Recent Research in Science and Technology 2010, 2(2): 49–53
ISSN: 2076-5061
www.recent-science.com

BIOTECHNOLOGY

BITTER GOURD (*MOMORDICA CHARANTIA*) PEROXIDASE IN DECOLORIZATION OF DYES FROM TANNERY EFFLUENT

Subramaniam Sivakumar[1*], Balasubramaniam Ramesh[2], Kuppuswamy Kavitha[3], Venkatesan Gopalakrishnan[2], Vasu Kanniyappan[1]

[1]Department of Biochemistry, Sri Sankara Arts and Science College, Kanchipuram - 631 561, India
[2]Department of Biotechnology, Sri Sankara Arts and Science College, Kanchipuram - 631 561, India
[3]Department of Microbiology, Sri Sankara Arts and Science College, Kanchipuram - 631 561, India

Abstract

Bitter Gourd (*Momordica charantia*) is a commonly available plant in India and its applications are limited to few medicinal properties in addition to being edible. The primary objective of this study is to evaluate the efficacy of *Momordica charantia* peroxidase in the degradation of dyes present in tannery effluents under various experimental conditions like pH, Temperature, Time intervals and Enzyme concentration on the basis of the one-factor-at-a-time (OFAT) method. The maximum decolorization was achieved at pH 5.0 – 6.0, 40°C temperature, in 4 hours with an enzyme concentration of 0.6 ml consisting of 4500 Units enzyme activity extracted from 0.5 g of Bitter Gourd. Present study results demonstrate that the *Momordica charantia* peroxidase is an effective biocatalyst for the treatment of effluents with dyes from tanning industries.

Key Words: *Momordica charantia;* Peroxidase; Dye decolorization; Tannery effluent.

Introduction

During leather production, dyeing of leather is one of the essential steps during post tanning operations. Dyes were used to stabilize the color and also to improve the fixation of three dimensional structure of fiber. The effluent from leather industry was found to show toxic effects to various aquatic ecosystems [1]. Some of the tanning and other industries are discharging their effluents without proper treatment to the environment and pollutants present in the effluent not only affect the color of the water and also toxic to aquatic and other forms of life [2]. The industrial tanning of leather usually produces considerable amounts of chromium-containing solid waste and liquid effluents and raises many concerns on its environmental effect as well as on escalating landfill costs. Actually, these shortcomings are becoming increasingly a limiting factor to leather industrial activity that claims for simple and cost effective method of dye disposals [3]. Effluent treatment methods based on removal of organic polluting agents are in many cases expensive and inefficient. Therefore, new methods and technologies of effluent treatment have being explored with the aim to reach complete removal of contaminants [4]. Conventional dye degradation physical and chemical methods are not in vogue. Biodegradation method was found to be a promising technology [5]. Recently an enzymatic approach has attracted much interest in the removal of dyes from effluent as an alternative method to the conventional chemical as well as microbial treatments [6]. Since enzymes can act in a wide range of substrates and remove organic pollutants even at low concentrations in the effluent with high rate of clearance, oxidoreductive enzymes such as peroxidases and polyphenol oxidases are used in degradation of pollutants [7].

Momordica charantia peroxidase is one of such enzyme used for degradation of phenols from waste water [8]. *Momordica charantia* is commonly known as bitter gourd, is widely planted in tropical areas. It has also been frequently used as medicinal herb, because of its anti-diabetic, anti-helminthic, abortifacient, anti-bacterial, antiviral, and chemopreventive functions [9]. *Momordica charantia* peroxidase was extracted from *Momordica charantia* fruit pulp. Several studies were carried out to study the effect of *Momordica charantia* peroxidase in pollutant removal in textile effluent, non-textile effluent, waste water and polluted water [10, 11].

An effort was made in the present study to optimize the experimental conditions like different pH, Temperature, Time intervals and Enzyme concentration and to assess efficacy of *Momordica charantia* peroxidase in the decolorization of tannery effluent.

* *Corresponding Author, Email: sivabio@gmail.com*

Materials and Methods

Materials

Momordica charantia fruits were collected from the environment of Enathur village (12^{0}51'45''N, 79^{0}44'08''E), Kanchipuram, Tamilnadu, India. The samples were aseptically transferred into sterilized plastic bags. Effluent water of tanning industry was collected from Ranipet (12^{0}56'17''N, 79^{0}19'10''E), Tamilnadu. The sample was aseptically transferred into sterilized bottle.

