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# CATALYTIC POTENTIAL OF GOURD PEEL PEROXIDASE FOR BIODEGRADATION OF SYNTHETIC RECALCITRANT DYES FUCHSIN ACID AND CRYSTAL VIOLET

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## ABSTRACT

Peroxidases can be used in decolorization processes and the treatment of textile effluents. In this context, the current study evaluates the potential of the partially purified gourd peel peroxidase in decolorization of triarylmethane class dyes; fuchsin acid and crystal violet. For this purpose, some important process parameters like pH, temperature, time of incubation, dye concentration, enzyme and $H_2O_2$ dose were optimized. Besides this, the effect of mediators was also evaluated on the performance of peroxidase. 98.25 % decolorization of fuchsin acid (50 mg/l) was achieved at 36 $Uml^{-1}$ enzyme dose and 1.6 mM $H_2O_2$ concentration, in the presence of 0.2 mM vanillin, whereas pH, temperature and time for maximum decolorization were recorded to be 2.0, 30 $^0$C and 30 min respectively. However, at the same $H_2O_2$ and vanillin concentrations, 85.21 % decolorization of crystal violet was achieved at a dye concentration of 40 mg/l and enzyme dose of 28 U/ml. The pH, temperature and time for maximum decolorization were reported to be 4.5, 50 $^0$C, respectively. Thus, the data indicates that gourd peel peroxidase could be a potential source for developing an inexpensive and efficient method for the treatment of recalcitrant fuchsin acid and crystal violet dyes that are potentially toxic.

**Key words:** Gourd peel peroxidase; Decolorization, Triarylmethane dyes, Crystal violet; Fuchsin acid; Vanillin.

## INTRODUCTION

Dye overload in the industrial effluents adversely affects the biological communities. Not only these compounds form toxic products, their strong colour causes turbidity so hinders the absorption of solar radiation, thus reducing the natural photosynthetic activity, causing changes in aquatic biota. Moreover, many of these dyes present acute or chronic toxicity on the ecosystems (Reis da Silva *et al*. 2010).

Worldwide over 10,000 different dyes and pigments are used in dyeing and printing industries. The total world colorant production is estimated to be 8.00.000 tons per year (Akdogan *et al*., 2014). Due to their chemical structure, most of the dyes are resistant to fading on exposure to light, water and many chemicals (Robinson *et al*., 2001). There are many structural varieties of dyes that fall into cationic, anionic or nonionic types. Anionic dyes are categorized into the direct, acid and reactive dyes. Reactive dyes are the only colorants designed to bond covalently with the fabrics.These dyes contain chromophoric groups such as azo, anthraquinone, triarylmethane etc. and reactive groups e.g., vinyl sulfone, chlorotriazine, trichloropyrimidine etc., that form covalent bonds with the fiber.

Conventional chemical/physical methods of dye decolorization including; coagulation, adsorption and oxidation with ozone are not only expensive, generate large volumes of sludge but usually require 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*., 2007). Although, biodegradation appears to be a promising technology but unfortunately the analysis of contaminated soil and water has shown that these toxic pollutants persist even in the presence of microorganisms that are completely capable of mineralizing the pollutants. Often the environment of the microorganisms is not optimal for rapid degradation. There is a need to find alternative treatments that are effective in removing dyes from large volumes of effluents and are low in cost.

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 (Arabaci and Usluoglu, 2014). Oxidoreductive enzymes such as peroxidases and polyphenol oxidases are participating in the degradation/removal of aromatic pollutants from various contaminated sites. They all share a common mechanism in which the heme groups are the main constituents and are responsible for catalyzing the reactions in the presence of hydrogen peroxide. Although peroxidases and polyphenol oxidases participate in the catalysis of a broad range of substrates and can also work at very low concentrations, they have generally not been used for the

degradation of dyes on a larger scale due to their low catalytic activity and high purification cost (Matto and Husain, 2008). However, recent studies employing redox mediators have shown very promising results (Jamal *et al*., 2011; Jamal *et al*., 2012)

Hence for the first time an attempt has been made to extract peroxidase from an economical source of gourd peel and utilize it in partially purified form for the decolorization of recalcitrant triaryl methane class dyes (FA and CV) under various experimental conditions. Promising results obtained by this method showed that this method can be further extended for the treatment of industrial wastewater.

