Removal of Phenols from Wastewater with Encapsulated Horseradish Peroxidase in Calcium Alginate

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Abstract - Horseradish peroxidase was encapsulated in calcium alginate for the purpose of phenol removal. Upon immobilization, pH profile of enzyme activity changes as it shows higher value at basic and acidic solution. Investigation into time course of phenol removal for both encapsulated and free enzyme showed that encapsulated enzyme had nearly similar efficiency in comparison with the same concentration of free enzyme; however the capsules were reusable up to four cycles without any changes in their retention activity.

Keywords: Horseradish peroxidase, Alginate, Gelation, Encapsulation

1. INTRODUCTION

Enzymes as biocatalysts have been used in many biological reactions but they mostly suffer from certain disadvantages. Enzymatic removal of phenolic compounds have been investigated by many researchers and it has been shown that peroxidases are able to react with aqueous phenolic compounds and form non-soluble materials that could be easily removed from the aqueous phase [1-7], however; these processes suffer from enzyme inactivation.

Therefore attentions came into immobilization of peroxidases for the purpose of phenolic compound removal. Among most abundant peroxidases investigated, horseradish peroxidase (HRP) has been successfully used to remove phenol from waste effluent and it is by far the most researched peroxidase. Using alternative peroxidase was also investigated due to need for cheaper catalyst and it has been shown that soybean peroxidase which is abundant in soybean seed hull can also remove phenolic compound from waste stream with acceptable removal efficiency comparable to HRP [8-10]. Media containing immobilized enzyme seems to be more suitable when large amount of wastewater need to be processed. Many materials and different methods have been used for HRP immobilization, glass beads, polymers, ion exchange resins, magnetite and aluminum-pillared clay [11-18].

In the present work, we attempted to use a new support for immobilization of HRP for the purpose of phenol removal from a synthetic wastewater. We used one step encapsulation method for immobilization of HRP in a semi permeable alginate membrane. The application of immobilized peroxidase for the removal of phenol from aqueous solution was studied at different pH, contact time and enzyme concentrations. From the results obtained in the present work, the possibility of continuous phenol removal was shown to be promising.

2. MATERIALS AND METHODS

Horseradish peroxidase, HRP (lyophilized powder, 200U mg $^{-1}$), Phenol 99% and H_2O_2 30% w/v were purchased from Merck also the analytical chemicals 4-aminoantipyrine (AAP) and potassium ferricyanide. Sodium alginate (reach in guluronic acid) from *Lamirania hyperborean* and calcium chloride hexahydrate were obtained from BDH (U.K). Catalase enzyme from aspergillus Niger (EC.1.11.1.6) (lyophilized powder 2993 U mg $^{-1}$) was purchased from SERNA. Other chemical were of analytical grade and were used without further purification.

The immobilization method was carried out according to the method described by Nigma[19]. The amount of protein initially offered, in the wash-liquid after encapsulation and also the protein content in capsule after leakage test were obtained by Lowry's procedure as modified by Peterson [20].

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Enzyme leakage measurement was carried out by placing capsules in a test tube filled with tris buffer (pH=8.0) for 18 hours. Then the capsules were removed, cut in half and put in phosphate buffer (pH=7.4) solution.

HRP activity was assessed by employing 4-aminoantipyrene method involving colorimetric estimation by using phenol and H_2O_2 as substrate and 4-aminoantipyrene (Am-NH_2) as chromogen [21]. Phenol concentration was determined using a colorimetric assay in which the phenolic compounds within a sample react with 2.08 mM AAP in the presence of 8.34 mM potassium ferricyanide reagent[21].

3. RESULT AND DISCUSSION

Biocatalytic properties including lower enzyme leakage and higher enzyme encapsulation and retention activity achieved when the calcium chloride hexa hydrate and sodium alginate solution were 4.5 %w/v and 0.75% w/v respectively.

3.1. Dependence of pH

The pH activity profile of free and encapsulated HRP was obtained by incubating both the free and immobilized enzyme at 25 ℃ for 15 min in 5 ml buffer solution followed by measuring the enzyme activity at 510 nm. Fig.1 depicts the results of these measurements. This behavior might be the result of interior microenvironment of capsule that is slightly cationic and separated from bulk with a semi-permeable membrane which is anionic in nature.

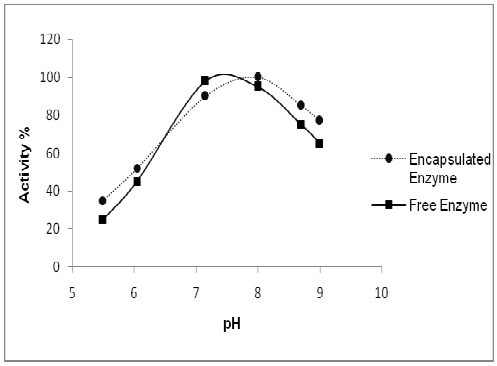

Fig.1. Effect of pH on the Activity of free and immobilized HRP; Free enzyme (■) Encapsulated HRP (●)

3.2. Optimum contact time

Initial experiments were performed in order to assess the optimum contact time required for phenol removal. To a series of beakers each one containing 100 ml of 2 mM phenol, 20 µl hydrogen peroxide along with enzyme concentration (0.8 units/ml) were added and reaction media (25℃ , pH=8.0) was agitated for a period of 4 hours. Every 20 minutes, a 1 ml sample was taken from solution and was analyzed for the residual phenol concentration. It was shown that 100 min is required to reach acceptable removal efficiency. Subsequent experiments were performed at a backer containing 100 ml phenol with definite concentration and lasted for 100 min. Further reactions with different phenol concentrations have shown that phenol removal follows the same trends (Fig.2). The phenol conversion against time was also studied for both encapsulated and free enzyme. Fig. 2, shows the comparison between free and encapsulated enzyme phenol removal efficiency versus time.

