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Immobilization of Alpha Amylase Using Dual Matrix Entrapment: Method for Commercial Usability

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Abstract: Industrial development of the enzyme reactors requires the use of immobilized enzyme in order to reduce the cost of the biocatalyst. To a large extent this method prevents enzyme losses and at the same time maintains activity of biocatalyst at a high concentration. α-amylase has been immobilized on different carrier matrix by different methods like entrapment, physical adsorption, covalent binding and ionic binding (Singh et al., 2012). In the present study after characterization of dual matrix immobilized beads, the effect of pH and temperature on efficiency of enzyme immobilization was evaluated by comparing the retained catalytic activity of the immobilized enzyme on dual matrix with that of the free enzyme. Method described for immobilization using sodium alginate: i-carrageenan dual matrix entrapment leads to high stability of α-amylase at low pH. Stability of α-amylase at low pH after immobilization was found to be consistent at 28°C, 37°C and 50°C.
Keywords*: *amylase, sodium alginate, carrageenan, immobilization

I. INTRODUCTION

Immobilization using different matrixes has been found to be useful for increasing enzyme stability and its reusability. Most widely used matrix for enzyme immobilization includes sodium alginate (C6H7NaO6)n and carrageenan (k-C24H36O25S2-2, i-C14H24O15S2, λ-C12H19O20S3-3). Amylases are enzymes that utilize starch molecules to give a variety of products such as dextrins and smaller polymers, which are composed of glucose units . Amylases are of great importance in biotechnology due to their applications in food, baking, brewing, fermentation processes, and detergent, textile desizing and paper industries. (Pandya et al., 2005 and Alva et al., 2007). Microbial amylases are available commercially and have almost completely replaced chemical hydrolysis of starch in starch processing industry (Gupta et al., 2003). Immobilization of alpha amylase has been carried out on different matrix by various methods like physical adsorption, covalent binding, entrapment and ionic binding. (Singh et al., 2012)Matrix used for immobilization such as sodium alginate (Dey et al., 2003), gelatin and gluteraldehyde (Sunita, 2014) has already been found to be effective in retaining. enzyme activity. The objective of the current study is to immobilize an industrially important enzyme; alpha amylase using one of the main directions of the present investigation is applied enzymology, to study the stability of amylase while using dual matrix immobilization. Dual matrix entrapment so as to make it stable at greater temperature and pH range and thus make it reusable for various industrial applications.

II. MATERIALS AND METHOD

A. Procurement of Alpha amylase

Commercially available α-amylase enzyme in dry powder form procured from Sigma Aldrich, USA was used for immobilization.

B. Preparation of dual matrix beads

Sodium alginate and carrageenan are mixed in 1:1 ratio to prepare 4% solution in distilled water. Briefly, carrageenan at 4% was heated in water bath until the slurry was formed and then 4% sodium alginate slurry was added to the above mentioned slurry. 100 ml of Enzyme extract samples in equal proportion were added to the slurry mix for preparation of loaded beads. The slurry was then loaded in a syringe and drop wise added to CaCl2 solution

C. *Characterization of bead*

D. Enzyme loaded and unloaded beads were characterized measuring diameter of beads, wet and dry weights of the beads using vernier caliper. Dye diffusion test was also performed using Trypan blue dye (0.4% w/v) prepared in distilled water. Briefly, beads were soaked in dye for one minute and 10 beads were put into 100 ml distilled water. Optical Density was measured after every five minute at 607 nm.

E. Scanning electron microscopy of beads with and without immobilized enzyme

The scanning electron microscopy has been used to determine shape and surface topography to examine the morphology of fractured or sectioned surface. Using ZEISS EVO-MA 10, Oberkochen, Germany, carried out SEM studies. Samples were placed on an electron microscope brass stub and coated with gold in an ion sputter. Picture of microspheres were taken by random scanning of the stub.

F. Preparation of enzyme solution

Enzyme solution was prepared by mixing 0.5mg/ml dry amylase powder in 100mM phosphate buffer saline. This solution was kept in incubator for 2h at 37°C to activate the enzyme.

