Journal of Multidisciplinary Engineering Science Studies (JMESS)
ISSN: 2912-1309
Vol. 2 Issue 1, January - 2016

Immobilization Of Catalase From Red Poppy On Chitosan Beads

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***Abstract*—Catalase enzyme from red poppy (*Papaver rhoeas*) plant was immobilized covalently with glutaraldehyde onto chitosan particles. The changes in the kinetic properties of the immobilized and free red poppy catalase were determined. The Km values were found to be 26 and 33 mM for free and immobilized red poppy catalase on chitosan. Furthermore, various characteristics of free and immobilized red poppy catalase, such as the temperature profile, thermal stability, optimum pH, reusability and storage stability were evaluated. When comparing, free and immobilized enzyme, change was observed in optimum temperature from 30°C to 40°C, respectively. Immobilized enzyme showed 10°C increase in temperature. The optimum pH was found 7.0 for free and immobilized catalase. Immobilized catalase showed higher storage stabilities than free catalase. Thermal stability increased with immobilization. Free catalase lost all its activity within 5 days, whereas immobilized catalase lost 25% of its activity within 60 days at 25 °C. The remaining activity of the catalase was about 50% after 6 cycles of batch operation.**

Keywords— catalase, immobilization, chitosan

I. INTRODUCTION

Catalase (EC 1.11.1.6) is a heme containing metalloenzyme which decomposes hydrogen peroxide to water and oxygen. This enzyme has many applications in various industries such as food, textile exct. This enzyme has been used for the degradation of hydrogen peroxide after textile bleaching and during the sterilization of milk [ ]. However, soluble enzymes used in industry are limited because of their properties such as instability in industrial conditions, high cost and non-reusability in order to increase their use in industry, enzymes are immobilized into solid matrixes. Immobilized enzymes becomes more thermo stabile, operational stabile, high quality and more favorable economic factors. Many methods for enzyme immobilization have been found in the literature. Organic or inorganic, natural or synthetic supporting materials have been used for enzyme immobilization Chitosan is the most attractive one for enzyme immobilization because of its cheapness, biocompatibility, nontoxicity, high mechanical strength [1-3].

Thus, chitosan has been widely used in the industrial and biomedical fields including enzyme immobilization, wastewater treatment and carriers for drug delivery [4–7]. It has been reported that various enzymes such as pectin lyase, carbonic anhydrase, invertase, catalase, laccase, and lipase were immobilized on chitosan particles and characterized [8]. Catalase was immobilized on chitosan and modified chitosan particles [12, 25] and different materials such as magnesium silicate, [9], Eupergit C. [10], microbeads with organic polymers [11]. Catalase for immobilization processes was usually used from different sources such as commercial one and microorganisms such as Aspergillus niger [13], Bacillus sp. [14]. The characterization and purification of catalase have been reported to exist in many plants, such as spinach, maize, cotton, sunflower, tobacco, van apple dill and *Malva sylvestris L.* [4-6,15-18]. The present study describes the immobilization of red poppy catalase in alginate beads and the effect of this immobilization on kinetic characteristics of immobilized red poppy catalase in comparison with free enzyme. The operational and storage stability of free and immobilized catalase were also determined.

II. MATERIALS AND METHODS

A. Materials

Red poppy (Papaver rhoeas) was collected from Sakarya region. Chitosan, glutaraldehyde, Hydrogen peroxide, polyvinylpolypyrolidone (PVPP), sodium phosphate buffer, (NH4)2SO4, acetic acid were obtained from Sigma Chemicals Ltd.