## MATERIALS AND METHODS

**Materials:** Fuchsin acid (FA) was purchased from Fluka Chemicals while crystal violet (CV) was a product of Applichem Chemical Co. Gourd was purchased from the local vegetable market. All other chemicals and reagents employed were obtained from Sigma-Aldrich Chemical Co. USA.

**Extraction of gourd peel peroxidase:** Fresh gourd peels (10 g) were homogenized with 100 ml of pre-cooled 100 mM phosphate buffer pH 7.0. The mixture was immediately filtered through the four layers of Muselin cloth (Nouren *et. al*. 2013). The filtrate was then subjected to 80 % ammonium sulphate fractionation by overnight continuous stirring in cold (placing in cold cabin). The precipitated proteins were then collected by centrifugation at 10,000 rpm for 15 min at 4 $^0$C using a Remi C-24 centrifuge and the pellets obtained were then redissolved in 25 ml of 100 mM phosphate buffer (pH 7.0) and then subjected to dialysis against 25 mM phosphate buffer (pH 7) (Bhatti *et al*., 2006 ; Khan and Husain, 2007).

**Determination of peroxidase activity:** Peroxidase activity was determined colorimetrically using spectrophotometer (Cecil 7200) following the formation of tetraguaiacol ($A_{max}$=470 nm, $\varepsilon$= 26.6 $mM^{-1}cm^{-1}$) with slight modification in the earlier reported assay method (Liu *et al*. 1999). The reaction mixture contained 1mL of 100 mM acetate buffer pH 5.0, 1 mL of 15 mM guaiacol, 1mL of 1.6 mM $H_2O_2$ and 60 µL of the peroxidase extract.

One unit of peroxidase activity was defined as the amount of enzyme catalyzing the oxidation of 1 µmol of guaiacol in 1 min.

**Effect of pH on the decolorization of FA and CV:** Each dye was treated with 20 EU/ml gourd peel peroxidase in the presence of different pH buffers (200 mM) ranging from 2.0–8.0 at 40 $^0$C for 30 min. Reaction was stopped by keeping it in boiling water bath for 10 min and then centrifuged with10,000 rpm for 10 min to remove insoluble products. Dye decolorization by gourd peel peroxidase was monitored at the specific wavelength respective to each dye (FA 543 nm and CV 590 nm). The % decolorization was calculated by taking untreated dye solution as control.

**Effect of temperature on the decolorization of FA and CV:** Both the dyes were treated with gourd peel peroxidase in the presence of 200 mM respective buffer (2.0 for FA and 4.5 for CV) at wide range of temperature (25- 65 $^0$C) for 30 min under the same conditions mentioned in section 2.4.

**Effect of time on the decolorization of FA and CV:** Dye solutions were treated with 20 EU/ml of gourd peel peroxidase in 200 mM buffer at their respective temperature optima (30 $^0$C for FA and 50 $^0$C for CV) for different time intervals (0-90 min) under the same mentioned conditions (section 2.4).

**Effect of gourd peel peroxidase concentration on the decolorization of FA and CV:** The dyes were decolorized with increasing concentrations (4–48 $EUml^{-1}$) of gourd peel peroxidase in the presence of 200 mM respective buffer solutions keeping other parameters as constant under the same conditions as mentioned earlier.

**Effect of dye concentration on the decolorization of FA and CV:** The extents of dye decolorization were checked with increasing concentrations (20-100 mg/l) of FA and CV violet. Reaction mixture was treated with 36 EU/ml in 200 mM of respective buffer at specific value of pH and temperature under the same mentioned conditions.

**Dye decolorization with varying concentration of $H_2O_2$:** Both the dyes were incubated with increasing concentrations of $H_2O_2$ (1.0–2.0 mM) in the presence of 36 and 28 U/ml of enzyme dose and 50 and 40 mg/l of dye concentration for FA and CV, respectively. Although other parameters i.e. pH (2 for FA and 4.5 for CV), temperature (30 $^0$C for FA and 50 $^0$C for CV) and time (30 min for FA and 40 min for CV) were kept as constant.

