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Original Research

Sweet Lime-Mediated Decolorization of Textile Industry Effluents

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Abstract

This study looks at using partially purified peroxidase extracted from peels of sweet lime (*Citrus limetta*) for decolorizing textile industry effluent. The ideal pH and thermal conditions of the enzyme were 7 and 35ºC. The K_m and V_{max} for guaiacol were 0.66 mM and 6666 µmol/mL/min, respectively. We found that sweet lime peroxidase was very effective in decolorizing textile industry effluent. Almost complete decolorization (>99 %) of effluent was attained at a pH of 5.0, temperature of 55ºC, H_2O_2 concentration of 2 mM, and enzyme dose of 40 U/mL within 60 minutes of incubation. The effluent was also analysed in terms of physicochemical parameters before and after treatment with sweet lime peroxidase. The reduction in toxicity after the enzymatic treatment was evidenced by chemical oxygen demand (COD) and total suspended solids (TSS) values.

Keywords: peroxidase, sweet lime, decolorization, K_m, V_{max}, effluents

Introduction

Rapid increases in world population, urbanization, and other activities of industrialization have led to extensive applications of chemicals such as dyes in our daily life. A major source of these dyes are our textile industries that utilize them extensively and possess a major threat to the environment by releasing a significant portion via wastewater. It is estimated that >10,000 dyes and pigments are utilized and more than 0.7 million tons of dyes of synthetic origin are produced annually [1-2]. Dye removal from industrial effluents is a concern, and the variety of physical, chemical, and biological techniques have been developed [3]. Traditionally it has been done by applying various processes like adsorption,

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ozonation, UV/NaOCl, electrochemical oxidation, ultrasonic irradiation, the photo-Fenton process, and UV/H_2O_2 [4-18]. These methods are not considered as effective due to overuse of various toxic chemicals that cause many environmental issues (although an eco-friendly technique like biological decolorization could be used as best alternative). These enzyme-based methods applied in dye degradation are cost-effective, easily controllable, and eco-friendly. In this regard, peroxidases (PODs) belonging to the class of oxidoreductases are considered very important [19].

PODs are the enzymes that are widely found in plants, microorganisms, and vertebrates. These are categorized as: prokaryotic and plant intracellular enzymes, extracellular fungal PODs, and higher plants PODs [20]. These PODs are single-chain proteins having multiple isomers and may differ in function, substrate specificity, or pH [21]. Owing to enzymatic activity, stability, and the variety of industrial and environmental applications, higher plant PODs have been deeply studied [22-23].

PODs extracted from different sources have been utilized for degradation of dyes, including horseradish peroxidase [24-27], soybean peroxidase [28], *Cucurbita pepo* [29], and white Spanish broom [30].

Most recent research is concerned with finding economical ways to treat dye-contaminated effluents. So in the current project an attempt has been made using somewhat purified POD extracted from sweet lime peels (*Citrus limetta)* to decolorize industrial effluent. The conventional methodology of one variable at a time (OVAT) is well accepted and is utilized in this study.

Material and Methods

All the chemicals used in this work were of analytical grade and purchased from Sigma-Aldrich Chemical Co. (USA). Isolation and partial purification was done following the method described in Nouren et al. [31]. Enzyme protein contents were determined with bovine serum albumin (BSA) as the standard protein [32].

POD activity was determined with slight modification in the earlier reported assay method [33]. The optimum pH for POD activity was determined by monitoring the activity of the enzyme as in the assay section using the following buffers: glycine HCL (pH 2.0-4.0), acetate (pH 5.0, 6.0), phosphate (pH 7.0, 8.0), and tris-HCl (pH 9.0). The optimal temperature was determined by assaying for the activity of the enzyme as in the assay section at different temperatures (25-60ºC). The K_m and V_{max} for guaiacol for sweet lime peroxidase were determined by taking different concentrations of guaiacol (0.5-45 mM) and following the assay for the activity of peroxidase as described in the assay section. The average of the data generated from the assay was used to construct the Line Weaver-Burk plot from which K_m and V_{max} were determined for guaiacol.

