilled water for 2-3 times to remove any dirt and contaminants. The tomatoes were then washed with 10% Sodium chloride (NaCl) solution and again washed with double distilled water. The tomatoes were peeled and the peel and pulp were kept in separate containers. The Polyphenol oxidase from peel and pulp of tomato were extracted by 0.1M Sodium phosphate buffer (pH= 6.5); (Lu *et al*., 2006). To the peel and pulp of tomato 0.1M Sodium phosphate buffer (pH= 6.5) in the ratio 1:20 (Sample: Buffer) was added and homogenized separately. The homogenate was then filtered and the filtrate was then centrifuged at 6000rpm for 10 minutes at 4 °C. The supernatant collected was the crude polyphenol oxidase enzyme fraction (Ziyan and Pekyardimi, 2004). The crude polyphenol oxidase enzyme fraction of both peel and pulp was purified by Ammonium salt precipitation method (45%) (Duong-Ly, Krisna, 2014) where the protein of interest was salted out from a mixture of proteins.

After salt precipitation, dialysis was carried out where the enzyme molecules were separated from the excessive salts and other contaminants by dialysis involving diffusion method (Berg, 2007) using Thermo Scientific™ Slide-A-Lyzer™ Dialysis Cas-

settes with 10k Molecular Weight cut off.

IonExchange chromatography: The dialysis sample (peel and pulp) were purified by Ion Exchange chromatography (anionic exchanger) using DEAE cellulose (as stationary phase) (Ninfa, 2010).

Gel-filtration chromatography: was also carried out for the tomato peel and pulp, where the stationary phase used for separation was Sephadex G 100 (Wang *et al*., 2010). The stationary phase Sephadex G 100 has particle size of 40-120 μm and the fractionation range of Molecular weight range from 1,000-200,000 kDa.

Immobilization of Purified enzyme from Peel and Pulp of Tomato

The immobilization of the purified enzyme from ion exchange chromatography and gel permeation chromatography was carried out by two methods: Alginate method (entrapment) and Celite545 (diatomaceous earth, 30–80 mesh) (Adsorption type)

Immobilisation of PPO using Alginate

Alginate solution (1,2, 3%, w/v) was prepared by dissolving sodium alginate in deionized water. Tomato peel and pulp PPO solution was mixed with 20mL of alginate solution at the enzyme/alginate ratio of 1:10 (v/v). This was taken in a 20 mL syringe and alginate-enzyme mixture was dropped into chilled 8% calcium chloride. As soon as the drop fell the free enzyme is entrapped in calcium alginate in the shape of a bead. After stirring for 4—5 h at 4 °C, beads of calcium alginate with entrapped enzyme were collected. Beads were washed with buffer to remove any calcium chloride solution and were stored suspended in phosphate buffer at 4 °C

Immobilisation of PPO using Celite 545

4g of Celite 545 was suspended in Phosphate buffer pH 7.0 and stirred at room temperature for 1hour. The fine particles present in the suspension were removed by decantation and this procedure was repeated thrice. The washed celite was stirred with enzyme solution at 4 °C overnight. The enzyme bound celite was then washed 4 – 5 times with phosphate buffer and then suspended in 20 mL of phosphate buffer (Amjad Ali Khan, 2005)

PPO Enzyme Activity

PPO activity was determined by measuring the absorbance at 420 nm using a spectrophotometer (Jasco). To determine the best concentration of enzyme preparation corresponding to the highest enzyme activity, the activity was assayed in 3 mL of reaction mixture consisting of 2.0 mL substrate 200mM catechol and different concentrations (0.1-0.3 mL) of the enzyme preparation. This mixture was topped-up to 3.0 mL with the phosphate buffer (pH 6.8). The blank consisted of 3.0 mL 0.1 M phosphate buffer (pH 6.8). PPO activity was calculated from the linear portion of the curve. The initial rate of PPO catalyzed oxidation reaction was calculated from the slope of the absorbance–time curve. An enzyme preparation of 0.2 mL showed the highest activity using catechol as a substrate which was used in all other experiments. Assays were carried out at room temperature and results are the averages of at least three assays and the mean and standard deviations were plotted. PPO activity was assayed in triplicate at room temperature and one enzyme unit represents the amount of enzyme that produces a rise of 0.001 absorbance in one minute at 420 nm.

