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PRODUCTION AND PARTIAL PURIFICATION OF PEROXIDASE FROM WATER HYACINTH PLANTS INDUCED BY TEXTILE DYEING EFFLUENT

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ABSTRACT

Water hyacinth is the "Cinderella" of the plant world. It has the ability to treat industrial effluents. In the present study, production and partial purification of peroxidase from acclimatized water hyacinth roots has been studied. Initially, the water hyacinth plants were gradually acclimatized to textile dyeing effluent from 5% to 50%. The production of enzyme was greatly induced by the strength of the effluent. The enzyme was purified by fractional precipitation using ammonium sulphate, dialysis and gel exclusion chromatography. The specific activity of peroxidase in crude extract was 6.25 x 10^{-2} and it was significantly increased to 2 in the purified enzyme sample. The results indicated that, acclimatized water hyacinth roots could be a simple and easily available source for cost effective industrial production of peroxidase.

INTRODUCTION

Industrialization has led to the introduction of a variety of synthetic organic (xeno-alien, biotic-nature) compounds which are generally toxic. One of the very pressing environmental problems of the textile industry is the removal of colour from effluents prior to discharge to local sewage treatment system. Brightly coloured water soluble dyes are problematic as they pass unaffected through conventional sewage treatment systems (Nikhath Kousar *et al*., 2000). Phenolic effluents which colour receiving waters are toxic to mammals and

fish (Peyton, 1984). To depollute the dye waste water, the physico-chemical methods, adsorption, chemical precipitation, flocculation, photolysis, chemical oxidation and reduction, electro chemical treatment and ion-pair extraction have proved to be costly and less effective (Zollinger, 1987; Boman *et al.*, 1988). Conventional biological treatment methods such as aerated lagoons and activated sludge processes require a long hydraulic detention time and long sludge retention time to overcome shock loads (Kumaran and Shivaraman, 1998). Emerging applications in Biotechnology include the search for enzymes that function well under extremes of conditions. Advantages of the enzymatic approach are the broad specificity of the enzymes, which enable them to react with a wide range of phenols and less sensitivity to the operational upsets. Peroxidases, laccascs and tyrosinase are now being.

MATERIAL AND METHOD

Acclimation of water hyacinth to textile dyeing effluent

Young water hyacinth plants were collected from Madurai. The plants were washed with water and then grown in 5% of textile dyeing effluent for one week. Then the plants were transferred to 10% of effluent for one week. Similarly, the percentage of effluent was gradually increased at an interval of 5% upto 50%.

Extraction of Peroxidase

l0g of water hyacinth roots acclimatized to 50% of textile dyeing effluent was extracted with 0.2 M potassium phosphate buffer of pH 7.0.

Assay of Peroxidase

Peroxidase activity was assayed using amino antipyrine and hydrogen peroxide (Boyer, 1992) 2.5 ml of amino antipyrine phenol solution and 0.1 ml of enzyme served as the reaction mixture. The reaction was initiated by the addition of 2.5 ml of hydrogen peroxide. The change in absorbance was noted at zero time and after 3 minutes at 5 10 nm. The enzyme activity is expressed as units/mg.

Partial Purification of Peroxidase

The enzyme was precipitated from the crude extract using different concentration of ammonium sulphate ranging from 10% to 90%. The extract showing maximum activity was dialysed with fresh 0.2 M potassium phosphate buffer for 5 hrs. The dialysate was further purified by column chromatography with saphadex G-100. The ten fractions collected were also assayed for peroxidase activity.

RESULTS AND DISCUSSIONS

During acclimation to the textile dyeing effluent the growth of water hyacinth plant was profuse. But above 50% of the effluent, the plant growth was inhibited. Trivedy (1998), discussed the role of acclimation in bio-augmentation

and enrichment of specialized cultures which treat the waste. Water hyacinth roots serve as a site for bacterial attachment. The activity of peroxidase is superior in water hyacinth (Shuangxi Shi and Xichung Wang, 1991). Increased enzyme activity indicated the phyto physiological metabolic activity and so the treatment of effluent. The specific activity of the crude extract was 6.25 x

Table - 1
Fractional precipitation of peroxidase with ammonium sulphate.