SLP was applied to check the decolorization of effluent collected from Arzoo Textile Industry of Faisalabad. It was centrifuged at 10,000 *g* for 15 min and supernatant diluted to 50 times with distilled water in order to get its absorbance within range of the spectrophotometer. Then the λ_{max} was scanned using a Cecil 7200 spectrophotometer and found to be 574.5 nm. The physicochemical parameters were estimated according to the methods prescribed in APHA [34].

The following parameters were optimized for decolorization of the effluent: optimum pH for decolorization of sweet lime peroxidase catalyzed textile effluent was monitored by using 375 µL of buffers (50 mM) of different pH ranging from 2-10 with 12 U/mL of SLP, and 0.25 mM H_2O_2 (375 µL) at 40ºC for 30 min. The absorbance was noted at 574.5 nm [35].

A similar series of experiments were performed through OVAT in order to optimize parameters like temperature (25-70ºC), enzyme dose (5.0-50.0 U/mL), and H_2O_2 concentration (0.5-10 mM). In the end, incubation time (5-240 min) was determined. After heating for 10 min the reaction was stopped, insoluble product was removed by centrifugation, and a decrease in absorbance was noted at specific λ_{max} of each effluent. Effluent decolorization was calculated as:

$$\text{Decolorization (\%)} = (A_0\text{-}A_t/A_0)*100 \qquad [1]$$

…where A_0 is the absorbance of the untreated effluent and A_t is the absorbance of the treated effluent.

Results and Discussion

Isolation and Partial Purification of SLP

PODs from peels of sweet lime (SLP) were isolated with 0.1 M phosphate buffer (pH 7.0) using a blender with short intermissions. The enzyme assay was performed and specific activity of crude SLP was recorded to be 443.75 U/mg of the protein. Then the crude enzyme was partially purified by 80% ammonium sulphate precipitation and then centrifuged. The residue was dissolved in 90 mL of 0.1 M phosphate buffer (pH 7.0) and then subjected to dialysis. The process of dialysis was conducted using dialysis tubing within 8 h by 5-6 changes of 25 mM phosphate buffer (pH 7.0). After performing a partial purification step, the specific activity of SLP increased to 2,819.71 U/ mg of protein with 6.35-fold purification. In previous literature, after ammonium sulphate fractionation 4.8-fold purification was obtained in the case of peroxidase extracted from peel of *Citrus jumbhiri* with an increase in specific activity from 751 to 2,925 U/mg [36], while in the case of peroxidase extracted from tubers of Jerusalem artichoke, 2.49-fold purification was attained after dialysis step with an increase in specific activity from 246.2 to 612.1 EU/mg [37].

Characterizing SLP

Various kinetic and thermodynamic parameters were employed for SLP characterization. The effect of pH on activity of SLP was determined by assaying the enzyme using buffers of different pH (2-9) and the result is shown in Fig. 1. It is apparent from Fig. 1 that SLP showed maximum activity at pH 7.0. There was a gradual decrease in activity with increasing pH from 8.0-9.0. The optimum activity at pH 7.0 shows that POD has better function in a neutral environment. The heme binding to the active site of enzyme was most stable at neutral pH [38]. Furthermore, pH change affects the dissociation of amino acids involved in substrate binding and enzyme catalysis. Therefore, optimal pH is needed for proper working of a biocatalyst.

Neutral pH was also reported as optimum in the previous literature for PODs extracted from two Salvia species viz., *S. virgata Jacq* and *S. viridis L.* using guaiacol as substrate. It was also revealed that activities of the PODs were also dependent on the concentrations of the buffers as well as the substrate used. The same optimum was also achieved for *Olea europaea* peroxidase [39] and horseradish peroxidase [40]. The temperature-activity profile of SLP is shown in Fig. 1, which shows that the optimum temperature for the activity of SLP was 35ºC and beyond, in which a decrease in activity took place, indicating low themostability. A wide variability regarding optimum temperature of peroxidase has been noticed from diverse sources. For example, PODs extracted from vanilla bean [41], cauliflower [42], *A. sativum* [43-44], and *Citrus jambherri* peel [22] showed optima at 16, 30, 35, and 40ºC, respectively.