Effect of temperature and pH on the activity of free and immobilized PPO

To determine the optimum temperature for PPO, the activity of the PPO free enzyme and immobilised enzyme from peel and pulp of tomato was measured at temperature range (20-65°C) using 50 mM of catechol as a substrate. The substrate solution was heated to the tested temperature, and then the enzyme solution was added. Temperature at which there was highest PPO residual activity was considered as the optimum temperature.

The optimum pH of the enzyme PPO was determined under the standard assay conditions by measuring enzyme activity of free PPO and immobilised PPO from peel and pulp using buffers at different pH values ranging from 3.5 to 8.0 (3.5–5.5 acetate buffer, 100 mM; 5.5–8.0 potassium phosphate buffer, 100 mM).

Polyphenol oxidase activity in dye decolorizing

The enzyme extracts (purified enzymes from peel and pulp of tomato) were analyzed for degradation or reduction of different azodyes. Five dyes Congo Red, Methyl Red, Methyl Yellow, Auromine, Methyl Violet were selected for the present study. Each dye (150μg mL^{-1}) was incubated with Tomato PPO in 50 mM phosphate buffer, pH 6.5 at 30 °C. The disappearance of the color by tomato PPO activity was

monitored at λmax of the respective dye solution. The percent decolorization was calculated by taking the maximum absorbance of each untreated dye solution as control (100%) (Saratale *et al*., 2006).

The azodye degradation carried out by purified Polyphenol oxidase enzyme (Catechol oxidase) against different azodyes were analysed by their percentage of degradation determined by:

$$\text{Percentage of decolorisation} = \frac{\text{Abs (Control)–Abs (Test)}}{\text{Abs (Control)}} \times 100$$

Where Abs (control) = Absorbance of untreated dye, Abs (Test) = Absorbance of dye after treatment with PPO enzyme

Results and Discussion

Polyphenol oxidase enzyme was isolated from tomato peel and pulp, purified by Ion Exchange and Gel permeation Chromatography. These fractions were used as enzyme extract for immobilization with Sodium Alginate and Celite 545 (Fig. 1).

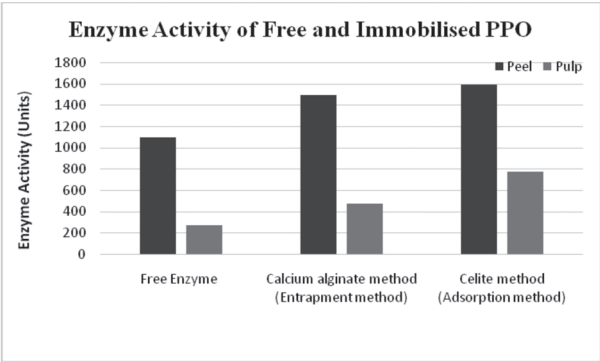

Fig. 1. Enzyme activity of free and immobilized enzyme

Optimum pH for free and immobilised PPO

Optimum pH for PPO activity with catechol as substrate was 6.5 both for free and immobilised tomato peel and pulp PPO. As the pH increased from 3.5 to 6.5, the enzyme activity increased, with maximal activity at pH 6.5, after which the activity started to decline (Fig. 2).

