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OPTIMIZATION OF HORSERADISH PEROXIDASE ENCAPSULATION WITHIN TYRAMINE-ALGINATE FOR PHENOL REMOVAL

Nevena Pantić[1*], Nikolina Popović[2], Miloš Prokopijević[1], Dragica Spasojević[1], Radivoje Prodanović[2], Daniela Đikanović[1], Olivera Prodanović[1]

[1]University of Belgrade, Institute for Multidisciplinary Research, 11000 Belgrade, SERBIA

[2]University of Belgrade, Faculty of Chemistry, Studentski trg 12, 11000 Belgrade, SERBIA

[*]nevena.pantic@imsi.rs

Abstract

Phenolic compounds are one of the most common pollutants in aqueous systems, so their removal from water is of major interest. Among biocatalysts used for phenol removal, horseradish peroxidase is the most investigated for this purpose. Enzyme inactivation is a major problem which could be successfully overcome by immobilization of the enzyme onto different polymers. Tyramine-alginate micro-beads were tested for the immobilization of horseradish peroxidase. Different concentrations of tyramine-alginate were used and their influence on specific activity of the enzyme was tested. Increasing concentration of oxidized alginate results in increase of specific activity. Immobilized HRP was tested for phenol removal in a batch reactor. Presented results were obtained with HRP immobilized within 10 mol% tyramine-alginate micro-beads. These biocatalysts can be used up to three cycles.

Keywords: horseradish peroxidase, immobilization, phenol removal, tyramine, alginate

INTRODUCTION

On a daily basis, large amounts of pollutants reach water courses leaving the consequences to the entire ecosystem. Phenol is one of the most common water pollutants, because of its toxicity even at low concentrations [1]. Phenols and phenolic compounds, commonly found in wastewaters, are derived from a number of industrial processes, including coal conversion, mining, petroleum refining, plastics and textiles. Methods used for the removal of phenolic compounds from waste effluents include many physical, chemical and biological processes [2,3].

Different enzymatic approaches have been used for the removal of phenolic compounds. Peroxidases are able to oxidize phenol like compounds in the presence of hydrogen peroxide, forming water-insoluble polymers that could be easily removed from aqueous phase using filtration or sedimentation. Among investigated peroxidases, horseradish peroxidase (HRP) was successfully used for the purpose of phenol removal from wastewaters. Inactivation of the enzyme is a major problem in the phenolic compound treatment. Immobilization on different materials was tested in order to overcome this difficulty and to increase operational stability of the enzyme [4].

In this work, horseradish peroxidase was encapsulated within tyramine-alginate hydrogels that we have previously developed and tested for the phenol removal in a batch reactor.

MATERIALS AND METHODS

Materials

Sodium alginate, horseradish peroxidase, phenol, hydrogen peroxide and other chemicals used for this research were purchased from Sigma-Aldrich, USA.

Synthesis of tyramine-alginates

Alginate was modified with sodium metaperiodate and tyramine hydrochloride as was previously described Prodanovic et al. [5]. Briefly, appropriate amount of sodium alginate (0.28 g) was dissolved in water to a final concentration of 1% (w/v). Sodium metaperiodate was added to this solution to a final concentration of 2.5 mM and 5 mM so that molarity ratio of periodate to C6 glycoside units in alginate was set to 5 mol% and 10 mol%. Reaction was stopped by adding glycerol at 50 mM concentration and oxidized alginate was precipitated by adding NaCl at 1% (w/v) and 2 volumes of 96% ethanol (v/v). The precipitate was separated, dried and dissolved in 0.1 M sodium-phosphate buffer pH 6. Modification of oxidized alginate was carried out by adding solid tyramine hydrochloride and sodium cyanoborohydride to this solution. Modified alginate was precipitated by adding NaCl at 1% final concentration and two volumes of 96% ethanol.

Immobilization of horseradish peroxidase

Tyramine-alginates were dissolved in 50 mM Tris HCl buffer pH 7.0 at final concentration of up to 15%. Horseradish peroxidase, glucose oxidase and glucose were added in tyramine-alginate solution. Micro-beads were formed by pouring this mixture into 600 µL of light mineral oil containing 3% Span 80 detergent. Reaction was stopped by adding 1 mL of 0.5% Triton X-100 in 5% calcium chloride solution.

Measurement of enzyme activity

Peroxidase activity was measured using pyrogallol and H_2O_2 as substrates. For immobilized enzyme 130 µL of suspension and 30 µL of 0.97 M H_2O_2 were poured into 3 mL of 13 mM pyrogallol solution. Reaction was performed under constant stirring for 7 min. Every 120 s aliquots were taken and filtrated and absorbance at 420 nm was measured. 1 U of enzyme activity was defined as the amount of enzyme that produces 1 mg of purpurogallin in 20 s.