G. Determination of enzyme activity

Enzyme activity was measured using Dinitrosalicylic acid method [8]. 1 ml enzyme was taken and mixed with 1% soluble starch solution. 1 ml phosphate buffer was added to

the above mixture and incubated at 50° C for 30 minutes. Samples were prepared by adding 3mL of DNS reagent to 3mL of test solution. This mixture was held in a boiling water bath (100°C) for five minutes. After cooling down, 1 mL of Rochelle salt solution (40%) was added to stabilise the colour reaction. Absorbance was measured at a wavelength of 540 nm. Glucose standard was prepared as reference.

The amylase production was determined in IU/ml/min by applying the standard formula.

Enzyme activity = micromoles of glucose released per ml per minute (IU/ml/min)

III. RESULT AND DISCUSSION

Results of characterization of beads on the basis of weight, size and dye diffusion ability are given in Table 1, 2 and Figure 1. Results indicate that after loading with enzyme there was 2 times increase in the dry and wet weight of beads. Maximum dye diffusion took place after a period of 80 minutes.

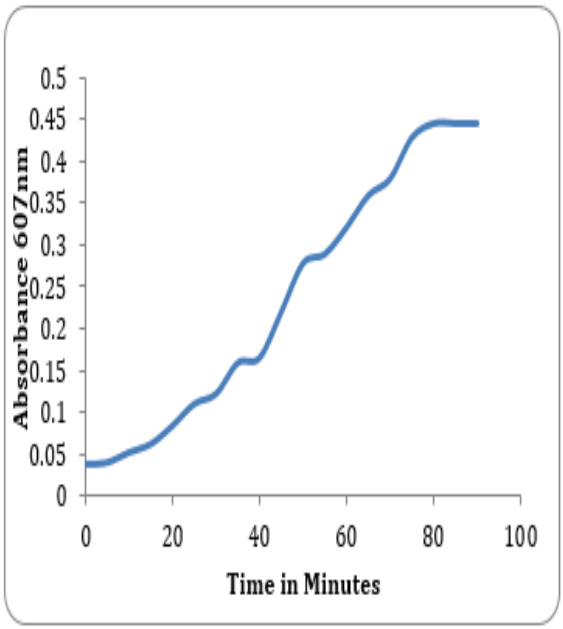

Fig. 1. Results of Dye diffusion test using beads

Table 1: Characterization of Beads on the Basis of Weight

Iota carrageenan beads	**Weight(in grams)**
Wet beads (blank beads)	0.213±0.003
Dry beads (blank beads)	0.045±0.004
Wet beads (with enzyme)	0.510±0.010
Dry beads (with enzyme)	0.400±0.015

Table 2: Size of dry and wet Bead

Type	Size (in cm)
Wet beads	0.49±0.001
Dry beads	0.37±0.003

Immobilized alpha amylase beads were tested for their stability with respect to pH and temperature range. The results of comparative analysis of enzyme activity using dual matrix loaded beads and free enzyme are given in figure 2-4. Effect of temperature at 28°C on enzyme activity of loaded beads and free enzyme was monitored over range of pH 3- pH 7 as shown in figure 2. It was found that the dual matrix beads protected enzyme activity at pH 3, however free enzyme was found to have minimal enzyme activity. From pH range 4-6, free enzyme activity was found to be 182 ± 4 IU/ml and dual matrix bead activity was 133.24± 4 IU/ml which was quite stable at this pH range. Both loaded beads and free enzyme showed decrease in enzyme activity at pH 7.