Optimum Temperature for free and immobilised PPO

Both free and Immobilized PPO enzyme samples of tomato peel and pulp was incubated with catechol at different temperatures that ranged from 20-65 °C. Results in the figure showed that 50 °C was the best

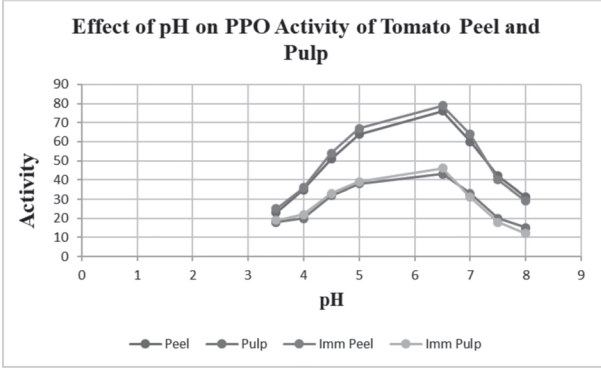

Fig. 2. Optimum pH for free and immobilised PPO

temperature for free enzyme both peel and pulp and 55 °C for immobilized PPO of tomato peel and pulp samples (Fig 3). It shows that immobilized PPO could withstand higher temperature conditions compared to free PPO.

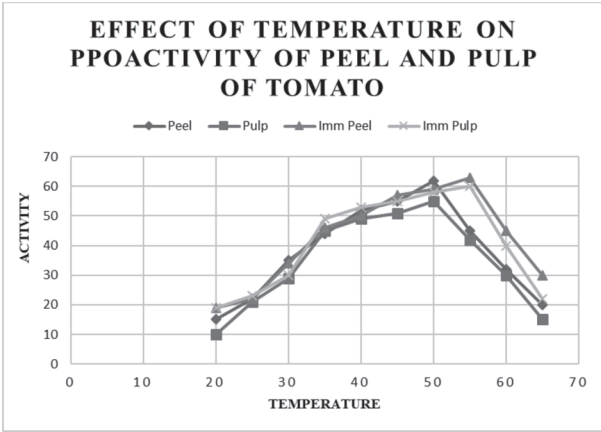

Fig. 3. Optimum Temperature for free and immobilised PPO

Decolourization assay of Purified Polyphenol oxidase and Immobilized enzyme

The enzyme extracts (purified enzymes from peel and pulp of tomato) were analyzed for degradation or reduction of different azodye (Fig. 4). The percent decolorization was calculated by taking the maximum absorbance of each untreated dye solution as control (100%) (Ghazi *et al*., 2018).

Immobilised PPO showed higher percentage of decolorization compared to the free enzyme. Immobilisation on Celite 545 gave better decolorization than with Sodium Alginate which may be due to the enzyme leaching out.

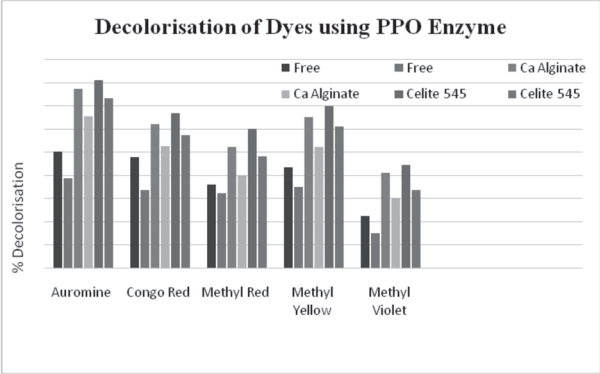

Fig. 4. Decolorisation of Dyes using PPO Enzyme

Conclusion

PPO is an enzyme that is responsible for the browning of the fruits and vegetables. It is present in nearly all plants. We have tried to extract the enzyme polyphenol oxidase from the pulp and peel of tomato and also to know the amount of the enzyme present in both. Our results showed that PPO enzyme was successfully partially purified from tomato and immobilized onto alginate beads. Immobilization of PPO increased its stability to pH and temperature that could be more useful in industrial applications. Free and immobilized PPO were applied to five different textile dyes for decolorization. Immobilized PPO enzyme was significantly more effective in decolorizing the dyes as compared to free enzyme at pH 4.0. Our results clearly demonstrated that the different textile dyes in wastewater could be easily decolorized by the partially purified plant PPO obtained from cheaper plant sources. The use of partially purified immobilized PPO may be extendable to the effluents coming out of industries and mixtures of dyes present in wastewaters.

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