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Immobilization and Characterization of Catalase Enzymes from Potato Extract by Sodium Alginate

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Abstract

To control oxidative stress, the eukaryotic cell yields various ROS-scavenging enzymes such as superoxide dismutase (which reduces O2 to H2O2), glutathione peroxidase, and catalase. In this work catalase was moderately purified from potato and immobilized against sodium alginate and its catalytic assets in view of the pH and temperature properties were examined. (NH4)2SO4 precipitation and dialysis were made in the extracted enzyme. The pH and temperature optima of the immobilized and free catalase were examined. Optimum temperature was detected higher than that of the free enzyme. The optimum pH was the same for both free and immobilized catalases (pH 7.50). The immobilized catalase was inhibited by anionic stabilizers and the immobilized enzyme presented higher strengths than the free enzyme.

Key words: Catalse, ROS, pH, Temperature, immobilization

INTRODUCTION

Reactive oxygen species (ROS) show significant role in existence of all living organisms. Highly reactive and reduced metabolites of O_2 such as superoxide anion and hydrogen peroxide (H_2O_2) are made during cellular respiration in organisms. When ROS are made extremely in the cell, they damage DNA, proteins, and lipids which bases to harm of cell function, oxidative stress, and programmed cell death (PCD) (Foyer et al, 2000). To control oxidative stress, the eukaryotic cell produces different ROS-scavenging enzymes such as superoxide dismutase (which reduces to H_2O_2), glutathione peroxidase, and catalase (Scandalios, 1993).

Catalase (oxidoreductase, EC 1.11.1.6; CAT) is a tetrameric heme containing intracellular enzyme that is extensively dispersed in animals, plants, and all aerobic microorganisms. The typical catalase response is the promptly deprivation of two molecules of H_2O_2 to water and molecular oxygen (Mhamdi et al, 2010). Catalase has been biochemically, genetically considered, purified, and characterized from many plants such as black gram (Vignamungo) seeds (Kandukuri et al, 2012), dill (Anethum graveolens) (Arabaci, 2-11), van apple (Yoruk et al, 2005), parsley (Ozturk et al, 2007), *Nicotiana tabacum* (Havir ett al, 1987), cotton (Ni et al, 1990), Pinus taeda (Mullen et al, 1993), sunflower (Eising eet al, 1990), and pumpkin (Yamaguchi et al, 1986). Catalase has been widely immobilized on abundant transporter materials such as chitosan (Cetinus et al, 2000), dextran (Marshall et al, 1976), asymmetric cellulose membrane (Selli et al, 1993), nylon membrane (Dagun et al, 1993), eggshell (Chatterjee et al, 1990), magnesium silicate (Tukel et al, 2004), Eupergit C (Alptekin et al, 2010), microbeads with organic polymers (Bayramoglu ett al, 2010), and characterizations of the immobilization enzyme were considered analytically. Catalase enzymes used in these immobilization revisions were frequently achieved from bovine liver and microorganisms (Aspergillus niger) (Tukel et al, 2004). Immobilization of catalase from plant origins has not been considered extensively yet. Therefore the objective of this effort is to study the immobilization and characterization of catalase enzymes from potato extract by sodium alginate.

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MATERIALS AND METHODS

Extraction and Isolation of Catalase

Catalase enzyme extraction was equipped as Aragão Börner et al, (2014) method. Potato's are acquired from local market and 15 grams of potato parts were added to 100 mL 50 mM sodium phosphate buffer (pH; 7.0) with 0.3 g polyvinylpolypyrolidone (PVPP). The combination was homogenized with blender. Then, the filtrate was centrifuged at 14.000 g for 30 min and supernatant was collected. Extraction was fractionated with $(NH_4)_2SO_4$, solid $(NH_4)_2SO_4$ 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 throughout dialysis. The dialyzed enzyme extract was centrifuged and equilibrated with extraction buffer and washed with the same buffer to remove unbound proteins. As the source of catalase enzyme, the eluate was used in the following experimentations.

Determination of Protein:

The quantity of catalase was accomplished according to method of Bradford with bovine serum albumin as standard (Bradford et al, 1976).

Immobilization of catalase:

25 ml each of sodium alginate solutions containing 2 % w/w and 4% w/w were arranged. 30 µl catalase was mixed to each sodium alginate solution as per Zaushitsvna et al (2013) and Wu et al (2013). The mixture was disturbed for 5 min. The resultant mixtures were transported one by one to a beaker with $CaCl_2$ and the material in the beaker was moved by means of a magnetic stirrer. A syringe with a 1.2-mm needle, positioned in an infusion pump was used for the drop wise transporting of the viscous sodium alginate solutions. The replacement of sodium ions with calcium ions caused in immediate gelation of the alginate drops. The resulting biocatalyst beads were detached from the $CaCl_2$ solution by means of a mesh and were kept in a fridge at 4ºC.

Activity Assays of Catalase

The activity of biocatalyst was studied during decomposition of H_2O_2 at an original concentration of 0.015 mol/dm^3. The biocatalyst beads (0.2 g) were mixed with 10 ml H_2O_2 solution with rapid stirring and, after 30 min, the concentration of H_2O_2 was determined by means of manganometry. Activity was articulated in µmol of decomposed H_2O_2 per 1 g biocatalyst per 1 minute.

