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Decolourization of synthetic dyes by laccase enzyme extracted from Broccoli

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Abstract: Urbanization and industrialization has adversely affected our surrounding environment. Industries like tanning and textile are responsible for discharging toxic synthetic dyes into the water bodies which affects aquatic life, causes water pollution, and has become a great environmental concern. In the present piece of work, laccase enzyme was isolated from Broccoli. The enzyme activity was measured and the crude extract was subjected to the partial purification by salt precipitate method and dialysis. The maximum enzyme activity of the dialyzed sample was found to be 148.96IU. The kinetic parameter of laccase was studied and the optimum conditions were as follows: pH 6; temperature 100^0C; incubation time was found to be 30 minutes. Decolourization of bromophenol blue by laccase was measured and determined to be 60% and decolourization of bromocresol green was 43%.

Keywords: Biodegradation, laccase, broccoli, bromophenol blue, bromocresol green.

1. INTRODUCTION

Dyes are made from synthetic resources such as chemicals, petroleum by-products and earth minerals. Textile industries up to 200,000 tons of dyes are lost to effluents every year during the process of manufacturing of clothes. The clothing looks more vibrant from synthetic dyes, but also indirectly affects the environment. The need for synthetic dyes are rapidly increasing due to their excessive use in textile industries and if the effluent is not treated prior to discharge, it causes adverse effects on water bodies, as well as human health. These dyes are carcinogenic and toxic constituents and have serious effects on human beings.

Laccases (EC 1.10.3.2) is benzenediol:oxygen oxidoreductases. They are multi-copper enzymes belonging to the group of blue oxidases. It is found to be present in bacteria, fungi, plants and insects. It is widely studied in fungi. Extraction of Laccase enzymes from plants are hardly been reported in literature (Vinit Kumar et al., 2013). The present work was based on the degradation of synthetic dyes from laccase enzyme extracted from Broccoli. Laccase enzyme was isolated from the florets of the plant Brassica oleracea var.italica (Broccoli) and dye degradation was analyzed. The dye decolorization studies showed 60.54% dye decolorization in bromophenol blue and 43.77% in bromocresol green.

2.MATERIALS AND METHODS

2.1 Buffer Extraction

10g of the floret of Brassica oleracea was taken and 100ml of 0.1M phosphate-citrate buffer ranging pH-5 was added and homogenized. It was centrifuged for 15 minutes at 10000 rpm in 4°C. Crude enzyme extract was used for further analysis. (Benjamin, 1997).

2.2 Enzyme assay

Substrates commonly used for laccase are syringaldazine, guaiacol, DMP, ABTS, etc. The substrate which we have used was guaiacol for our investigation. Assay was carried out by adding 3 ml of phosphate-citrate buffer (100mM, pH-5), 1ml of guaiacol (10mM) and 1ml of the crude enzyme. Blank was prepared by adding 3ml of buffer, 1ml of guaiacol and 1ml distilled water. Incubation was done for 10 minutes and absorbance was read at 470nm using UV-visible spectrophotometer (Jadhav et al., 2009). Enzyme activity was expressed in the international unit IU (µmol/min). Laccases enzyme activity was determined by the formula:

$$\textbf{Enzyme activity} = \frac{\mathbf{A \times 4 \times V_t \times D.F}}{\mathbf{E \times V_s}}$$

Where, A = absorbance at 470 nm, 4 = derived from unit definition and principle, V_t= final volume of reaction mixture, D.F = Dilution factor, E = Extinction coefficient of guaiacol (0.6740µm/cm), V_s = sample volume.

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2.3 Enzyme Kinetics

2.3.1 Effect of pH

3 ml of Phosphate-citrate buffer (0.1M) of pH ranging from 4, 5, 6, 7, and 8 were used, 1 ml of assay was carried out and tubes were incubated at room temperature for 30 minutes and absorbance was read at 470nm using UV-Visible spectrophotometer.

2.3.1 Effect of Temperature

Assay was carried out by adding 3 ml of phosphate buffer (0.1M), 1 ml of Guaiacol (100mM) and 1 ml of crude enzyme was added, the tubes were incubated at varied temperatures 4°C, room temperature(25º C), 37°C, 40°C, boiling water bath(100ºC) for 30 minutes and absorbance was read at 470nm using UV- Visible spectrophotometer.

2.4 Partial purification of laccase:

2.4.1 Ammonium Sulphate Precipitation

Using Whatman filter paper No.1 crude enzyme was filtered and the total volume measured was 100ml. This was followed by 80% saturation of crude enzyme. Ammonium sulphate salt was added to the sample kept on the magnetic stirrer and allowed to continuously stir. The sample was incubated at 4°C overnight. Centrifuged for 15 minutes at 10000rpm, and the pellet was dissolved in Tris-HCl (10mM) and supernatant was discarded. (Distasioe et al., 1976)

Assay of the partially purified enzyme was done by taking 0.5mL of guaiacol (10mM), 0.5mL of partially purified enzyme sample and 1.5mL of the buffer. Subsequently, a blank was prepared without enzyme extract. Both the tubes were incubated for 10 minutes. The absorbance was then read at 470 nm using a UV-visible spectrophotometer.

