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REMOVAL OF DYES IN TEXTILE EFFLUENT USING BITTER GOURD, POTATO AND BRINJAL ENZYMES

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ABSTRACT

In this study an effort has been made to use per oxidases enzyme of bitter gourd (*momordica charantia)* and plant polyphenol oxidases enzymes of potato (*solanum tuberosum) and* brinjal *(solanum melongena)* for the treatment of various important dyes used in textile industries. Potato, brinjal and bitter gourd is a commonly available plant in India. The primary objective of this study is to evaluate the efficacy of peroxidase and poly phenol oxidases enzymes in the degradation of dyes present in textile effluents under various experimental conditions like pH, Time intervals and Enzyme concentration on the basis of the one-factor-at-a-time (OFAT) method. And also evaluated the physico-chemical parameters of effluent after addition of enzyme extract. The maximum decolourisation of bitter gourd, potato and brinjal enzyme activity was achieved at pH 6.0, 10.0 and 3.0 with 4, 2 and 3 hrs of incubation and with an enzyme concentration of 0.8,0.6 and 1 ml. The maximum decolourisation percentage obtained by potato enzyme extract is 95% and hence this study results demonstrate that, among these three enzyme extracts the potato (*Solanum Tuberosum)* poly phenol oxidase enzyme is an effective biocatalyst for the treatment of effluents with dyes from textile industries and also most of the physico-chemical parameters values has also been reduced after addition of this enzyme extract.

KEY WORDS: Decolourisation; Textile Dyes; Polyphenol Oxidases; Peroxidases; Potato; Bitter Gourd; Brinjal.

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 textile industry was found to show toxic effects to various aquatic ecosystems. Some of the textile 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. The industrial dyeing of textile usually produces 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 textile industrial activity that claims for simple and cost effective method of dye disposals. 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. Conventional dye degradation physical and chemical methods are not in vogue.
A number of different physical methods are used such as membrane-filtration processes (Reverse osmosis, electro-dialysis, nano-filtration) and various adsorption techniques. The major disadvantage associated with the membrane filtration processes is that they have a limited lifetime, the problem of membrane fouling and the cost of periodic replacement must thus be included in any analysis of their economic viability. Adsorption techniques has also been used for the decolourisation and it is been practiced in many fields. It is found to be efficient, if the sorbent is inexpensive & does not require an additional pre-treatment step before its application. Chemical methods includes a number of processes such as coagulation or flocculation combined with flotation and filtration, precipitation-flocculation with Fe(II)/$Ca(OH)_2$, electro-kinetic coagulation, conventional oxidation methods by oxidizing agents (ozone), irradiation, electro-flotation or electrochemical processes. These chemical techniques are helpful in the

removal of dyes from the wastewater but are expensive and hence not much used and having one disadvantage of disposal problem because of the accumulation of the concentrated sludge. These methods are efficient for waste water treatment purposes contaminated with different pollutants in them, but are not that much used due to some reasons of high electrical energy demand, large consumption of the chemical reagents and high cost.

Biodegradation method was found to be a promising technology. 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. 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, oxido-reductive enzymes such as peroxidases and polyphenol oxidases are used in degradation of pollutants.

Bitter gourd, potato and brinjal are widely planted in all areas. Bitter gourd peroxidase was extracted from bitter gourd fruit pulp and potato & brinjal poly phenol oxidase enzymes were extracted from their respective pulps. Several studies were carried out to study the effect of potato, brinjal and bitter gourd enzymes for pollutant removal in textile effluent, non-textile effluent, waste water and polluted water. An effort was made in the present study to optimize the experimental conditions like different pH, Time intervals and Enzyme concentration and to assess efficacy of potato, bitter gourd and brinjal enzyme extracts in the decolourisation of textile effluent.

MATERIALS AND METHODS

Sample collection

Bitter gourd, potato and brinjal vegetables was collected from the nearby local market areas and the effluent has been collected from the textile industry located in Arakkonam town. Both materials are kept safely under the sterile condition.

Extraction of per-oxidase enzyme from Bitter gourd (*momordica charantia)*

A 100gm of *Momordica charantia* fruit pulp was homogenized using 200ml of 0.1M sodium acetate buffer. 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 4ºC. Precipitate was obtained by again centrifuging at 10,000g in Remi C-24BL cooling centrifuge. The obtained precipitate was re-dissolved in appropriate volume of 0.1M sodium acetate buffer (1g/ml).

