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IMMOBILIZED POLYPHENOLOXIDASE FOR WASTEWATERS TRATEMENT

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ABSTRACT. A simple and efficient method for immobilization of crude polyphenol oxidase from potato by entrapment in calcium alginate was developed. The obtained enzyme preparate was tested for bioremediation of wastewaters in batch and packed bed reactor.

Keywords: *immobilized polyhenoloxidase, phenol removal, bioremediation, calcium alginate, batch reactor, packed bed reactor*

INTRODUCTION

The use of enzymes in the bioremediation processes is a protective methodology for the environment which can reduces the damages caused by industrial polluting effluents. The presence of phenolic compdunds in drinking and irrigation water represents a significant health and environmental hazard and, therefore, the development of methods for their removal and transformation have received increased attention in recent years. The most results dealing with the fundamental and applied aspects of free and immobilized polyphenoloxidases (PPO) for food industry wastewater processing was presented by Chiacchierini [1]. The enzyme was isolated from a large variety of plants and fungi [2], but the costs for obtaining pure enzyme preparation are still high. Many cheep available sources were tested. Recently, PPO was extracted from tomato fresh pulp and different trademarks of tomato puree sold in supermarkets by using sodium phosphate buffer at different pHs. The best pH values was 7.5 [3].

Polyphenol oxidase from mango (*Mangifera indica*) peel and green tea (*Camellia sinensis*) leaves were immobilized on various supports, e.g., polyacrylamide gel, DEAE-Sephadex, DEAE-cellulose, collagen, arylamine glass,

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and alkylamine glass and some important properties (optimum temperature, pH, Km, substrate specificity, thermal stability, storage stability and reusability) were studied and compared with those of native enzyme [4].

Gusarova and co. studied [5] the immobilization pf PPO from higher basidiomycetes (*Coriolus sp*.) on polyvinylalcohol fibers and the use of immobilized enzyme for purification of wastewater from hydrolysis-yeast industry.

The gel-entrapped tyrosinase and laccase prepared by immobilization of enzymes in gelatine [6] were capable of removing naturally occurring and xenobiotic aromatic compounds from aqueous suspensions and with different degrees of efficiency. A column packed with gel-immobilized tyrosinase was used to demonstrate that enzymes immobilized with this technique may be reused several times in the same reaction without loosing their efficiency. Moreover, this immobilization procedure enhanced the enzymes stability to thermal inactivation.

In this work, with the aim to improve the phenol removal from wastewaters, the immobilization of crude polyphenole oxidase from potato using a simple and efficient method, entrapement in calcium alginate, is presented. The immobilized enzyme was tested for phenol oxidation in batch and in packed bed reactors.

RESULTS AND DISCUSSION

Immobilization of crude PPO from potato, activity and stability of enzyme

Many methods are available for immobilization of enzymes and cells. Since the method greatly influence the properties of the resulting biocatalyst, the selection of the most suitable strategy determines the process specifications for the catalyst, such as overall catalytic activity, efficiency of the enzymatic process, deactivation and regeneration kinetics and cost.

In order to immobilize PPO in alginate beads, we used the entrapement of enzyme in calcium alginate. The immobilization procedure was studied with the aim to obtain the most performant biocatalyst for phenol removal from wastewaters. The quantity of included enzyme was determined from the protein content of enzyme solutions before and after immobilization, which was determined by Bradford method with Comassie Blue.

The enzymatic activity of each enzyme preparate was determined as previously described [7], with 4-aminoantipyrine (4-Amino-1,2-Dihydro-1,5-Dimethyl-2-Phenyl-3H-Pyrazol-3-One, AAP) in presence of potassium ferricyanide. As observed in Figure 1, the activity depends linear on the quantity of immobilized enzyme. In conclusion, the ability of alginate to include the entire crude enzyme corresponde our goal.

Next the stability of immobilized PPO in time was tested in comparison with those of the free enzyme. As observed in Figure 2, the most unstable is the free (soluble) enzyme. The immobilized enzyme preparates with < 0.5 g crude PPO are stable in time and retains more than 50% from their initial activity. The most suitable seems to be the calcium alginate beads obtained from 0.4 g enzyme.