Partial Purification of *Momordica charantia* peroxidase

A 50gm of *Momordica charantia* fruit pulp was homogenized using 100ml of 0.1M sodium acetate buffer with pH 5.6. Four layers of cheesecloth were used to filter homogenate. Filtered homogenate was then centrifuged at 10,000g in Remi C-24BL cooling centrifuge for 15 minutes. By adding 20-80% (w/v) of ammonium sulphate, salt fractionation was carried out with the clear supernatant. The content was stirred overnight to get maximum precipitate at 4^{o}C. Precipitate was obtained by again centrifuging at 10,000g in Remi C-24BL cooling centrifuge. The obtained precipitate was redissolved in appropriate volume of 0.1M sodium acetate buffer (1g/ml) with pH 5.6 [12].

Momordica charantia peroxidase assay

Peroxidase activity present in the partially purified extract was estimated from the change in absorbance at 470nm in 0.1M sodium acetate buffer (pH 5.6) at 37^{o}C [13]. Reaction mixture contained 0.05ml of 25mM guaiacol, 0.05ml of 10mM Hydrogen peroxide, 0.01ml of enzyme extract and 0.1M sodium acetate buffer was added in a final volume of 4.0ml. All reactions were started by the addition of hydrogen peroxide to the reaction mixture. The color developed was read for 2 minutes in every 30seconds intervals. The rate of guaiacol peroxidation reaction was linear function of time for 1.5 to 2.0mintues in all assay procedures.

One unit of enzyme activity was defined as the amount of enzyme required to increase the absorbance by 0.001 per minute [14].

Effluent Processing

Effluent collected was centrifuged and the clear supernatant was diluted with distilled water till it exhibited an absorbance around 0.55 at 470nm for control tube measurement [15].

General Procedure for Effluent treatment

Tanning effluent water was incubated with *Momordica charantia* peroxidase in 0.1M sodium acetate buffer, pH 5.6 at 37^{o}C in the presence of 10mM hydrogen peroxide for 4 hours. Dye decolorization was monitored at 470nm and the percent decolorization was calculated by taking untreated dye solution as control 100% [8].

Effect of pH, Temperature, Time and Enzyme concentration

The effects of pH in dye degradation action of *Momordica charantia* peroxidase was studied by treating tanning water at different pH values from 3.0 – 8.0 in the presence of hydrogen peroxide at 37°C. The molarity of each buffer was 0.1M.

The effect of temperature in dye degradation action of *Momordica charantia* peroxidase was studied by treating *Momordica charantia* peroxidase with tanning water at different temperature values (4°C - 80°C) in 0.1M sodium acetate buffer, pH 5.6 at 37^{o}C in the presence of 10mM hydrogen peroxide for 4hrs.

The effect of time duration in dye degrading action of *Momordica charantia* peroxidase was studied by treating *Momordica charantia* peroxidase with tanning water at different time intervals (1 – 5hrs) in 0.1M sodium acetate buffer, pH 5.6 at 37^{o}C in the presence of 10mM hydrogen peroxide.

Tanning effluent was treated with increasing concentrations of soluble *Momordica charantia* peroxidase (0.2ml – 1.0ml) in 0.1M sodium acetate buffer, pH 5.6 at 37^{o}C in the presence of 10mM hydrogen peroxide for 4hrs [8].

Calculation of percent dye decolorization

The decolorization was calculated for tanning dye as described previously [16]. Parameter percent decolorization was defined as

$$\text{Percent decolorization} = \frac{\text{Absorbanc of control -- Absorbance after treatment}}{\text{Absorbanc of control}} \times 100$$

Statistical Analysis

Statistical analysis was carried out by Mean and standard deviation. Data were analyzed using one sample "t" test by applying mean comparison method. Significant difference were defined as two tailed $p<0.05$. Data from all experiments were processed in the program Microsoft Excel to represent tables and graphs.

Results

The results of *Momordica charantia* peroxidase activity and percentage dye decolorization under various conditions were reported in table 1 and table 2 respectively.