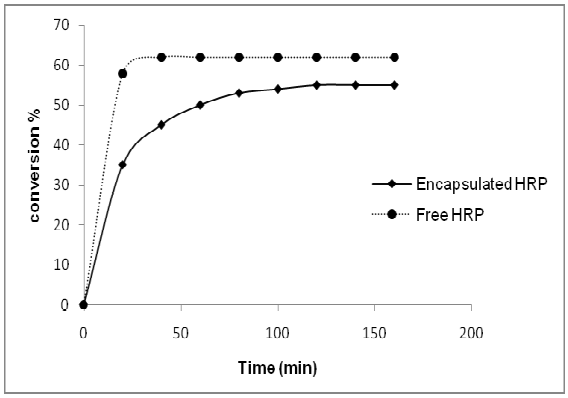

Fig. 2. Comparing time course of phenol conversion of immobilized and free enzyme, Phenol concentration 2 mM. Free enzyme (●) Encapsulated HRP (■)

3.3. Influence of enzyme concentration

Since the biocatalyst has a finite lifetime and also the conversion is found to be dependent on the contact time, normally removal of phenol is dependent on the amount of catalyst added. To study the effect of enzyme concentration on phenol removal, five different enzyme concentrations were used to compare the

efficiency of encapsulated enzyme. The phenol and hydrogen peroxide concentration along with the physical condition of reaction remained unchanged (phenol concentration 2mM, pH=8.0). Fig.3 depicts the effect of enzyme concentration on phenol removal. It is found that for a 2 mM phenol solution, increasing enzyme concentration from 0.15 units/ml to 0.8 units/ml results in gradual increase in phenol removal. Further increases in enzyme concentration have no significant effect on phenol removal. The enzyme concentration of 0.8 Units/ml was found to be the optimal dose for the experiment condition.

3.4. Reusability

The immobilized enzyme could be easily removed and assessed for its remained catalytic activity. To demonstrate the reusability of encapsulated enzyme, capsules were separated after 100 min of reaction time and then rinsed thoroughly with distilled water. The capsules used for subsequent batches. After 5 repeated test, the phenol removal efficiency was reduced to half of its initial value (Fig.4). The later might be the result of plugging of the membrane pore and

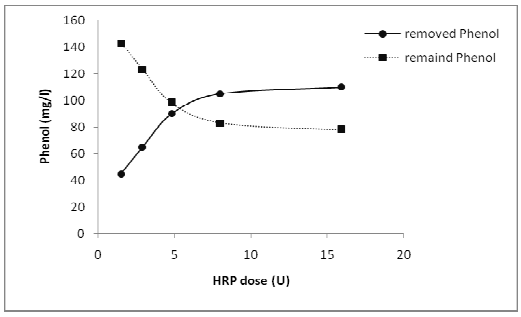

Fig 3. Effect of encapsulated HRP dose on phenol removal

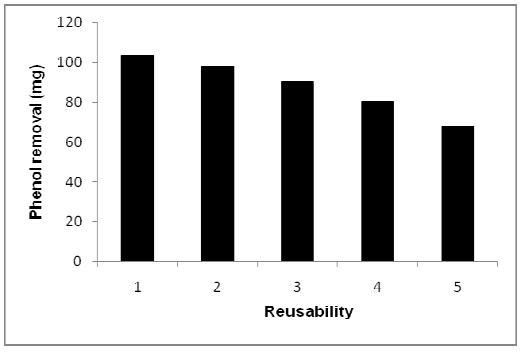

Fig. 4. Reusability of capsules, Phenol concentration 2 mM and enzyme content 7.5 units/ml

accumulating of radicals and dimmer in the interior environment of each capsule which entrapped the active site of enzyme or even enzyme molecules resulting in enzyme inactivation.

4. CONCLUSION

The experimental results obtained in the present work revealed the effectiveness of the encapsulated peroxidase in phenol removal. The performance of phenol removal was found to be highly dependents on, aqueous pH, contact time, and enzyme dose. The encapsulated enzyme activity shows higher relative activity in acidic and basic solutions which are the most common conditions appeared in waste stream. Enzyme Retention activity, encapsulation and leakage percentage of enzymes are influenced by gel preparation condition and finding a proper value for above quantities totally depends on alginate species used. The reusability experiment showed that these biocatalysts can be used up to five cycles without serious deficiency in their catalytic performance.

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