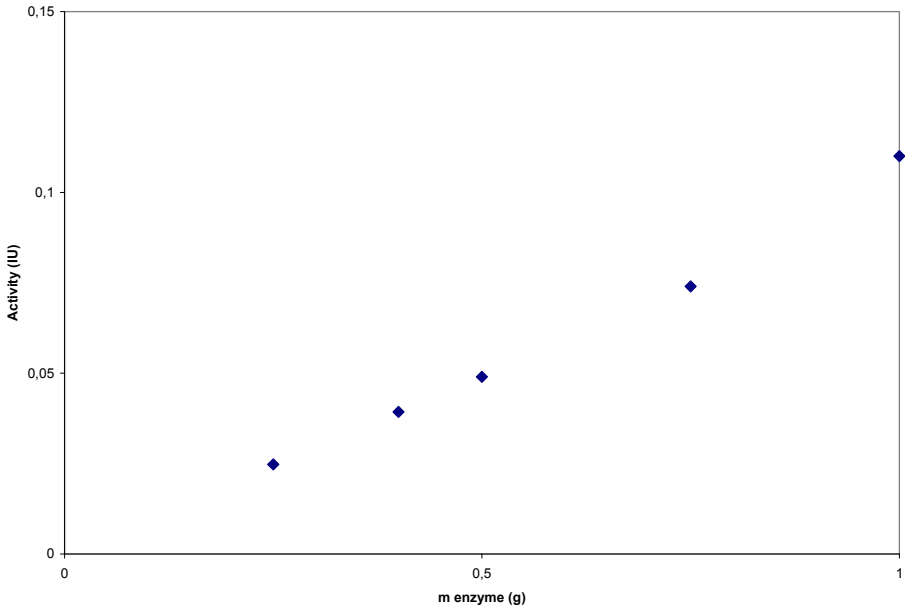

Figure 1. The influence of crude enzyme quantity on the enzymatic activity

Phenol removal with immobilized PPO in packed bed reactor

The bioremediation process was realized with the most efficient enzyme preparate (o.4 g crude PPO) and a solution of phenol (13 mM) in 0.2M phosfate buffer (pH 6.15). The phenolic solution was passed over the alginate beads which contain the calcium alginate beads included enzyme, until the concentration of phenol remain constant. After each step, the phenol concentartion was determined as described in section 1. As observed in Figure 3, in the first cycle the efficiency of process was approx. 25%. In the next cycles, the efficiency strongly decrease to 2-3%. In conclusion, due to the absence of stirring the diffusion of phenol through the alginate reduced the efficiency of bioremediation process.

