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WASTEWATER TREATMENT BY AMINATED PEROXIDASE IN ALGINATE HYDROGEL

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Abstract

Phenols are highly toxic organic compounds found in wastewater due to various industries' pollution. Its removal is of great importance for human and animal health. Enzymatic wastewater treatment has several advantages over traditional methods. Enzyme immobilization onto solid carriers enables its reusability and lowers the cost of treatment. In this work, immobilized horseradish peroxidase on chemically modified alginate hydrogel was tested for phenol removal. The reusability of the tested immobilizate was monitored in repeated cycles. After five consecutive cycles, the remaining activity of the immobilized enzyme was 54%. The obtained result shows the potential for using this immobilizate for wastewater treatment.

Keywords: phenol, alginate, aminated HRP, immobilization.

INTRODUCTION

Phenolic compounds are hazardous substances commonly found in the wastewater of various industries, such as petrochemicals, pharmaceuticals, paper and wood industries, textiles, plastic and paint manufacturing, etc. As it is found highly toxic to human, animal and aquatic life, its removal or neutralization is of great importance. Different methods are developed for dealing with this problem, like adsorption and extraction, membrane separation, bio-degradation, evaporation/distillation, chemical oxidation [1]. Enzymatic wastewater treatment has advantages over conventional treatments. This process is eco-friendly, not dependent on energy, highly selective, and requires mild working conditions. The immobilization of enzymes onto different carriers allows its reusability, which makes this process cost-effective [2].

Peroxidases are well-known enzymes found in all living organisms. Horseradish peroxidase (HRP) is a commercially available and extensively used peroxidase, that first appeared in scientific literature 200 years ago [3]. HRP uses H_2O_2 to oxidase organic substrates. Since green chemistry is one of the main focuses in modern science, HRP found its place as a useful tool [4]. Natural polymers are non-toxic, biocompatible, and biodegradable and therefore are particularly suitable for enzyme immobilization. Alginate is a natural polysaccharide from brown seaweed, composed of two monomeric units: β–D-mannuronic

acid and α–L-guluronic acid. Alginic acid can easily form hydrogels by ionic (in a solution of many divalent cations) or covalent cross-linking methods. Covalent cross-linking improves the physical properties of alginate hydrogels [5].

The aim of this research was to test the chemically crosslinked alginate hydrogel with immobilized HRP for synthetic wastewater treatment. The reusability of obtained immobilizate was monitored in a batch reactor during five repeated cycles.

MATERIALS AND METHODS

Materials

Sodium alginate (medium viscosity), HRP (150–250 U/mg), ethylenediamine dihydrochloride (98%), and sodium borohydride ($NaBH_4$) (≥98.0%) were purchased from Sigma-Aldrich (St. Louise, Mo, USA). Phenol (p.a. 99.5%), was obtained from Centrohem (Stara Pazova, Serbia). 4–aminoantipyrene and potassium ferricyanide were purchased from Fluka (Buchs, Switzerland) MP Hemija (Belgrade, Serbia), respectively.

Chemical modifications and immobilization

Immobilizate of aminated HRP in oxidate alginate beads was prepared as previously described by Spasojevic *et al.* [6]. Briefly, sodium alginate was activated by oxidation with sodium periodate in the dark. Peroxidase was firstly oxidized with periodate and then added into a solution of ethylenediamine. $NaBH_4$ was used as a reducing agent.

A 2% solution of activated alginate in $NaHCO_3$ was made and aminated HRP was added at a concentration of 0.01 mg/mL. After 24 hours Tris buffer was added to quench any remaining noncoupled carbonyl group. Immobilizate was mixed with native alginate to improve the mechanical properties of the gel. The beads were made by dripping the immobilizate solution from a syringe into a 5.5% solution of $CaCl_2$ (in distilled water) and stirring it for 1 h. The obtained beads were stored in a fridge in a HEPES buffer with 5 mM $CaCl_2$.