B. Extraction of Enzyme

Red poppy (Papaver rhoeas) catalase enzyme extraction was prepared as described in a perious work [20]. 15 g of Red poppy (Papaver rhoeas) was obtained from local Sakarya region. After that samples were added to 100 ml 50mM sodium phosphate buffer (pH; 7.0) with 0.3 g polyvinylpolypyrolidone (PVPP), and extraction was prepared. The mixture was homogenized with blender. After the filtrate was centrifuged at 14.000 g for 30 min and supernatant was collected [21]. Extraction was fractionated with (NH4)2SO4, solid (NH4)2SO4 was added to the supernatant to obtain 80% saturation. The mixture was centrifuged at 14,000 g for 30 minutes and the precipitate was dissolved in a small amount of phosphate buffer and then dialyzed at 4°C in the same

buffer for 24 h with three changes of the buffer during dialysis. The dialyzed enzyme extract was centrifuged and loaded onto Sephadex G200 column (1x10 cm) previously equilibrated with extraction buffer, and washed with the same buffer to remove unbound proteins. The eluate was used as the CAT enzyme source in the following experiments. The protein content of catalase solution was determined by the method of Bradford [23].

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C. Preperation of Chitosan Beads

3 grams of chitosan powder were suspended into 99 ml of distilled water by stirring for 10 min. One milliliter of glacial acetic acid was then added and stirring allowed for 3 h at room temperature. The solution was filtered and dried. Some 2.0 wt.% NaOH aqueous solution was added to neutralize the acetic acid in the chitosan film. The films were repeatedly washed with deionized water and finally dried again.

D. Reinforcement of chitosan beads by glutaraldehyde treatment

The crosslinking was carried out by immersing the dried chitosan into 0.05% (w/v) glutaraldehyde solution in 0.05M cold phosphate buffer (pH 7.0) for 1 h. The brownish reinforced beads were washed several times by 0.05M cold phosphate buffer (pH 7.0) to remove the excess of glutaraldehyde [22].

E. Immobilization of Catalase Into Chitosan Beads

Chitosan beads were mixed with 2 mg ml−1pepsin red poppy catalase solution in 0.05M phosphate buffer (pH 7.0) for 5 h with slight stirring and finally washed at 4°C. Then the beads dried in a vacuum incubator at room temperature and stored at 4°C. The amount of immobilized enzyme was estimated by subtracting the amount of protein determined in the supernatant after immobilization from the amount of protein used for immobilization. The protein content in solutions was determined by the method of Bradford [23].

F. Activity Assays of Catalase

Catalase (CAT) activity was determined at 25^{o}C according to Aebi [21] The reaction mixture contained 40 mM H_2O_2 in a 50 mM phosphate buffer pH 7.0, and 0.1 ml enzyme in a total volume of 3 ml. Catalase (CAT) activity was estimated by decreased in absorbance of H_2O_2 at 240 nm. Activities were carried out at optimum conditions. Approximately 100 mg of catalase immobilize chitosan beads were mixed 10 ml of 10 mM H_2O_2 solution in 0.05M phosphate buffer (pH 7.0) 30°C. After 5 min, the reaction was terminated by removal of the chitosan beads from the reaction mixture. The absorbance of the reaction mixture was determined and the immobilized catalase activity was calculated. The effect of substrate concentration on the activity was tested by using increasing concentrations of H_2O_2 and Vmax and Km values of immobilized and free catalase were determined.

G. Influence of pH

The activity assays were carried out over the pH range 3,5–9.0. Reaction rates of free and immobilized enzyme preparations depending on pH were investigated using 50 mM. Acetate buffer pH 3.5, 4.0, 5.0 50 mM. Phosphate buffer at pH 6.0, 7.0, 7.5, 8.0 and 9.0. Activity of pH profiles was determined at various pH in 10mM H_2O_2 solution at 30°C.

H. Influence of Temperature

The effect of temperature on enzyme activity was investigated in the range of 4 – 70 °C for both free and immobilized catalases. Activity of temperature profiles was determined at indicated temperatures in 10 mM H_2O_2 solution (pH 7.0)

I. Storage stability of free and immobilized catalase

The activity of free and immobilized catalase after storage in 50 mM. Phosphate buffer (pH 7.0) at 25°C and 4°C was investigated.