In order to determine the effect of substrate concentration and kinetic parameters, substrate specificity (K_m and V_{max}) values for guaiacol were determined by Line Weaver-Burk plot. The effect of guaiacol on POD activity was determined by varying the concentration of guaiacol, keeping a fixed concentration of the second substrate of H_2O_2.

The K_m and V_{max} values for SLP were calculated respectively from Fig. 1. It is obvious from the data that K_m value for SLP was reported to be 0.66 mM with corresponding V_{max} value of 6666 µmol/mL/min. The lower value of K_m for SLP depicted its higher affinity for guaiacol.

PODs have different K_m and V_{max} depending upon sources, e.g., in the case of *Citrus jambherri* peel POII [22], Turkish black radish [45], and horseradish cv. Balady [27], K_m values of 5, 0.036, and 16.4 mM with corresponding V_{max} values of 18, 38,728.17, and 0.71 µmol/mL/min were respectively found for guaiacol as substrate.

Physicochemical Portrayal of Textile Runoff

Textile industry runoff was characterized before and after treatment with SLP for different physicochemical parameters, the values of these parameters are presented in Table 1. pH is one of the parameters for assessing water quality. Generally, water having pH <6.5 and >8.5 is not recommended for public consumption. In our study, pH is changed from 6.5 to 7.5. Electrical conductivity is changed from 3.31 to 2.98. The results indicate an almost 50% decrease in chemical oxygen demand and almost 11% decrease in total dissolved solids after the treatment of textile runoff

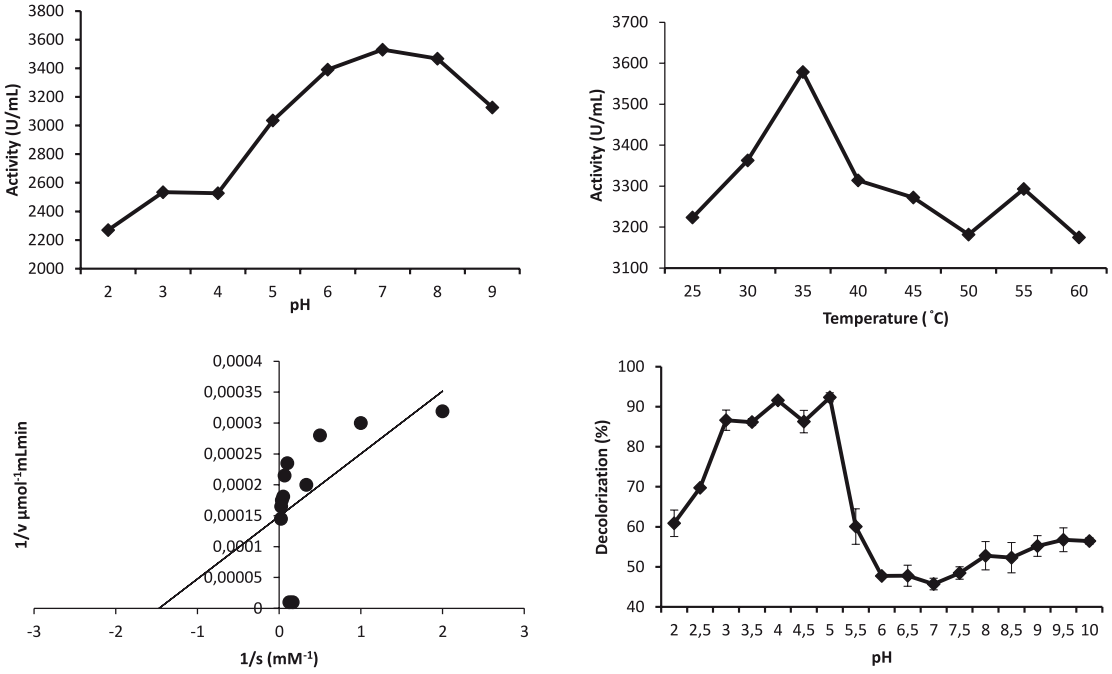

Fig. 1. Effects of pH, temperature, and guaiacol concentration on the activity of SLP and effect of pH on percentage decolorization.

Table 1. Physicochemical characterization of textile effluents.