Ammonium Sulphate %	Specific activity
10	$4.20 \times 1\ 0^{-3}$
20	$5.70 \times 1\ 0^{-3}$
30	$8.80 \times 1\ 0^{-3}$
40	2.30×10^{-2}
50	6.30×10^{-2}
60	7.50×10^{-2}
70	9.69×10^{-2}
80	6.50×10^{-2}
90	4.00×10^{-2}

Table - 2
Fraction profile of peroxidase by column chromatography

Fraction	specific activity
1	5.95×10^{-1}
2	6.23×10^{-1}
3	7.10×10^{-1}
4	7.90×10^{-1}
5	1
6	2
7	8.57×10^{-1}
8	5.00×10^{-1}
9	3.00×10^{-1}
10	1.30×10^{-1}

Table - 3
Purification of peroxidase

Step	Total Activity	Total Ptotein (mg)	Specific Activity	Purification fold	Yield (%)
Crude Extract	9.38×10^{-1}	15.00	6.25×10^{-2}	-	100
Ammonium Sulphate purified	7.56×10^{-1}	7.80	9.69×10^{-2}	1.50	80.00
Dialysate	5.60×10^{-1}	1.00	5.60×10^{-1}	8.99	59.70
After chromatography	2.00×10^{-1}	1.00×10^{-1}	2.00	32.00	21.32

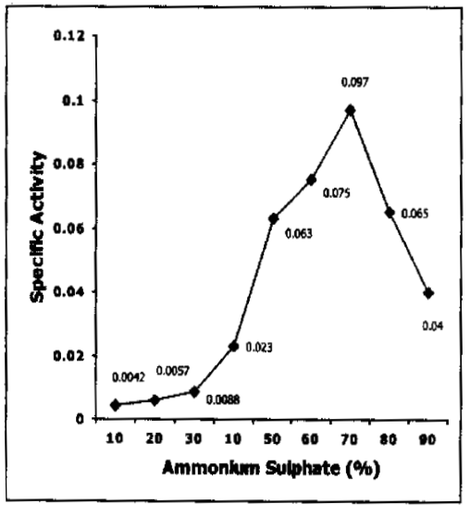

Fig.- 1 Fractional precipitation of peroxidase with ammonium sulphate

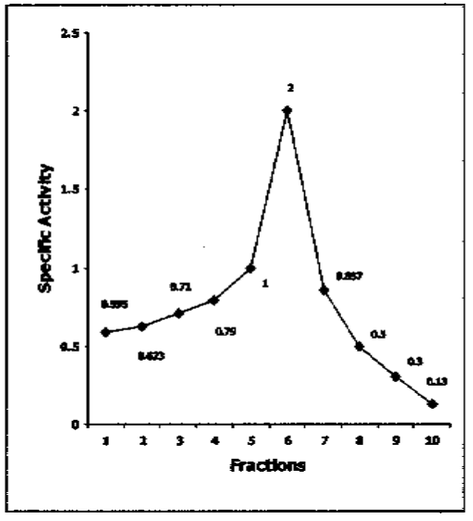

Fig.- 2 Fraction profile of peroxidase by column chromatography

1993).

CONCLUSION

The production of peroxidase from water hyacinth can be undertaken economically. Acclimation of water hyacinth is a simpler technology for the

10^{-2} . The specific activity of the enzyme during the precipitation with ammonium sulphate is presented in **Table 1** and **Fig. 1**. 70% of ammonium sulphate precipitated source showed maximum activity of 9.70 x I0″^{2}. The specific activity of the dialysate was 5.60 x 10′. The fraction profile of peroxidase by column chromotography is presented in **Table 2** and **Fig. 2.** 6^{lh} fraction exhibited a higher most activity of 2. Peroxidase has been purified and characterized from a number of higher plant systems including horse radish (Shannon et al., 1966), potato (Espelie and Kolattukudy, 1985), tobacco (Mader, 1980), turnip (Mazza *et al*., 1968) and barley (Saeki *et al*.. 1986). Production of peroxidase from *Inonotus Weirii* (Annikka Mustranta, 1987) and Aspergillus niger (Mellon. 1991) were also reported. Removal of phenolic compounds by peroxidase has been discussed by Alberti and Klibanov (1981); Klibanov and Morris (1981); Hakulinen (1988); Claus and Filip (1990). The enzyme converts soluble phenolics into insoluble polyphenolic precipitates which can be removed by filtration (Sun *et al*., 1992; wada *et al*.,

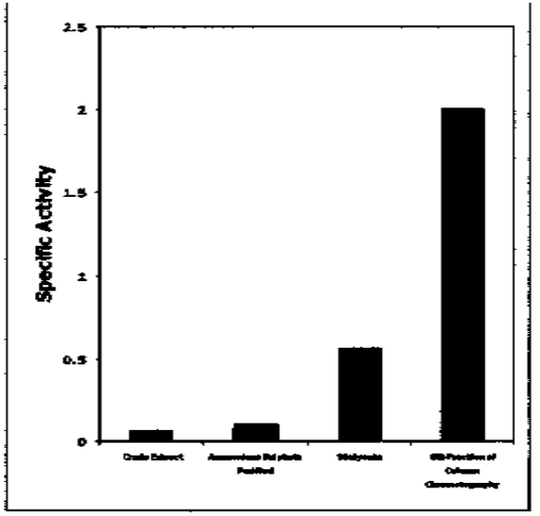

Fig.- 2 Fraction profile of peroxidase by column chromatography

maximum production of peroxidase.

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