Table 1: Soluble *Momordica charantia* peroxidase activity

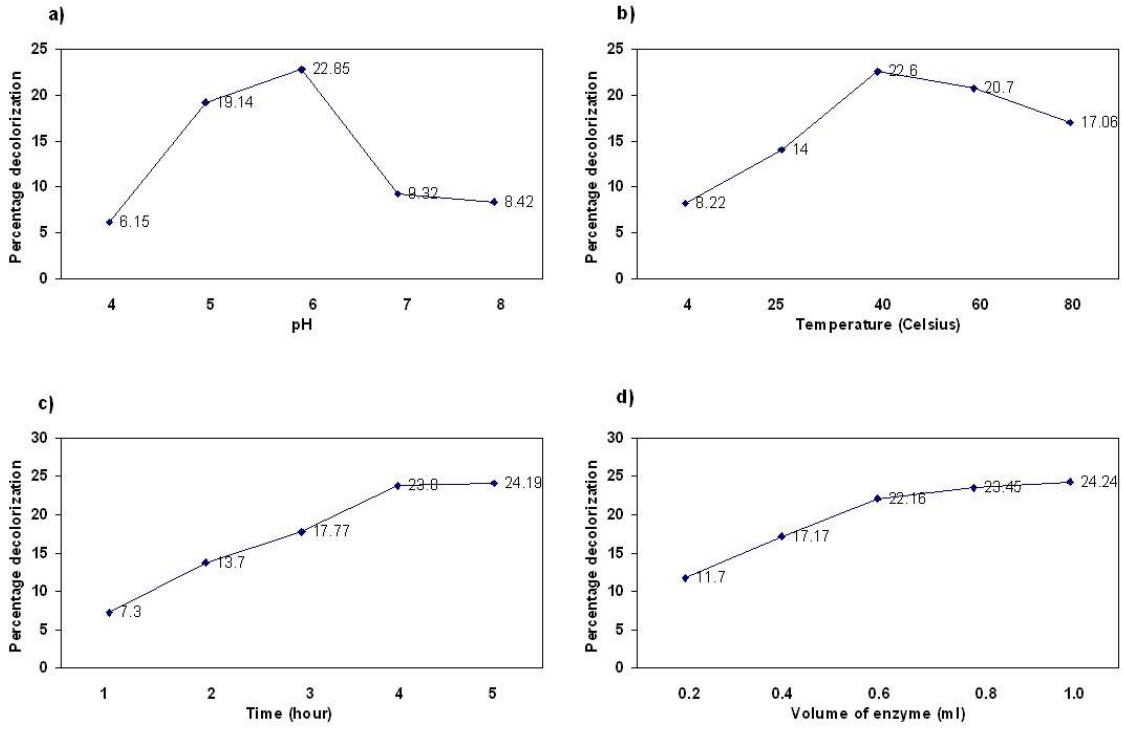
Activity in crude extract (units/min)	40.3 ± 1.0
Activity in fractionated extract (units/min)	75.0 ± 2.5

Table 2: Percentage decolorization in tanning effluent by *Momordica charantia* peroxidase was showed in different conditions

S. No	Effects	Parameters	Values				
1.	Effect of pH[a]	pH Value	4	5	6	7	8
		Percentage decolorization (%)	6.15 ± 0.13	19.14 ± 0.14	22.85 ± 0.02	9.32 ± 0.28	8.42 ± 0.38
2.	Effect of Temperature[a]	Temperature in Celsius	4	25	40	60	80
		Percentage decolorization (%)	8.22 ± 0.78	14.00 ± 1.10	22.60 ± 1.26	20.70 ± 0.95	17.06 ± 1.00
3.	Effect of Time[a]	Time in hour	1	2	3	4	5
		Percentage decolorization (%)	7.30 ± 0.63	13.7 ± 1.14	17.77 ± 0.71	23.80 ± 1.16	24.19 ± 1.86
4.	Effect of Enzyme Concentration[a]	Enzyme concentration (ml)	0.2	0.4	0.6	0.8	1.0
		Percentage decolorization (%)	11.70 ±0.99	17.17 ± 1.37	22.16 ± 0.93	23.45 ± 0.66	24.24 ± 0.24

a - All the values showed significance at $p < 0.05$ level

Figure 1. Effects of pH, Temperature, Time and Enzyme concentration on dye decolorization activity of *Momordica charantia* peroxidase.



a) pH-activity profile. Partially purified peroxidase was incubated in the buffer of varying pH (4.0 – 8.0) and percentage dye decolorization was measured. The molarity of each buffer was 0.1M. b) Temperature-activity profile. Dye decolorization percentage by partially purified peroxidase was measured in 0.1M acetate buffer, pH 5.6 at various temperatures (4 – 80°C). c) Time-activity profile. Percentage of dye decolorization was measured in 0.1M acetate buffer, pH 5.6 at 40°C for various incubation time intervals (1 - 5hrs). d) Enzyme-activity profile. Varying volumes of enzyme source (0.2 – 1.0ml) was utilized for dye decolorization in 0.1M acetate buffer, pH 5.6 at 40°C for 4hrs.