Extraction of poly phenol oxidase enzyme from Potato and Brinjal (solanum tuberosum and momordica melongena*)*

A 100gm of Potato and brinjal were homogenized with 200ml of pre-cooled 50 mmol/L sodium citrate buffer, in the presence of benzoic acid (1.8 mg/L). Benzoic acid was used to stop the enzymatic browning during enzyme extraction. The mixture was immediately filtered through the four layers of cheesecloth. The extract was then centrifuged at 3000 g for 10 min at 4°C in a Remi Cooling Centrifuge C-24. The supernatant was then subjected to 0–60% ammonium sulphate fractionation by overnight continuous stirring in cold. The precipitated proteins were then collected by centrifugation at 1000*g* for 20 min at 4°C and the pellets obtained were then re-dissolved in 50 ml of 50 mmol/L sodium citrate buffer. The dissolved proteins were dialyzed against the assay buffer containing benzoic acid.

Effluent processing

Effluent collected was centrifuged and the clear supernatant was diluted with distilled water for control tube measurement.

General procedure for Effluent treatment

Textile effluent water was incubated with potato, brinjal and bitter gourd enzyme extracts at 70 rpm with sodium acetate and sodium citrate buffer and in the presence of hydrogen peroxide in case of bitter gourd enzyme. Dye decolourisation was monitored at 470nm and the percent decolourisation was calculated by taking untreated textile effluent as control 100%.

Effect of pH, Time and enzyme Concentration

- The effects of pH in dye degrading action of bitter gourd peroxidas*e* and potato, brinjal poly phenol oxidase enzyme was studied by incubating those enzyme extracts with textile water at different pH values of 3,5,6,7 & 10 .

●The effect of time duration in dye degrading action of bitter gourd peroxidase and potato, brinjal poly phenol oxidase enzyme was studied by incubating those enzyme extracts with textile water at different time intervals from 1to 5hrs.

● Textile effluent was incubated with increasing concentrations of soluble bitter gourd peroxidase and potato, brinjal poly phenol oxidase enzyme extracts from 0.2ml to 1.0 ml.

Calculation of percent dye decolourisation

The decolourisation was calculated for textile dyes by using the below formula. Parameter percent decolourisation was defined as,

$$\textbf{Decolourisation (\%)} = \frac{\textbf{Absrorbance of control} - \textbf{Absorbance after treatment}}{\textbf{Absorbance of control}} * \mathbf{100}$$

Analysis of physico-chemical parameters

Various physico-chemical parameters has been found out for the raw textile effluent and it is been compared with textile effluent after addition of enzyme extract. The various parameters are; pH, colour, odour, Turbidity, Total Hardness (calcium & magnesium), Chlorides, Electrical conductivity, Total solids, Total dissolved solids, Total suspended solids, Dissolved oxygen, Bio-chemical oxygen demand, Chemical oxygen demand.

RESULTS AND DISCUSSION

Decolourisation of effluent

Bitter gourd (*Momordica charantia)* peroxidase enzyme and potato(*solanum tuberosum),*brinjal (*solanum melongena)* has been widely used in removal of dyes from textile effluent under various experimental conditions like varying pH, time and concentration which showed a significant result and it has been summarized below.

Effect of pH

Textile effluent was treated with equal volume of per oxidase and poly phenol oxidases enzyme of different pH values of 3, 5, 6, 7, 10. Most of the dyes was maximally decolourised in the pH 6.0 for bitter gourd per oxidase enzyme, pH 10.0 for potato poly phenol oxidase enzyme and pH 3.0 for brinjal poly phenol oxidase enzyme.As pH of the decolourising sample was increased up to pH 10, and after that the rate of decolourisation decreased in the study sample. The pH effect was graphically represented in the figure 1,2 and 3.The role of pH on the dye degradation by soluble peroxidase enzyme and poly phenol oxidase enzymes has been tabulated in table 1.

