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Comparison of horseradish peroxidase, soybean peroxidase and radish peroxidase for the polymerization of phenol

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ABSTRACT:

Polymerization of Phenol was carried out in laboratory using peroxidase enzyme extracted from three different plant sources namely, horseradish roots, soybean hulls and radish roots. The ambient room temperature during the study period was between 27-32°C. Phenol concentration of 100 - 300 mg/L was given to the free enzymes. Phenol polymerization of 84% was achieved at 100 mg/L phenol using free HRP enzyme. Similarly, 72% and 76% phenol polymerization was obtained using free SBP and RP respectively at 100 mg/L phenol. Peroxidase isolated from horseradish roots was able to polymerize phenol more efficiently than the enzymes isolated from soybean hulls and radish roots. As the concentration of phenol increased, reduction in phenol polymerization efficiency was observed for all the three enzymes.

Keywords:
Horseradish peroxidase (HRP), soybean peroxidase (SBP), radish peroxidase (RP), phenol, enzymes for phenol degradation.

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INTRODUCTION

Phenol (C_6H_5OH) is one of the most widely used organic compounds, the discovery of phenol is attributed to Runge in 1834 who isolated several components of coal tar including carbolic acid (Tyman, 1996). Phenolic compounds are found in wastewaters of various industries such as petroleum refining, coal conversion, plastics, resins, textiles, iron and steel manufacturing as well as pulp and paper manufacturing (Ensuncho *et al.,* 2005). Due to the toxic nature of some of these compounds, the Environmental Protection Agency, EPA, has set a water purification standard of less than 1 μg L–1 of phenol in drinking waters. Moreover, EPA studies have shown that the usage of chlorine for disinfection of phenol-containing water may yield toxic 2-chlorophenol. Phenol is toxic to fish at a level of 0.05 mg/l, therefore the removing of phenols from waste water is therefore of great importance (Mossallam *et al.,* 2009). Department of Transportation's Hazardous Material Regulation classifies phenol as a Class B poison. Drums and packages are to be labeled ''Poison'' and must comply with the regulations and bulk containers must be properly marked.

Enzymes as biocatalysts have been used in many biological reactions but they mostly suffer from certain disadvantages. Enzymatic treatment has been proposed by many as an alternative treatment technology to traditional methods (Mossallam *et al.,* 2009). Enzymatic removal of phenolic compounds has been investigated by many researchers and it has been shown that peroxidases are able to react with aqueous phenolic compounds (Iran and Siamak, 2009).

Peroxidases are oxidoreductases produced by a number of microorganisms and plants. Peroxidases catalyse a variety of reactions in the presence of peroxides; (eg) Hydrogen peroxide (Hamid M and Rehman K, 2009). Reduction of peroxides at the expense of electron donating substrates, make peroxidases useful in a number of biotechnological applications. Peroxidase has a potential for soil detoxification, while HRP as well as soybean and turnip peroxidases have been applied for the bioremediation of wastewater contaminated with phenols, cresols, and chlorinated phenols (Carlos *et al.*, 2004).

Horseradish peroxidase (HRP) is effective for the removal of phenols and phenolic compounds from aqueous solutions by catalyzing the formation of insoluble, polymeric phenolic aggregates (Lai and Lin, 2005). Soybean seed hulls have been identified as a rich source of a peroxidase, the soybean peroxidase (SBP), being a by-product of the soybean food industry, they provide a cheap and abundant source of peroxidase (Wilberg *et al.,* 2002). Radish root juice and pieces containing Radish peroxidase (RP) have a potential activity for the removal of phenol from a synthetic wastewater (Naghibi *et al.,*2003).

Phenolic wastewater is a major issue when disposed untreated. The present investigation would be a step towards the reduction of phenol using free enzymes.

MATERIALS AND METHODS

Enzyme extraction

Horse Radish Peroxidase (HRP) enzyme was extracted from horseradish roots in the laboratory. The horseradish roots were soaked in buffer of pH 6.8; later it was crushed using blender for 10 min. The extract was filtered using whatman filterpaper and the filtrate was centrifuged at 8,000 rpm, 25^{o}C for 30 min. The supernatant obtained after centrifugation was used as crude enzyme source. This enzyme solution was stored at 4^{o}C and warmed to room temperature immediately prior to use.