Physicochemical parameter	Before treatment	After treatment with SLP
pH	6.7	7.5
EC (mS/cm)	3.31±0.1655	2.98±0.149
TDS (mg/L)	1550±69.75	1380±62.10
COD (mg/L)	211±11.605	106±5.83

with SLP, indicating that enzymatic treatment is an effective method for the cause.

Application of Sweet Lime POD for Decolorizing Industrial Effluent

Binding the heme group to the enzyme active site is strictly pH dependent, and a loss or decrease of enzyme activity at low and high pH was due to instability of heme binding to the enzyme active site [46-51]. Furthermore, pH change affects the dissociation of amino acids that are involved in substrate binding and enzyme catalysis. Therefore, optimal pH is essential for proper working of a biocatalyst [52-53].

The decolorization percentage was plotted as a function of pH and the results obtained are shown in Fig. 1. It is obvious from the data that acidic range of pH was most favourable for maximum decolorization of textile industry effluents. Maximum decolorization was attained at pH 5.0. The decrease in percentage decolorization in the alkaline medium was observed. In accordance with our findings, direct yellow 4 was excellently decolorized at pH 5.0 by *Citrus limon* POD [54-55]. This study was in good agreement with [25], while in another report the best pH for decolorization of industrial effluent dyes by fenugreek peroxidase was also 5.0 [38].

Temperature significantly affects the enzyme-catalysed decolorization/degradation of dyes. High temperature increases the activity of the enzyme and the reaction speeds up until an optimum level, where the enzyme behaves most efficiently. The enzymatic activity starts decreasing either due to the reaction between H_2O_2 and intermediates of the enzyme's catalytic cycle or irreversible reactions between the enzyme and free radicals.

Lowering the temperature reduces the reaction rate and leads to a reduction of free radical generation, thus slowing down enzyme deactivation [56-57]. Experiments were conducted at various temperatures (25-70ºC) to find the suitable temperature range for significant percentage decolorization, and results are shown in Fig. 2. It is revealed in the figure that the maximum decolorization of textile effluent was obtained at 55ºC (although an increase in temperature above optima resulted in decreased decolorization). In contrast to our results, 40ºC was recorded to be the optimum temperature for maximum decolorization of textile carpet effluent, red (75 %), and blue (80%) by turnip peroxidase [50], whereas crystal violet was maximally decolorized at 50ºC by gourd peel peroxidase [58], and drimarene