Discussion

The *Momordica charantia* peroxidase activity determination in crude and partially purified fraction showed that the enzyme activity almost doubled due to fractionation procedure.

Effect of pH

Tanning effluent was treated with equal volume of enzyme in the buffers of different pH values. Most of the dye was maximally decolorized in the range of pH 5.0 – 6.0. As pH of the decolorizing sample was increased up to pH 8.0, the rate of decolorization decreased in the study sample. The pH effect was graphically represented in the figure-1a. The role of pH on the dye degradation by soluble *Momordica charantia* peroxidase, showed maximum activity observed in the buffer of pH 6.0. Akhtar and Husain reported that bitter gourd peroxidase preparation was capable of removing remarkably high percentage of phenols from polluted water. Maximum removal of phenols was found in the buffers of pH values 5.0 – 6.0 [8]. The present study concurs with previous findings that the decolorization was maximum at pH 6.0.

Effect of Temperature

The effect of different temperatures (4°C – 80°C) on the dye decolorization was monitored and it was observed that all the dyes were decolorized maximally at 40°C. Above and below this temperature rate of decolorization was gradually decreased. The enzyme effect was graphically represented in the figure-1b. Akhtar *et al.* reported that peroxidase from *Momordica charantia* is highly effective in decolorizing reactive textile dyes and decolorization was maximal at 40°C [11]. Fatima and Husain reported that *Momordica charantia* peroxidase activity maximum at 40°C [17]. Matto and Husain were reported that the activity found to be maximal at 5.0 pH and 37°C [18]. The present study once again proves that 40°C is temperature optima for *Momordica charantia* peroxidase.

Effect of Time

Tanning dye was incubated with 0.5ml of *Momordica charantia* peroxidase for increasing time period. The effect of time was graphically represented in the figure-1c. Effective decolorization was found to be achieved at 4hrs time interval. Although more color decolorized when effluent dye was incubated for longer time duration, however rate of decolorization was slow after 4hrs. Matto and Husain were reported that *Momordica charantia* peroxidase decolorized more than 90% effluent after 3hr at pH 5.0 and 40°C [18].

Effect of Enzyme Concentration

Tanning effluent was treated with increasing volume of *Momordica charantia* peroxidase extract (0.2ml – 1.0ml) for 4hr at 37°C. The enzyme effect was graphically represented in the figure-1d. It showed that the decolorization effect increases as the volume of enzyme increases but it attained saturation i.e. increase become insignificant when above 0.6ml of enzyme used. It clearly showed that 0.6 ml might be good volume for the effective decolorization of the *Momordica charantia* peroxidase.

The optimal conditions for peroxidase from *Momordica charantia* in dye decolorization are summarized in the Table 3.

Table 3: Study for the determination of the optimum conditions for the dye degradation

S. No	Parameters	Range evaluated	Optimized parameters
1.	pH	3.0 – 8.0	6.0
2.	Temperature (Celsius)	4 – 80	40
3.	Time (hr)	1 - 5	4
4.	Enzyme concentration (ml)	0.2 -1.0	0.6

Conclusion

The main aim of the decolorization process was to eliminate their harmful effect on the environment. The optimization of decolorization of tanning dye by *Momordica charantia* peroxidase was achieved with the present study through one-factor-at-a-time (OFAT) method by using four factors namely pH, Temperature, Time and Enzyme concentration. The optimized factors, pH 6.0, 40°C temperature, minimum of 4hrs of incubation and 0.6 ml of enzyme (1gm/ml) were determined through the present study. These conditions can be used for dye decolorization by *Momordica charantia* peroxidase in tanning effluent treatment plants. The present study conclusively demonstrates that *Momordica charantia* peroxidase may be effectively and economically used in tannery dye decolorization, particularly in tropical countries like India where *Momordica charantia* is in abundance.

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