The similar procedure was followed for the extraction of Soy Bean Peroxidase (SBP) enzyme from soybean seed hulls and Radish Peroxidase (RP) enzyme from edible part of radish roots.

Peroxidase activity measurement

The activity of Horse Radish Peroxidase (HRP), Soybean Peroxidase (SBP) and Radish Peroxidase (RP) were assayed at 25^{o}C using phenol, 4-Amino antipyrine (AAP) and hydrogen peroxide as substrates. The assay mixture contained 1.5 ml of a 20 mM phenol solution, 0.75 ml of a 9.6 mM AAP solution, 0.3 ml of 2 mM H_2O_2 solution, 0.45 ml of enzyme solution, and 0.3 ml phosphate buffer. The total volume of the assay mixture was 3 ml. The enzyme active concentration is proportional to the color development rate measured at 500 nm, during a period of time in which the substrate concentration is not significantly reduced (Sadasivam and Manickan, 2004).

The color development rate during this period was converted to activity using an extinction coefficient of 7,100 $M^{-1}cm^{-1}$ based on hydrogen peroxide. One unit of enzymatic activity is defined as the amount of enzyme which transforms 1 micromol of hydrogen peroxide per minute at 25^{o}C.

Determination of Phenol Concentration

The phenol concentration was detected using

4-Amino anti pyrine (4-AAP) method. This method can be used for wastewaters containing phenol concentrations from 0.5 to 5 mg/l. 100 ml of sample was taken, 2.5 ml of Ammonium hydroxide (0.5 N) was added and the pH was adjusted to 7.9 using phosphate buffer (pH 6.8). 1 ml of 4-AAP (2%) was added and mixed well, 1 ml of potassium ferri cyanide was added and optical density (OD) was measured at 500 nm after 15 minutes. The OD was converted to phenol concentration using a calibration curve (Dannis, 1951, Standard methods 1995).

Assay using free enzyme

Experiments were carried out in 100 ml Erlenmeyer flasks at room temperature. Reaction medium was prepared by adding 100 to 300 mg/L with an increment of 100 mg/L phenol, enzyme (4%) and H_2O_2 (1.1 to 5.5 mM) into the phosphate buffer (pH 6.8). The volume of assay mixture was 50 ml. The reaction was initiated by H_2O_2 which was added discretely for every 30 minutes to assure phenol conversion. The conical flasks containing the assay mixture was placed on a rotary shaker to ensure complete mixing. The sample was drawn for every 30 minutes and phenol concentration was measured. The phenol concentration was varied when there was no considerable difference in two consecutive readings.

RESULTS

The free HRP, RP and SBP enzymes were used for conversion of phenol. All the three free enzymes showed effective phenol conversions. The highest phenol conversion percentage was obtained at the concentration of 100 mg/L and the phenol polymerization efficiencies obtained were 84, 76 and 72% for HRP, RP and SBP respectively, as shown in **Fig 1.**

The percentage of phenol polymerization efficiencies at the concentration of 200 mg/L were 76, 69 and 66 for HRP, RP and SBP respectively. At the concentration of 200 mg/L, HRP proved to be most efficient among three and SBP being the least effective as shown in **Fig 2.**

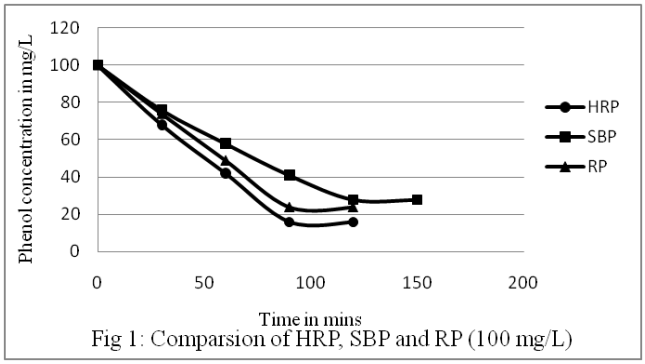

Fig 1: Comparsion of HRP, SBP and RP (100 mg/L)