2.4.2 Dialysis:

The dialysis bag was cut into 8 cm and kept for activation. 100ml of water was boiled and 2g of sodium-bi-carbonate was added and stirred for 10 minutes. The water was changed after 10 minutes and again boiled with 100 ml of distilled water. Then the bag was allowed to cool. Salt precipitated enzyme sample was poured into the activated bag and tied on both sides. The bag was kept in water and incubated at 4ºC overnight. After overnight incubation, the beakers were placed on the magnetic stirrer for 2 hours, and every half an hour the water was changed.

2.4.3 Dye Degradation:

For the process of dye degradation we chose bromophenol blue and bromocresol green synthetic dyes. The stock solution (1mg/ml) was prepared by dissolving 0.025g of dye in 1 liter of distilled water. 0.25ml of stock was taken, made upto 100ml to obtain 25ppm concentration. Dye decolourization was carried out by incubating dye solution and enzyme samples together. Absorbance was read at 470nm (UV-visible spectrometer).

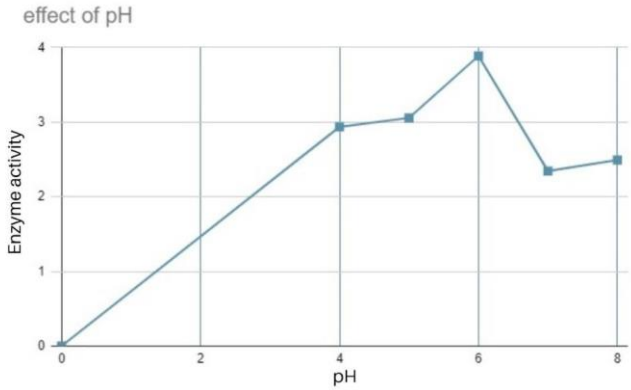
$$\%\ \text{Decolourization} = \frac{\text{Initial decolourization} - \text{Final decolourization}}{\text{Initial decolourization}} \times 100$$

RESULTS

3.1 Table.1. Enzyme activity

Sample	Absorbance at 470 nm	Enzyme activity (IU)
Crude	0.059	1.75
Salt precipitated	0.073	2.19
Dialyzed sample	0.130	3.88

3.2Enzyme Kinetics



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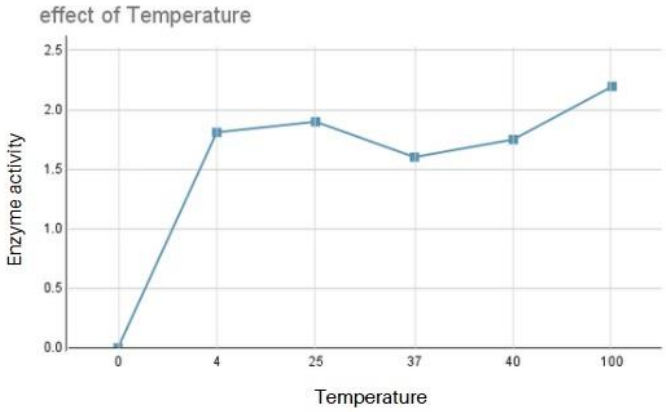
The optimum pH was found to be 6.



The optimum temperature was found to be 100°C.

3.3Dye Degradation:

Table 5: Dye Degradation Percentage

Dye	Crude %	Partial purified sample%
Bromocresol green	58.82	43.77
Bromophenol blue	45.47	60.54

Effective degradation of bromophenol blue dye was measured and was found to be 60.54% and dye degradation of bromocresol green was recorded to be 43.77%,from laccase enzyme extracted from Brassica oleracea.

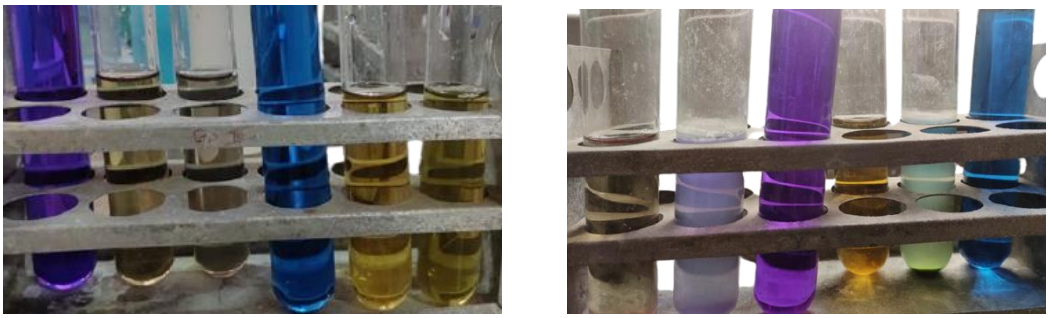


Fig. 1. Degradation of Bromophenol blue and Bromocresol green

CONCLUSION

Laccase enzyme from Brassica oleracea has shown effective degradation on bromophenol blue andbromocresol green synthetic dyeswhich are found to be carcinogenic and toxic dyes to the animals, aquatic life as well as humans. These dyes are non-biodegradable and hence increase water pollution through discharge of untreated effluents. Hence an attempt was made to degrade these dyes using laccase extracted from Brassica oleracea. More research is needed in this arena for commercial use.

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