J. Reuse of the immobilized catalase

The residual activity of the immobilized enzyme was determined under standard assay conditions. The same immobilized enzyme was reused after it was thoroughly washed.

III. Results and Methods

A. Immobilization and characterization of Red Poppy catalase on chitosan beads

İmmobilize enzymes have been heavily used in several industries such as food, textile animal feed, detergent and paper industries. Catalase has been used especially in the textile industry as a bleaching material [12]. Many studies on immobilization of catalase have been reported [11, 15, 16, 25, 26]. In this study, we determined immobilization of red poppy catalase onto chitosan beads with gluteraldehyde.

There is no report yet about red poppy catalase immobilization in the literature.

Catalase enzyme firstly was isolated and characterized from red poppy plant (*Papaver rhoeas*). Then, the catalase was effected with glutaraldehyde crosslinked chitosan particles for 5 hours and the amount of the bound enzyme and kinetic properties of free and immobilized catalase were determined as shown in table 1.

The immobilized catalase enzyme in alginate showed an apparent Km value (33 mM) higher than the free enzyme (26 mM). Similar results were reported by Arabaci et al and Akkus et al. [15, 16, 25]. An increase in Km after immobilization shows that the immobilized enzymes have less affinity for their substrate than the free enzyme. This may be made by the steric hindrance of the active site. This steric hindrance might cause the loss of enzyme flexibility which is necessary for substrate binding.

TABLE I. KINETIC PARAMETERS OF FREE AND IMMOBILIZED RED POPPY CATALASE.

Catalase	Km (mM)	Vmax. (U/ml)	Bound Protein (mg/g chitosan)	Optimum pH	Optimum Temperature
Free	26	3611,1	-	7.0	30°C
Immobiled	33	4714.2	.095	7.0	40°C

B. . Effect of pH on free and immobilized catalase

The optimum pH of free and immobilized red poppy catalase was studied at various pH values (4.0–9.0) (Figure 1). At the end of the time the activity measurements of the enzymes were made under the optimum assay conditions (Fig. 1). As it is shown from the figure pH stability of the both enzymes shown a similarity. The results indicated that the optimum pH value of free and immobilized red poppy catalase was 7.0 respectively. However, the immobilized catalase sowed much broader pH stability than the free enzyme. This could be micro environment which could be by immobilization of enzyme.

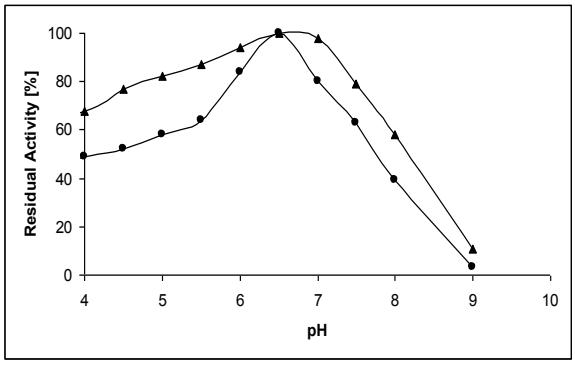

Fig. 1. *Effect of pH on activity: —■— free catalase, —▲— immobilized catalase.*

C. Effect of temperature on free and immobilized catalase

Effect of temperature variations on free and immobilized enzyme activity was investigated. Reactions were carried out at pH 7.0 and temperature influence was investigated within the 4–70°C range (Figure 2). The optimum temperature of the free enzyme was 30°C but after the immobilization of enzyme a shift in such temperature was observed with 10°C increase and the immobilized enzyme exhibited the highest activity at 40°C. Our result is similar to the literature work [15,16, 25, 26].