Phenol assay

Phenol concentrations were determined photometrically in an oxidative coupling reaction with 2.08 mM 4-aminoantipyrine (4-AAP) and 8.34 mM potassium ferricyanide ($K_3Fe(CN)_6$) in bicarbonate buffer pH 8 (Emerson reaction) [7]. After 10 minutes phenols fully reacted with 4-AAP in the presence of ferricyanide ion and form a stable reddish-brown antipyrine dye. Maximum absorbance was measured at 510 nm, and a calibration curve was made with a series of aqueous standards of phenols.

Phenol removal

200 mg of immobilizated HRP–alginate beads (with specific activity 0.43 U/g) was added in 3 mL phenol in water solution (2 mM). The solution with beads was placed on magnetic stirrer and the reaction started with the addition of 2.4 mM H_2O_2. After specified time intervals, aliquots were taken and the concentration of remaining phenol was determined using Emerson's reagent. The elimination efficiencies of phenol were calculated using the initial and residual concentrations. Each cycle lasted 2 hours, after which the beads were

filtered and washed. This procedure was repeated five times in the batch reactor at 25 °C with constant steering.

RESULTS AND DISCUSSION

Obtained immobilizate of aminated HRP onto oxidized alginate beads demonstrates the ability for phenol removal. To test the reusability of the immobilizate, hydrogel beads were separated from the batch reactor after 2 hours, rinsed with water, and used in the next cycle. The immobilized HRP could be easily separated and manipulated during repeated use, which is the main advantage over free enzyme. The phenol contents in the solution were calculated using equation (1) obtained from the standard curve.

$$y = 0.0122x + 0.0138 \quad (1)$$

The enzyme activity obtained in the first cycle is considered to be 100%. After the 5th cycle, the enzyme retained 54% of its original activity (Figure 1), which was similar to or better result compared to some existing studies [8–10].

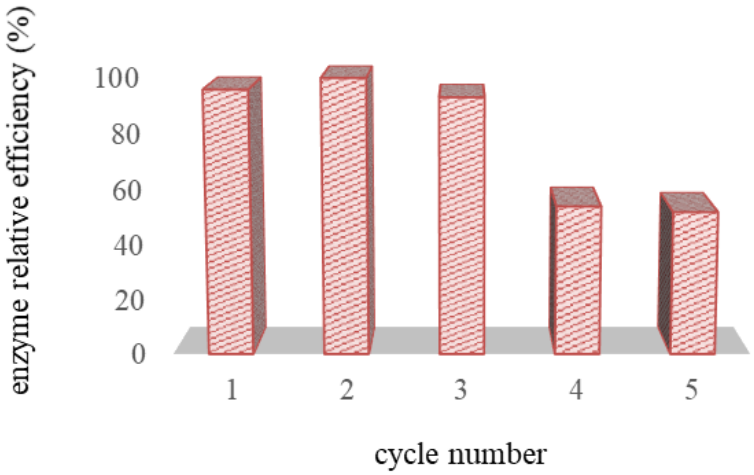

Figure 1 *Reusability of aminated HRP encapsulated within oxidized alginate*

The decrease in enzyme activity, which can be seen during cycles, is probably a result of the accumulation of polymerized phenol products onto alginate beads, which become brown upon utilization (Figure 2). A possible solution to this issue would be washing the hydrogel beads with organic solvents (like ethanol) after each cycle [11].

a) *b)*

Figure 2 *Hydrogel beads with immobilized HRP before a) and after b) reaction with phenol*

CONCLUSION

In this study, we tested the immobilizate of aminated HRP within oxidated alginate, that we have developed in our previous work, for phenol removal and its reusability. The results showed that the immobilized enzyme didn't lose its activity during the 3 cycles, and at the end of the 5th cycle activity loss was 46%. This confirmed that the hydrogel of chemically modified alginate and HRP has the potential for wastewater treatment or other phenolic removing processes. Also, this hydrogel system can be used for the immobilization of various enzymes and for removing pollutants from different mediums.

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