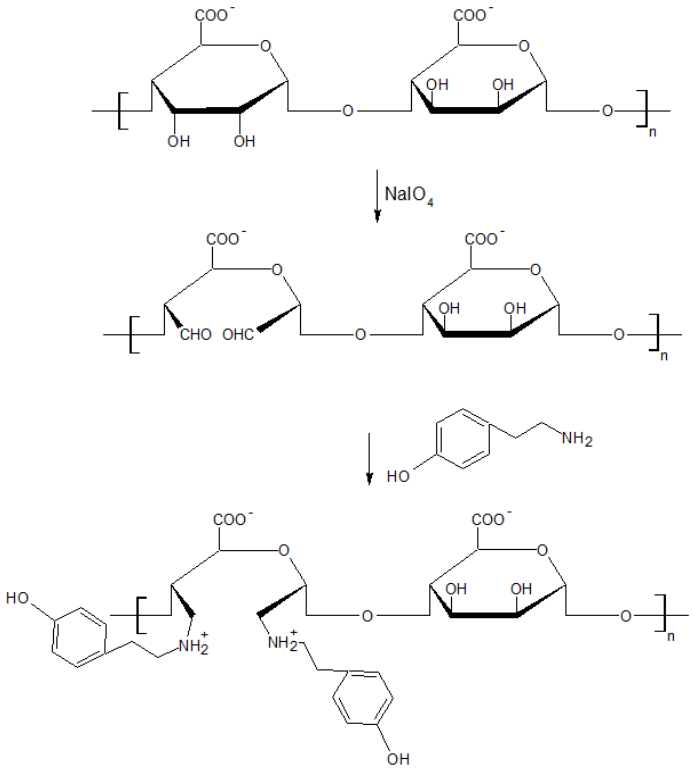
Phenol removal

Phenol removal was carried out in a batch reactor by adding alginate-tyramine-beads into 3 mL of 2 mM phenol solution in 50 mM Tris HCl buffer. For the delivery of hydrogen peroxide we tested system composed of glucose oxidase and glucose. Reaction mixture composed of phenol solution, glucose oxidase and glucose, stirred for 6h using magnetic stirrer. Aliquots were taken every 30 minutes. Phenol concentration was determined using a colorimetric assay in which phenol reacts with 4-aminoantipyrene (AAP) in the presence of potassium ferrocyanide ($K_3Fe(CN)_6$). Absorbance was measured at 510 nm after 10 min of color development.

Concentration of removed phenol was measured using calibration curve. Removal of phenol was followed for three days within 120 minutes, until decrease of the immobilized enzyme was noticed.

RESULTS AND DISCUSSION

Alginate was oxidized with various molar ratios of sodium metaperiodate and modified further with tyramine as it is shown on Scheme 1.



Scheme 1 *Tyramine-alginate synthesis: reaction of periodate oxidation and reductive amination with tyramine*

Influence of tyramine-alginate concentration (w/v) on specific activity of immobilized HRP was tested. The results presented in Table 1 show that specific activity of the enzyme is increasing with increase of tyramine-alginate concentration.

Table 1 *Influence of tyramine-alginate concentration (w/v) on specific activity of immobilized peroxidase*

Tyramine-alginate concentration (w/v)	5%	10%
Specific activity (U/mL)	0.66	2.04

Obtained micro-beads with encapsulated HRP were used for the removal of phenol in a batch reactor. The reusability studies showed that after three times of repeated use, these biocatalysts show decrease in their catalytic performance (Figure 1).

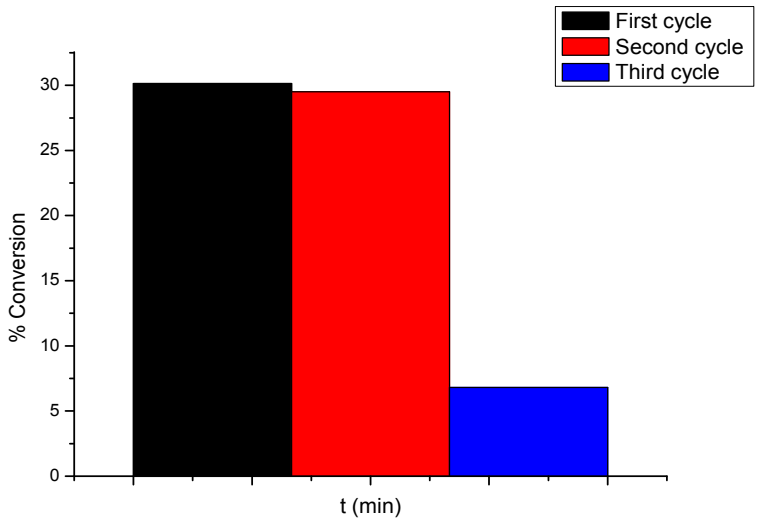


Figure 1 *Reusability studies of micro-beads, 10 mol% tyramine-alginate*

CONCLUSION

The preparation and application of immobilized horseradish peroxidase within tyramine-alginate micro-beads was investigated. Tyramine-alginates were used for the encapsulation of horseradish peroxidase within hydrogel micro-beads in a coupled emulsion polymerization reaction. Application of immobilized peroxidase for phenol removal was tested. Obtained experimental results revealed the effectiveness of the peroxidase encapsulation in the removal of phenol.

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