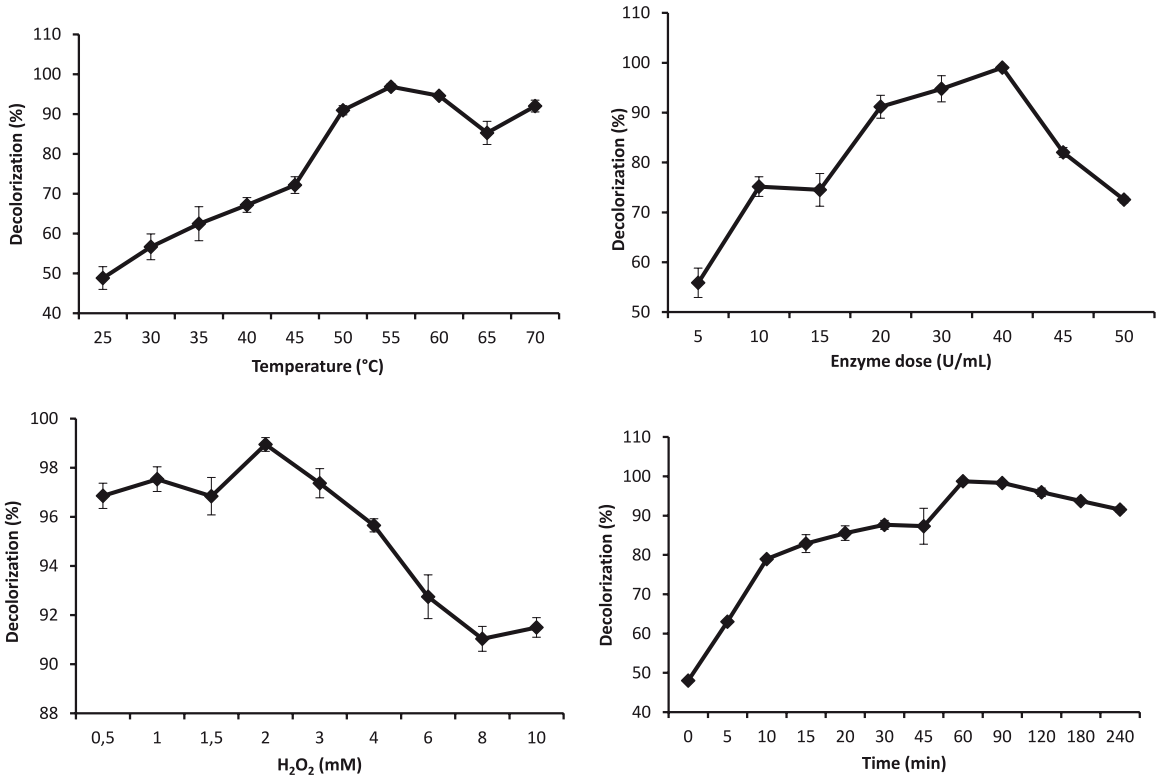

Fig. 2. Effect of temperature, enzyme dose, H_2O_2 concentration, and incubation on percentage decolourization.

orange KGL has shown significant decolourization at 60ºC [59].

Optimal concentration of enzymes is necessary to decolourise the effluents. Experiments were conducted by changing the enzyme dose (5.0-50.0 U/mL) and keeping other conditions constant to highlight the effect produced by enzyme dose. The results obtained are summarized in Fig. 2. We can conclude from the data that 40 U/mL of SLP was required for 99% decolorization of textile industry effluent. After the optimum, any increase in SLP did not result in an increase in percentage decolorization, i.e., saturation was attained. Similar to our previous investigation, 18 U/mL of citrus lemon peroxidase was required for almost 100% decolorization of the same effluent [35].

H_2O_2, co-substrate of POD plays its role in the catalytic mechanism of POD and thereby accepts the aromatic substrates and converts them into radicals. These radicals may polymerize or degrade into small products. Low concentration of H_2O_2 reduces the enzyme activity and high concentration can irreversibly cause enzyme inactivation [60]. The results are shown in Fig. 2.

It is said that H_2O_2 is a key factor for decolorization as depicted by the data. The efficiency of decolorization was enhanced with the increase in H_2O_2 concentration from 0.5-2.0 mM but slightly decreased hereafter. This might be due to the reason that H_2O_2 irreversibly oxidized the enzyme to reduce POD activity. The negative effect of high H_2O_2 concentration on decolorization efficiency was also perceived in our latest investigation for direct yellow 4 dye decolorized by *Citrus limon* peroxidase and GYPRA dye decolorized by *Citrus reticulate* var Kinnow peroxidase [54-55].

Enzyme/substrate contact time is also one of the most important parameters for decolorization of dyes by PODs. It has been reported that reaction time has a direct link to the structure of different dyes [55]. Textile industry effluent was independently incubated with SLP for increasing time period and the results thus obtained are shown in Fig. 2. It is obvious from the data that almost 99% decolorization was obtained after 60 min of incubation. However, after this time period no increase in percentage decolorization was recorded. In our previous investigation 100% decolorization was attained using the same effluent after 20 min by *Citrus limon* peroxidase [35], although in other reports much more time was required for maximum decolorization of textile effluent, e.g., 180 min by fenugreek peroxidase [38] and 240 min for tanning effluent by bitter gourd POD [56].

Conclusions

In this paper, efficient degradation of textile industry effluents were presented by sweet lime peroxidase. The enzyme was characterized in terms of pH and temperature. The enzyme showed good substrate affinity and high-activity 6,666 µmol/mL/min. Taking advantage of its activity, its potential was checked toward decolorizing textile industry effluent. SLP almost completely decolorized (>99%) textile industry effluent within 60 min time interval at a pH of 5.0, temperature of 55°C, H_2O_2 concentration of 2 mM, and enzyme dose of 40 U/mL. The assessment of physicochemical parameters of the textile industry effluent showed decreased toxicity after treatment with SLP.

Conflict of Interest

The authors declare no conflict of interest.

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