**Dye decolorization in the presence of different redox mediators:** Both the dyes were incubated with gourd peel peroxidase in the presence of different redox mediators including; 6-dimethoxy phenol, 1-hydroxy benzotriazol, *p*-cumeric acid, pyrocatechol, syringaldehyde, syringic acid, vanillin and veratryl alcohol (1.0 mM) keeping all the other conditions as constant as mentioned in section 2.9.

**Calculation of percent dye decolorization:** The percent dye decolorization was calculated for each dye according to the following equation

$$\% \ Decolorization = A_u - A_t / A_u \times 100$$

Where, $A_u$ is absorbance of the untreated dye, $A_t$ is absorbance after treatment.

**Statistical analysis:** The results were reported as mean ± standard deviation.

## RESULTS AND DISCUSSION

**Effect of pH on the decolorization of** FA and CV**:** Fig.1 represents the effect of pH on the decolorization of FA and CV dyes by soluble gourd peel peroxidase. Both of the dyes were maximally decolorized in acidic media i.e., pH 2.0 (FA) and 4.5 (CV).There are several earlier reports regarding the maximum decolorization of dyes by various plant peroxidases (Bhunia *et al*., 2001; Akhtar *et al*., 2005a; Mohan *et al*., 2005, De Souza *et al*., 2007) in the buffers of acidic pH values. Khan and Husain (2007) worked on potato and brinjal polyphenol oxidases and also reported that the decolorization rate was significantly high in the buffers of lower pH around 3, it was continuously decreased on increasing the pH of the buffers.

**Effect of temperature on the decolorization of** FA and CV: Fig. 2 showed that, 30 and 50 $^0$C were reported to be as the optimum temperatures for FA and CV, respectively. While above and below these optima the extent of decolorization decreased significantly. At 65 $^0$C FA and CV showed almost 50 % and 25 % of decolorization. It means that gourd peroxidase was less stable above 50 $^0$C, which might be due to unfolding of quaternary structure of peroxidase thus showing less activity towards higher temperature. Consistent with our work, maximum degradation of direct dyes and disperse red 343 with turnip POD and horseradish POD has been observed to be around 50 $^0$C (Schmitt *et al*., 2012) and 30 $^0$C (Matto and Husain; 2007) respectively.

**Effect of contact time on the decolorization of** FA and CV: Fig. 3 shows the decolorization of FA and CV as a function of contact time with the gourd peel POD. The data showed that the dye decolorization in both the cases increased with increase in reaction time. The optimum result was obtained at 30 and 40 min for FA and CV dyes, respectively. Then the increase in extent of decolorization was negligible. These results are in close agreement with earlier published work on HRP, BGP and TP catalyzed decolorization of textile dyes (Akhtar *et al*., 2005a; Mohan *et al*., 2005, Silva *et al*., 2012).

**Effect of gourd peel peroxidase concentration on the decolorization of** FA and CV: The decolourization effect of groud peel peroxidase on FA and CV dyes was shown in Fig.4. The rate of dye decolorization was continuously enhanced with increasing the amount of enzyme. At the concentration of 36 EU/mL and 28 EU/ml, the gourd peel peroxidase were sufficient to decolorize 50mg/l of FA and CV, respectively. After the optima there was no increase or decrease in activity was recorded. Mohan *et al*. (2005) found that dye decolourization was gradually increased as the concentration of enzyme was enhanced up to an appropriate extent and after that there was no significant increase or decrease in % decolorization.

**Effect of dye dose on the decolorization of** FA and CV: FA and CV dye solutions ranging from 10-100 mg/l were incubated with 36 and 28 EU/ml of gourd peel POD respectively. Under these conditions 50 mg/l of FA and 40 mg/l of CV were decolorized effectively. There was a decrease in % decolorization following this concentration, as shown in Fig. 5. This may be due to all the active sites of the enzyme are saturated for binding more dye molecules. Khan and Husain (2007) observed the same optimum dose (40 mg/l) of crystal violet sould be decolorized by bitter gourd peroxidase. The similar trend was observed by Mohan *et al*. (2005) for decolorization of acid black 10 BX by horseradish peroxidase.