Table 1. Percentage decolourisation of textile effluent by bitter gourd, potato and brinjal enzymes

pH values	Percentage of Decolourisation using bitter gourd enzyme(%)	Percentage of Decolourisation using potato enzyme (%)	Percentage of Decolourisation using brinjal enzyme (%)
3	41.1	12.5	25.2
5	40.8	20.4	20.1
6	61	22.5	22.6
7	55.8	30.7	12
10	45	33.5	17

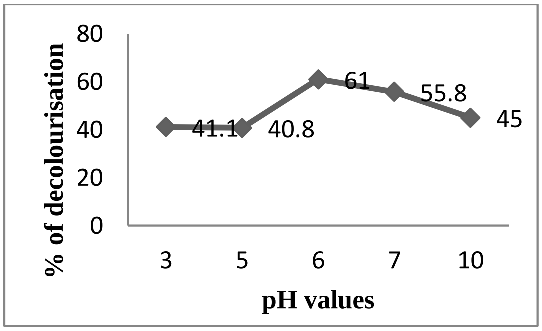

Fig. 1 Percentage of Decolourisation on the Effect of pH using Bitter gourd enzyme

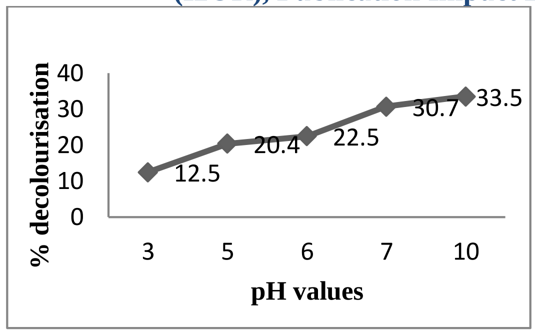

Fig. 2 Percentage of Decolourisation on the Effect of pH using potato enzyme

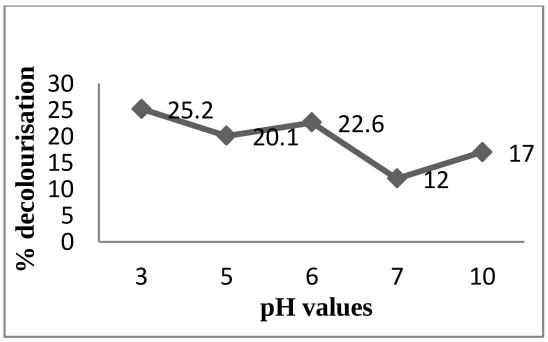

Fig. 3 Percentage of Decolourisation on the Effect of pH using Brinjal enzyme

Effect of Concentration

Textile effluent was treated with per oxidase and poly phenol oxidases enzyme of different volumes of 0.2 to 1ml. Most of the dyes was maximally decolourised in the concentration of 0.8 ml for bitter gourd per oxidase enzyme, at concentration 0.6 ml for potato poly phenol oxidase enzyme and at concentration of 1.0 ml for brinjal poly phenol oxidase enzyme. The concentration of the decolourising sample was increased up to 1 ml, and after that the rate of decolourisation decreased in the study sample. The concentration effect was graphically represented in the figure 4, 5 and 6.The role of concentration on the dye degradation by soluble peroxidase enzyme and poly phenol oxidase enzymes has been tabulated in table 2.

Table 2. *Percentage Decolourisation of textile effluent by Bitter gourd, Potato and Brinjal enzymes*

Concentration values(ml)	Percentage of Decolourisation using bitter gourd enzyme (%)	Percentage of Decolourisation using potato enzyme (%)	Percentage of Decolourisation using brinjal enzyme (%)
0.2	28	23.2	10.4
0.4	35.7	26.1	12.8
0.6	36.9	51.3	14.9
0.8	42.4	28.1	6.6
1	39.1	24.1	22.7

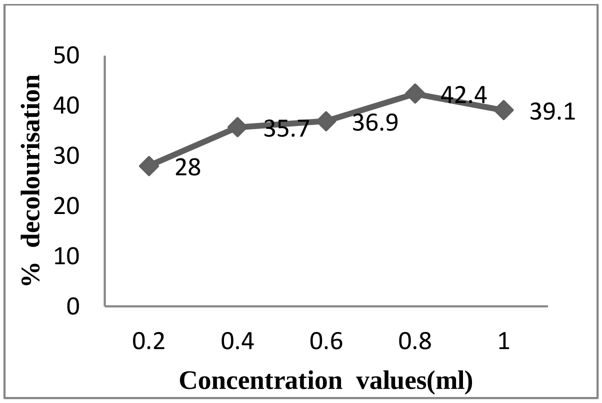

Fig. 4 Percentage of Decolourisation on the Effect of Concentration using Bitter gourd enzyme