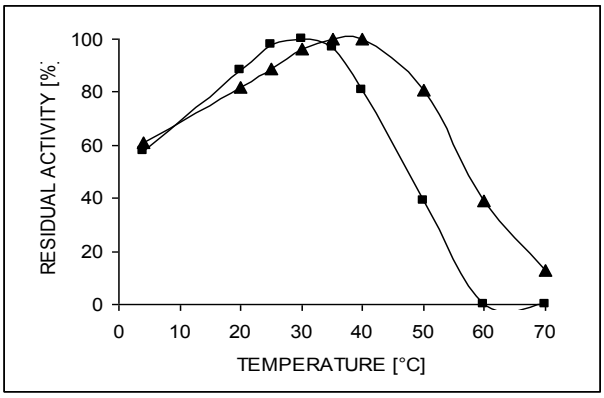

Fig. 2. *Effect of Temperature: —■— free catalase, —▲— immobilized catalase.*

D. Storage stability of catalase

Storage stability for the immobilized enzyme is an important property over the free enzymes, because free enzymes can lose their stability and activities quickly in the same conditions with immobilized enzymes. Free and immobilized red poppy catalase were stored in a phosphate buffer (50mM, pH 7.0) at 25°C and the activity measurements were carried out for a period of 60 days. After 4 days, only 50% of the activity was remained in free enzyme. However, immobilized enzyme was preserved %98 activity in the same time. During this incubation period, no enzyme leakage from the support was detected in the storage solution. The free enzyme lost all of its activity within 10 days. On the other hand, immobilized catalase lost 100 % of its activity during the incubation period. After 3 days, only 50% of the activity was remained. Immobilized enzyme was preserved %93 activity. The result shows that the immobilized catalase exhibits an increased stability over that of the free enzyme.

E. Reuse of Immobilized Enzyme

Figure 4 shows that immobilized enzyme could be reused. The activity of immobilized enzyme remained 50% after it was reused 6 times. The results confirmed that the stability of immobilized red poppy catalase enzyme is good for the future application.

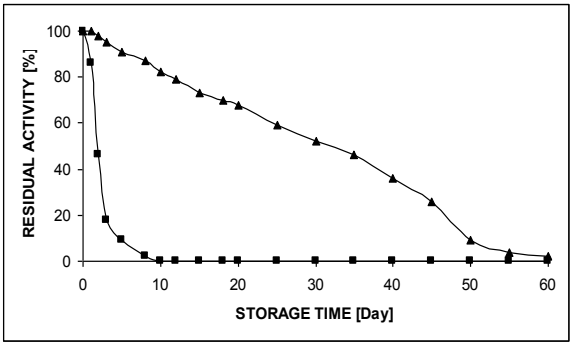

Fig. 3. *Storage stability at 25°C: —■— free catalase, —▲— immobilized catalase.*

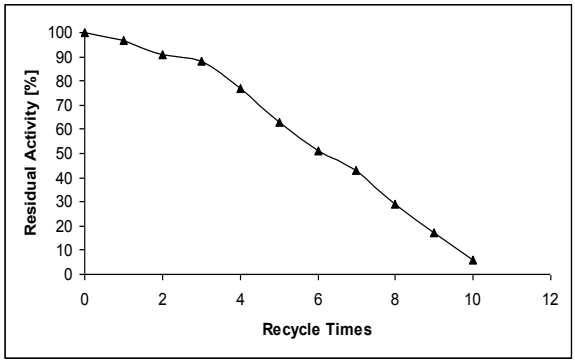

Fig.4. Reuse of immobilized red poppy catalase.

IV. CONCLUSION

Catalase enzyme form red poppy was successfully characterized and immobilized onto chitosan with glutaraldehyde. Our results showed that the Km value of immobilized catalase was higher than that of the free one which may be due to the decreased affinity of enzyme towards the substrate. The immobilized catalase demonstrated a shift in optimum temperature from 30°C to 40°C. It was found that the storage stability of immobilized catalase were much better than that of free catalase. It can be suggested that the immobilization of red poppy (*Papaver rhoeas*) catalase on chitosan makes the enzyme more useful for industrial applications.

ACKNOWLEDGMENT

This research was financed by Sakarya University. Author would like to thank Sakarya University for financial support (Project No.BAP 2012-02-04-040 and FBDTEZ, 2011-50-02-021).

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