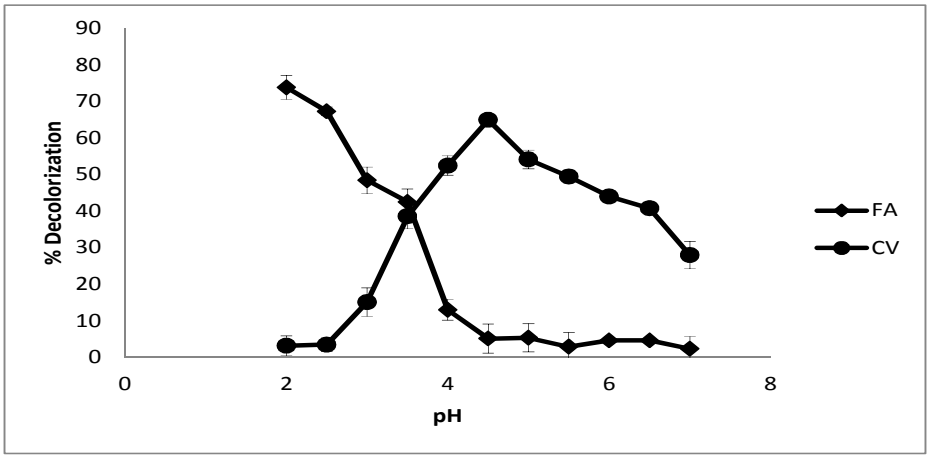


**Fig. 1: Effect of pH on % decolorization of FA and CV by gourd peel POD**

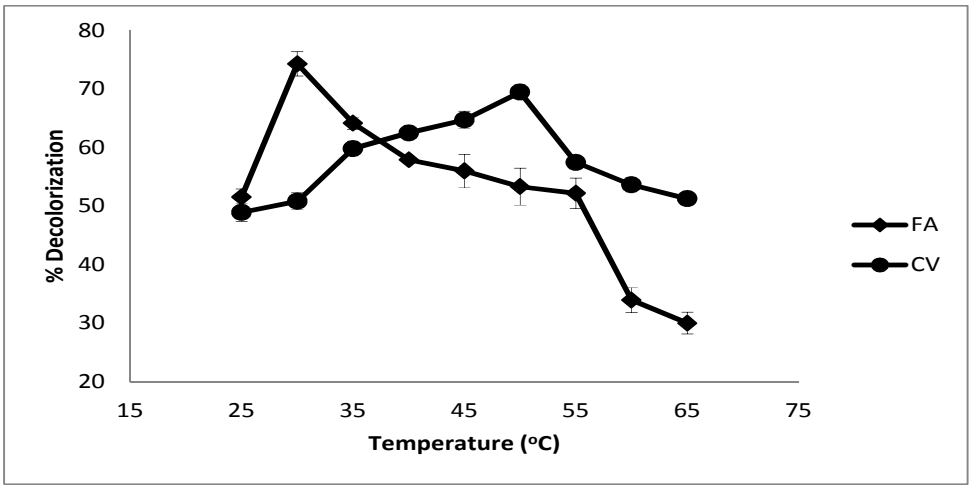


**Fig. 2: Effect of temperature on % decolorization of FA and CV by gourd peel POD**

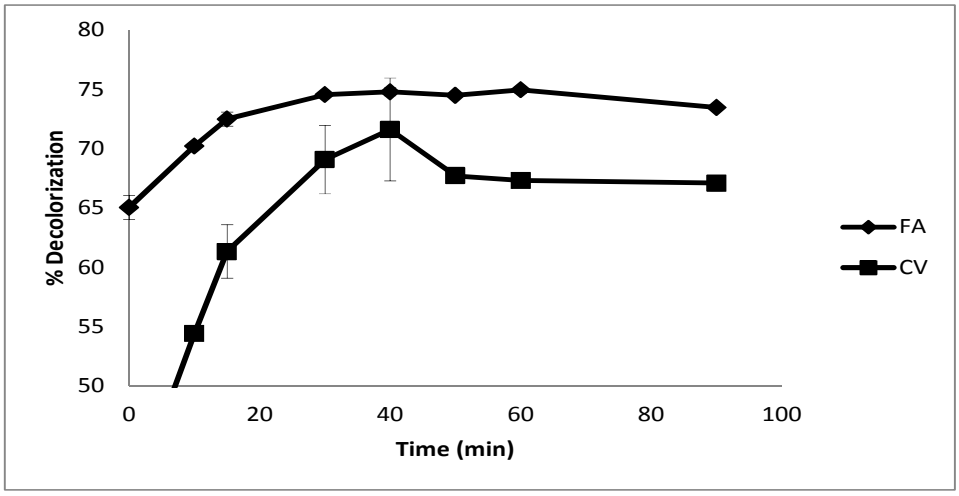


**Fig. 3: Effect of time of incubation on % decolorization of FA and CV by gourd peel POD**

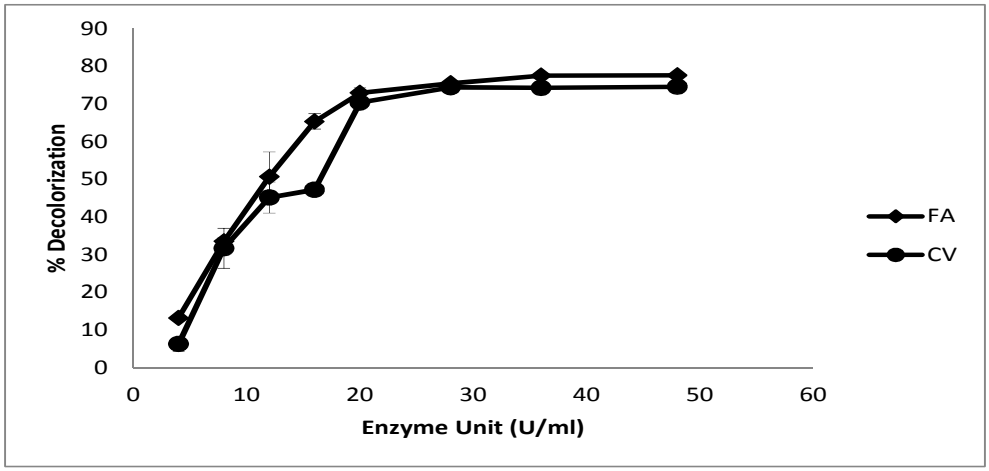


**Fig. 4: Effect of gourd peel POD concentration on % decolorization of FA and CV.**

**Effect of Hydrogen peroxide on decolorization of** FA and CV: $H_2O_2$ acts as co-substrate to activate the enzymatic action of peroxidase radical. It contributes in the catalytic cycle of peroxidase, to oxidize the native enzyme to form an enzymatic intermediate which accepts aromatic compound to carry out its oxidation to a free radical form. 1.6 mM of $H_2O_2$ was sufficient for both of the dyes to be decolorized optimally, Fig. 6. Comparing

the previous reports, less $H_2O_2$ was required for decolorization mediated by peroxidases in most cases e.g. Bhatti *et al*. (2012) reported that maximum decolorization of Solar blue A and Solar flavine G was reported at 0.8 mM concentration of $H_2O_2$.