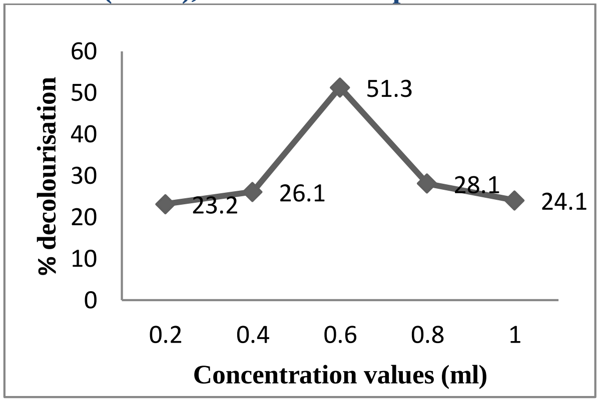

Fig. 5 Percentage of Decolourisation on the Effect of Concentration using Potato enzyme

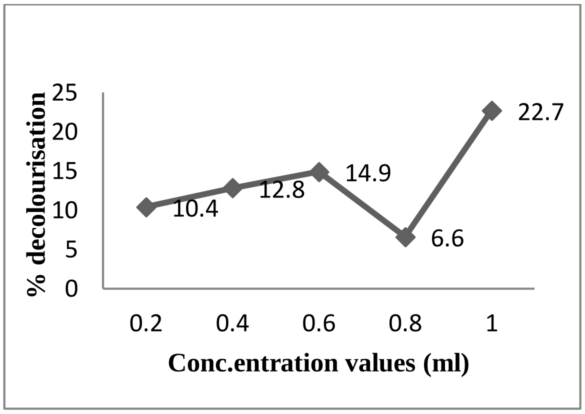

Fig. 6 Percentage of Decolourisation on the Effect of Concentration using Brinjal enzyme

Effect of Time

Textile effluent was treated with per oxidase and poly phenol oxidases enzyme of varying time from 1 to 5 hrs. Most of the dyes was maximally decolourised in the time of 4 hrs for bitter gourd per oxidase enzyme, at the time of 2 hrs for potato poly phenol oxidase enzyme and at the time of 3 hrs for brinjal poly phenol oxidase enzyme. The incubation time of the decolourising sample was increased up to 5hrs, and after that the rate of decolourisation decreased in the study sample. The effect of time was graphically represented in the figure 7, 8 and 9.The role of time on the dye degradation by soluble peroxidase enzyme and poly phenol oxidase enzymes has been tabulated in table 3.

Table 3. ***Percentage Decolourisation of textile effluent by Bitter gourd, Potato and Brinjal enzymes***

Time(hr)	Percentage of Decolourisation using bitter gourd enzyme (%)	Percentage of Decolourisation using potato enzyme (%)	Percentage of Decolourisation using brinjal enzyme (%)
1	38.1	45.6	12.3
2	35.9	47.2	14.2

3	44.1	37.1	18.8
4	48.1	44.7	6.5
5	36.8	39.4	8.6

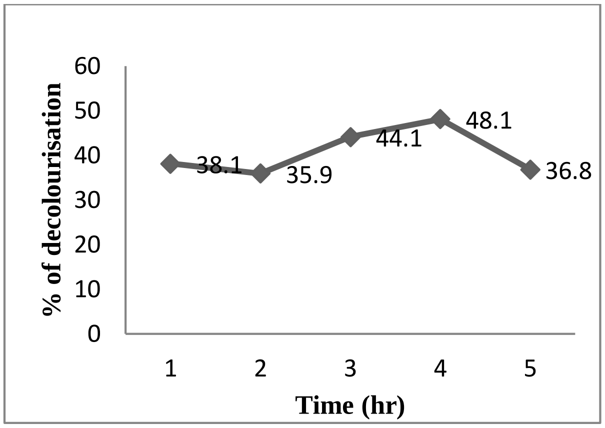

Fig. 7 Percentage of Decolourisation on the Effect of Time using Bitter gourd enzyme