Influence of pH and Temperature

Extents of the action of biocatalyst at various pH values in the range from 5 to 9 were conducted at 30ºC. Reaction amounts of free and immobilized enzyme arrangements contingent on pH were examined using 50 mM acetate buffer pH 3.5, 4.0, and 5.0 50 mM phosphate buffer at pH 5.0, 6.0, 7.0, 8.0, and 9.0. Activity of pH profiles was examined at various pH in 10 mM H_2O_2 solution at 25°C. Independent quantities of activity were performed at a pH of 7 at 10ºC, 20ºC, 30ºC, 40ºC and 50ºC for both free and immobilized catalases.

RESULTS & DISCUSSION

Effect of pH on the activity of catalase

The effect of pH on the free and immobilized enzyme for hydrogen peroxide degradation was considered in the pH range between 5.00 and 9.00 in acetate or/and phosphate buffers at 25°C. The results are obtainable in Figure-1. At the end of the time, the activity dimensions of the enzymes were made below the optimum assay

Figure-1. Effect of pH on activity of free catalase and immobilized catalase

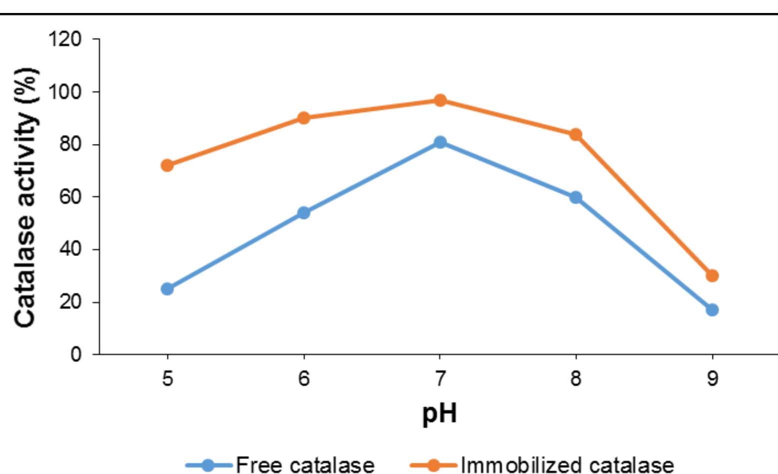

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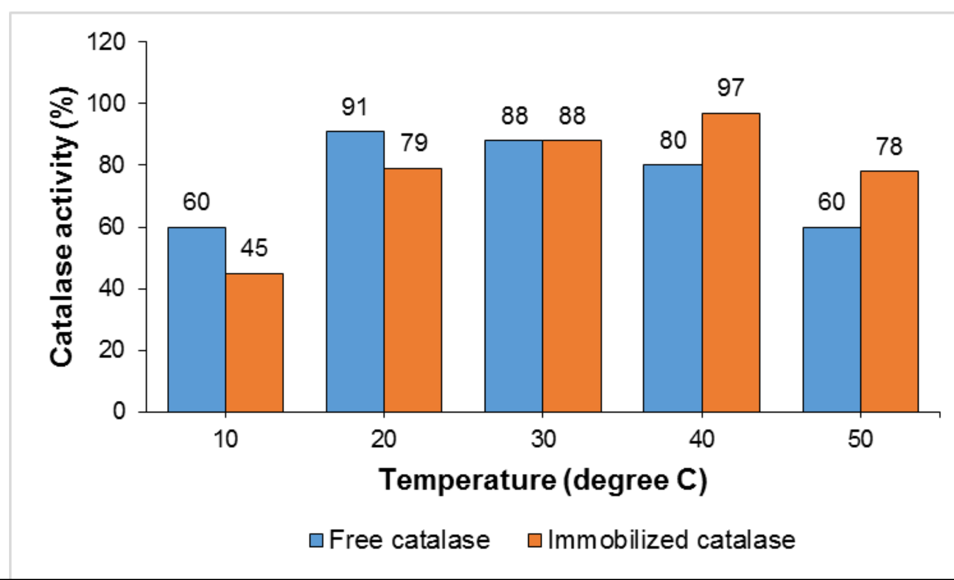
Figure-2. Effect of temperature on activity of free catalase and immobilized catalase



circumstances (Figure-1). As it was exposed from the figure, pH stabilities of the both enzymes had some resemblances. Both free and the immobilized catalase gave an optimum at pH 7.50; however, the immobilized catalase gave a much broader pH constancy than the free enzyme. This advised that immobilized enzyme was less sensitive to pH changes than free enzyme.

Effect of Temperature on Catalase activity

Temperature profiles of free and immobilized catalase are shown in Figure-2. Optimum temperature was initiate at about 30°C for free and 40°C for immobilized catalase. Figure-2 presented that the activity of immobilized catalase was more constant than the free catalase around 20–40°C. The activity defeat of immobilized enzyme was fewer than the free enzyme for advanced temperature. Immobilization of catalase in chitosan beads can basis growth in the enzyme rigidity which is commonly reflected by rise in constancy towards denaturation by raising the temperature (Jiang et al, 1993 and Abdel-Naby et al, 1993).

Conclusion

This present research work exhibited that the catalase enzyme was first time isolated and characterized from potato as a plant source. Then, this plant catalase effectively immobilized on sodium alginate. Thus, the plant enzyme was expanded strength against thermal denaturation by this successful immobilization of plant catalase on sodium alginate. It is possible to growth the deprivation of hydrogen peroxide and can be used numerous times in the procedures. The final results illustrated that the immobilization of potato catalase makes the enzyme additional useful in deprivation of hydrogen peroxide in various industrial applications such as food and textile.

Competing interests

The authors have declared that no competing interests exist.

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