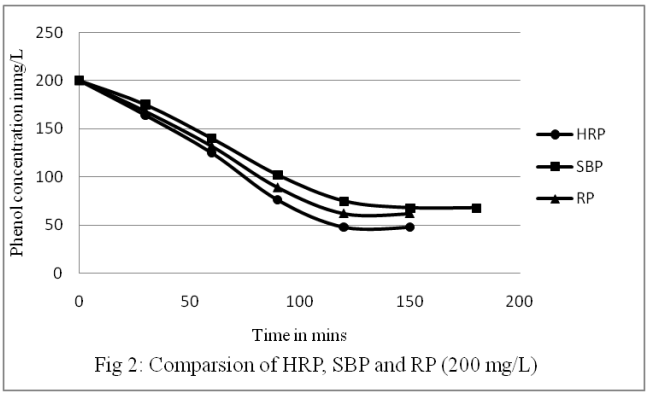

Fig 2: Comparsion of HRP, SBP and RP (200 mg/L)

At the concentration of 300 mg/L, HRP proved to be most efficient among three and RP being the least effective as shown in **Fig 3.** The percentage of phenol polymerization efficiencies at the concentration of 300 mg/L were 62, 58 and 57 for HRP, RP and SBP respectively.

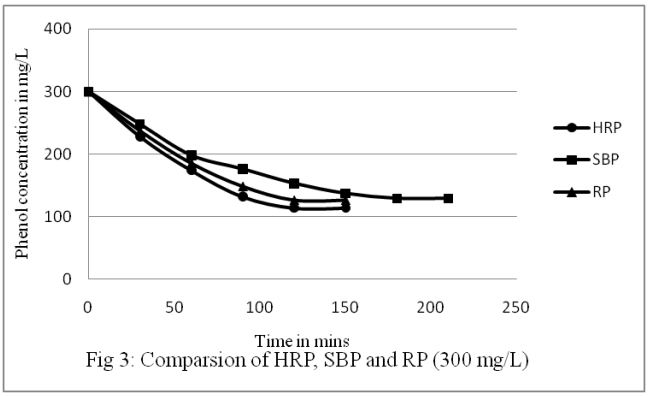

Fig 3: Comparsion of HRP, SBP and RP (300 mg/L)

DISCUSSIONS

All the three enzymes proved their efficiency in polymerizing phenol, HRP proved to be most efficient and SBP proved to least efficient among the three peroxidase enzymes. As the concentration of phenol increased, the phenol conversion efficiency reduced for all the peroxidase enzymes.

Wilberg *et al*., (2000) reported 99% phenol conversion efficiency using free HRP enzyme. Iran and Siamak (2009) attained 60% conversion of phenol using free HRP enzyme. Naghibi *et al.,* (2003) reported 98% phenol conversion using radish peroxidase enzyme extracted from the juice of *Rapahanus sativus*. Caza *et al.,* (1999) determined optimal conditions to achieve 95% phenol conversion using soybean peroxidase. They concluded the optimal pH of 7 as best suited for

phenol conversion. They attained 95% phenol conversion at 100 mg/L phenol solution.

Humaira and Qayyum (2009) used radish peroxidase and achieved 30% p-chlorophenol and 62% p-bromophenol removal from polluted water. Monika and James (2002) used HRP enzyme and removed upto 95% of phenol from aqueous solutions.

Findings of this study agree with the results published by Bodalo *et al.*, (2005) who compared HRP enzyme and SBP enzyme for phenol removal and reported that the HRP acts faster than SBP but is more susceptible to inactivation.

Guoping and James (2000) obtained a better p-chlorophenol transformation at pH 4 using HRP enzyme.

CONCLUSION

The following conclusions are made for phenol degradation using free enzymes:

1. Horse Radish Peroxidase (HRP) found to be efficient in phenol degradation with a removal of 84% at 100 mg/L using free HRP enzyme.
2. Free Radish Peroxidase (RP) degraded 76% phenol at 100 mg/L.
3. Free Soybean Peroxidase (SBP) achieved 72% phenol removal at 100 mg/L
4. The Horse Radish Peroxidase proved to be effective in degrading phenol at the concentrations from 100 mg/L to 300 mg/L when compared with Radish Peroxidase and Soybean Peroxidase.
5. As the concentration of phenol increased, reduction in phenol degradation efficiency was observed.

Enzymatic treatment using peroxidase is a viable option for the degradation of phenol. The results obtained indicate that enzymes isolated from various plant sources can be used for removal of phenol from wastewater.

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