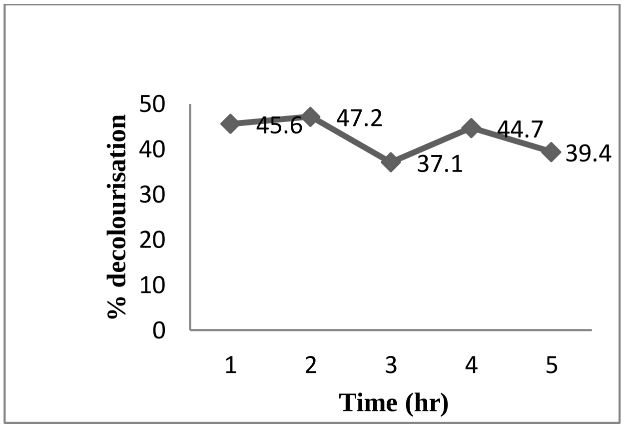

Fig. 8 Percentage of Decolourisation on the Effect of Time using Potato enzyme

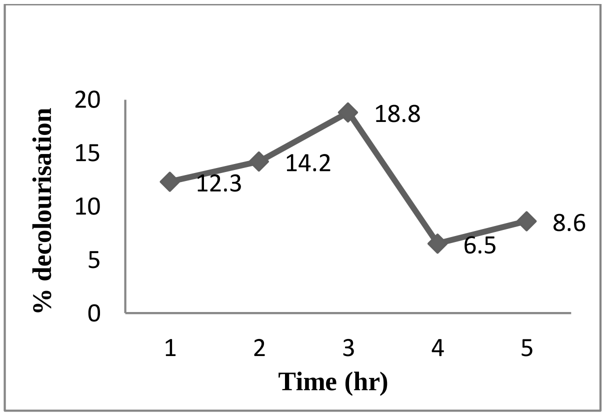

Fig. 9 Percentage of Decolourisation on the Effect of Time using Brinjal enzyme

Optimal conditions

The optimal conditions for peroxidase and poly phenol oxidase enzyme from bitter gourd, potato and brinjal in dye decolourisation of textile effluent are summarized in the Table 4.

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Table 4. Optimal Conditions for removing dyes from textile effluent

Parameters	Optimized parameter values for Bitter gourd enzyme	Optimized parameter values for Potato enzyme	Optimized parameter values for Brinjal enzyme	Maximum percentage removal of dyes using optimized parameter values (%)
pH	6	10	3	91
Time (hr)	4	2	3	95
Concentration (ml)	0.8	0.6	1	90

Physico-Chemical parameter values

The various physico-chemical parameters has been found out in raw textile effluent and after the addition of enzyme extract to the effluent. It is observed that some of the harmful physico-chemical parameter values has been reduced after the addition of enzyme extract. The resultant value has been tabulated in the table 5.

Table 5.Physico-Chemical parameter values of textile Effluent

Parameters	Raw effluent	After addition of bitter gourd enzyme extract	After addition of Potato enzyme extract	After addition of Brinjal enzyme extract
pH	9.8	9.5	11.5	11
Colour	greenish	colourless	colourless	colourless
Odour	unpleasant	unpleasant	unpleasant	unpleasant
Turbidity (NTU)	65	90	100	95
Chloride (mg/l)	3500	719.7	1500	905
Electrical Conductivity (ms/cm^2)	46	13	39.8	41
Dissolved Oxygen (mg/l)	17	14	30	20
Total Hardness (mg/l) Ca	830 270 560	800 300 500	650 330 320	710 510 200

Mg				
Total Solids (g/l) Total Suspended Solids Total Dissolved Solids	35.8 17.6 18.2	45.6 24.3 21.3	48.5 25.3 23.2	50.2 30.5 19.7
Bio-chemical Oxygen Demand(mg/l)	210	300	250	220
Chemical Oxygen Demand(mg/l)	110	80	75	92

CONCLUSION

The main aim of the decolourisation process was to eliminate their harmful effect on the environment. The optimization of decolourisation of textile dye by bitter gourd peroxidase enzyme and potato & brinjal poly phenol oxidase enzyme was achieved with the present study through one-factor-at-a-time (OFAT) method by using three factors namely pH, Time and Enzyme concentration. The optimized factors are, pH 6.0, with min. of 4 hrs of incubation and 0.8 ml of enzyme extract for bitter gourd enzyme and pH 10.0,with min. of 2 hrs incubation and 0.6 ml of enzyme extract for potato enzyme and pH 10.0,with min. of 2 hrs incubation and 1ml of enzyme extract for brinjal enzyme were determined through the present study. These conditions can be used for dye decolourisation by using these vegetable enzyme extracts in textile effluent treatment plants. The present study conclusively demonstrates that comparing to all these three vegetable enzyme extracts , potato*(solanum tuberosum*) poly phenol oxidase enzyme can be effectively and economically used in textile dye decolourisation. And also found out that some of the harmful physico- chemical parameters has also been reduced after addition of these three enzyme extracts to the textile effluent and in tat also potato poly phenol oxidase enzyme gives much reduced values while comparing to bitter gourd and brinjal enzymes.

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