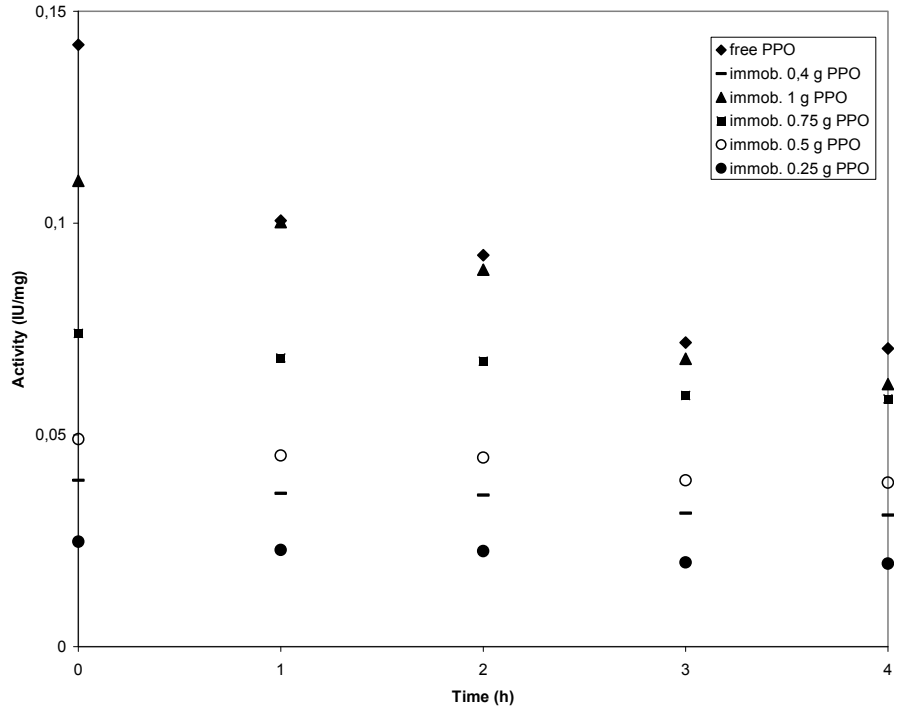

Figure 2. The stability of immobilized and free PPO in water

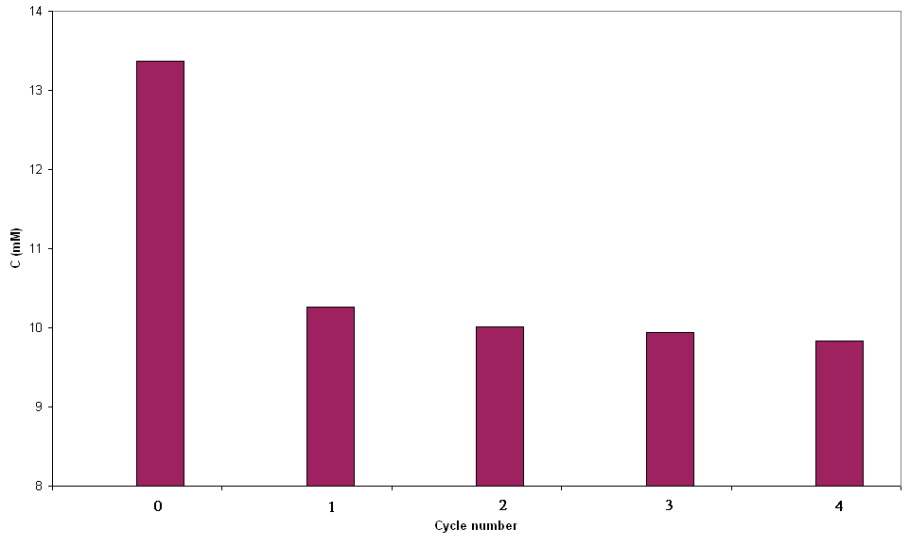

Figure 3. Phenol removal with immobilized PPO in packed bed reactor

Phenol removal with immobilized PPO in batch reactor

Next the process was realized in a batch reactor, under continously stirring, in the same conditions (initial phenol concentration, pH of buffer, quantity of immobilized PPO). Samples were tacken every hour and phenol concentration was determined colorimetrically. As ilustrated in Figure 4, after 5 hours 25% of phenol was removed and the process stopped.

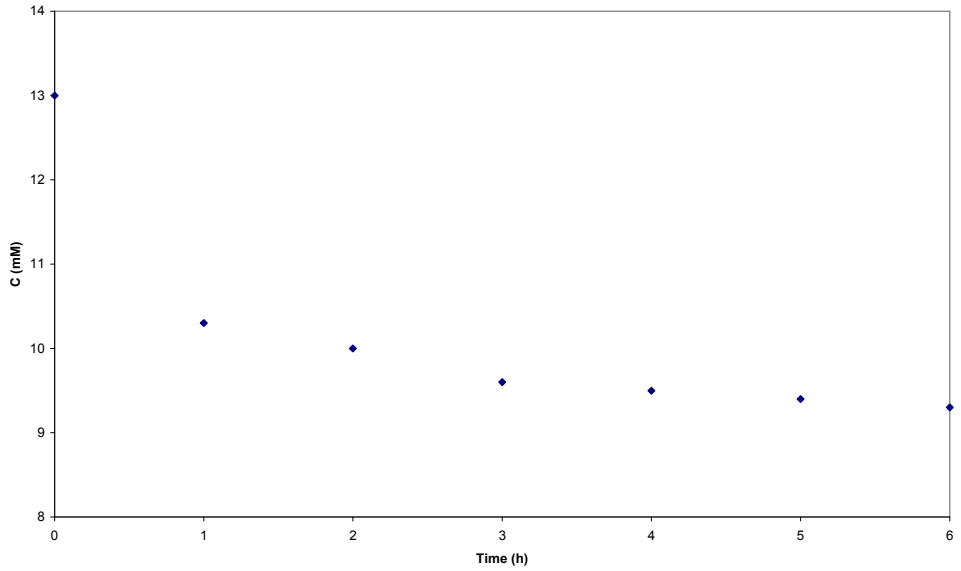

Figure 4. Phenol removal with immobilized PPO in stirred batch reactor

CONCLUSION

Polyphenol oxidase from potato with low purity immobilized by entrapement in calcium alginate showed a good potential for phenol removal from synthetic wastewaters. The entrapement in calcium alginate is a simple and cheep immobilization method which occurs with high yields (>90%). The enzyme preparates were tested for phenol removal from wastewaters in packed bed and batch reactors. In both cases, the phenol removal process occurs fast in the first period, with an approx. 25% yield. To increase the yield of phenol removal with this immobilized PPO, the use of packed bed reactor cascade can be an efficient solution.

EXPERIMENTAL PART

Materials and methods

Potato *(Solanum tuberosum*) tubers were obtained from commercial fields in Romania at 3- to 4-week intervals. All inorganic and organic reagents were products of Aldrich or Merck. The UV-VIS spectra were recorded on a Agilent 8453 spectrophotometer at room temperature. Crude PPO was prepared as earlier described by us [7]. Phenol concentration was measured using the colorimetric method with 4-aminoantipyrine (4-Amino-1,2-Dihydro-1,5-Dimethyl-2-Phenyl-3H-Pyrazol-3-One*,* AAP) in presence of potassium ferricyanide.

Immobilization of crude polyphenol oxidase in calcium alginate

A quantity of freshly obtained crude PPO from potato was dissolved in demineralized water (10 ml) and mixed with sodium alginate solution (2%) in 1:1 ratio. The mixture was added dropwise into calcium chloride (0.2 M) solution with continuous shaking. As soon as the drop of enzyme-alginate solution mixed with $CaCl_2$ solution, Na^+ ions of Na-alginate were replaced by the Ca^{+2} ions of $CaCl_2$ solution, which finally formed Ca-alginate beads. The beads thus formed were washed 3-4 times with deionized water and finally with 0.2 M phosphate (pH 6.15). These beads were used for further studies.

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