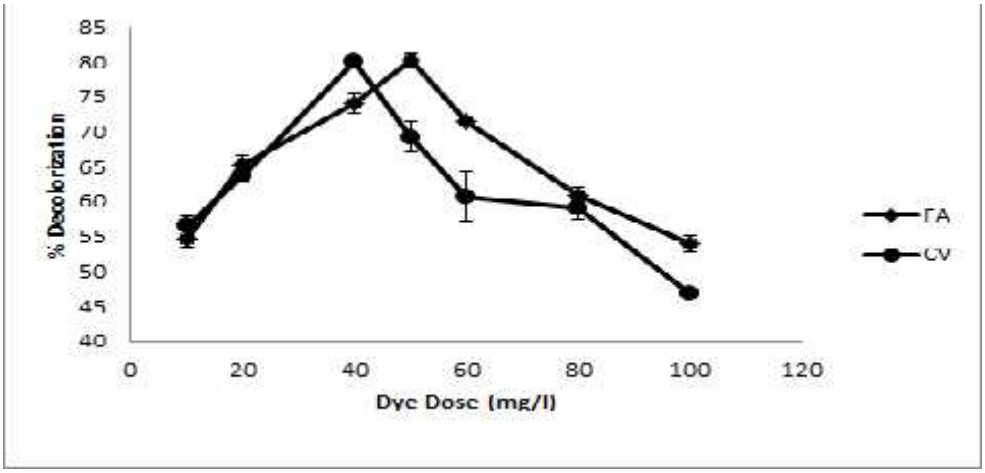


**Fig. 5: Effect dye dose on % decolorization of FA and CV**

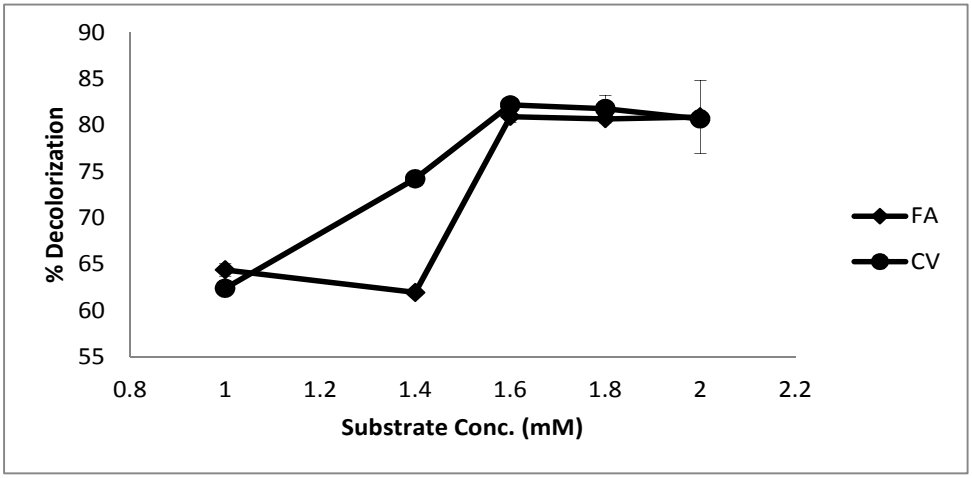


**Fig. 6: Effect of hydrogen peroxide on % decolorization of FA and CV**

**Dye decolorization in the presence of different redox mediators:** Mediators play a dual role first increase the substrate range of dyes for enzyme and secondly enhance the rate of oxidation. The data analysis showed that among the eight different redox mediators studied for dye decolorization, only vanillin enhanced the dye decolorizing ability of gourd peel peroxidase in both cases. The extent of decolorization was 98.25 and 85.21 % for FA and CV dyes respectively. Previously, Jamal *et al*. (2010) reported 84 and 89.1 % decolorization of naphthol blue black and rodamin 6G in presence of vanillin and by pointed gourd peroxidase, respectively.

**Conclusion:** The present study showed that gourd peel is one of the potential sources of peroxidase enzyme. It showed a great potential in the decolorization of triphenyl methane class dyes. FA and CV were decolorized up to 98.25 and 85.21 %, respectively in the presence of 1.6 mM $H_2O_2$ and 0.2 mM vanillin. The other conditions of pH, temperature, time, enzyme dose and dye concentration for FA and CV were reported to be; 2 and 4.5, 30 and 50 $^0$C, 30 and 40 min, 36 and 28 EU/ml, 50 and 40 mg/l. The promising results showed that this method may be extendable for the treatment of effluents coming out of industries and mixtures of dyes present in wastewaters. Furthermore, these plant peroxidases in their immobilized form may also be applied at the large-scale treatment of wide range of structurally dissimilar dyes present in the industrial effluents.

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