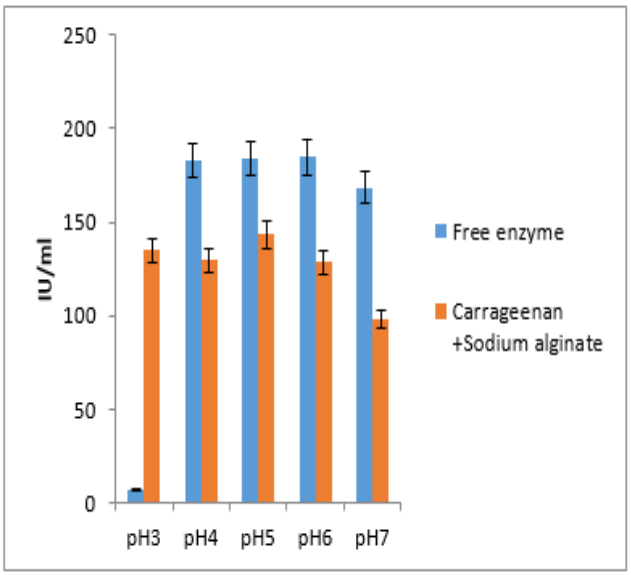

Fig. 2. Comparison of alpha amylase enzyme activity of free enzyme and loaded beads at 28°C.

Previous studies have described that optimum temperature for amylase is near 37°C, enzyme activity of loaded beads and free enzyme was monitored over range of pH 3- pH 7 at 37°C (figure 3). It was found that dual matrix beads protected enzyme activity at pH 3 at 37°C. From pH range 4-7, there was no significant difference between enzyme activity of loaded beads and free enzyme. These results indicate that dual matrix does not interfere with the transport of substrate as enzyme is functioning at maximum activity at 37°C.

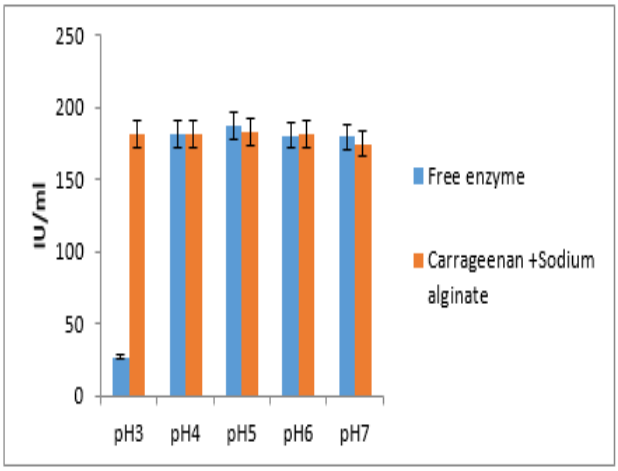

Fig. 3. Comparison of alpha amylase enzyme activity of free enzyme and loaded beads at 37°C.

Results indicating change in enzyme activity at 50°C of loaded beads and free enzyme was monitored over range of pH 3- pH 7 are given in figure 4. It was found that dual matrix beads protected enzyme activity at pH 3, however the activity was found to be significantly lower than at 28°C and 37°C. From pH range 4-6, free enzyme activity was found to be 180 ± 4 IU/ml and dual matrix bead activity was 146± 4 IU/ml which was quite stable at this pH range. The results of the study at 50°C indicate that immobilization using dual matrix is effective only at low pH range.

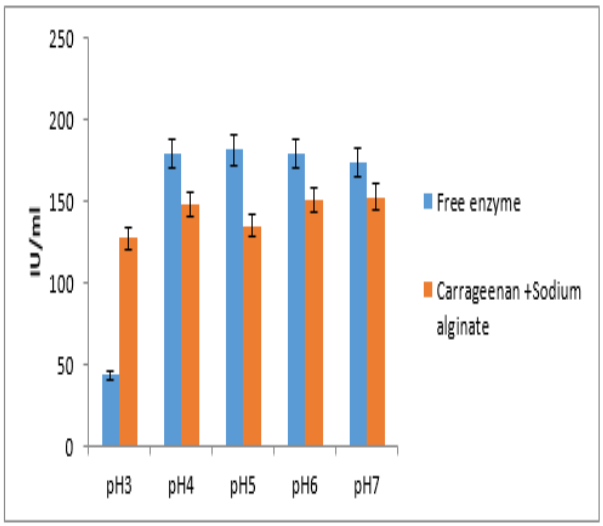

Fig. 4. Comparison of alpha amylase enzyme activity of free enzyme and loaded beads at 50°C.

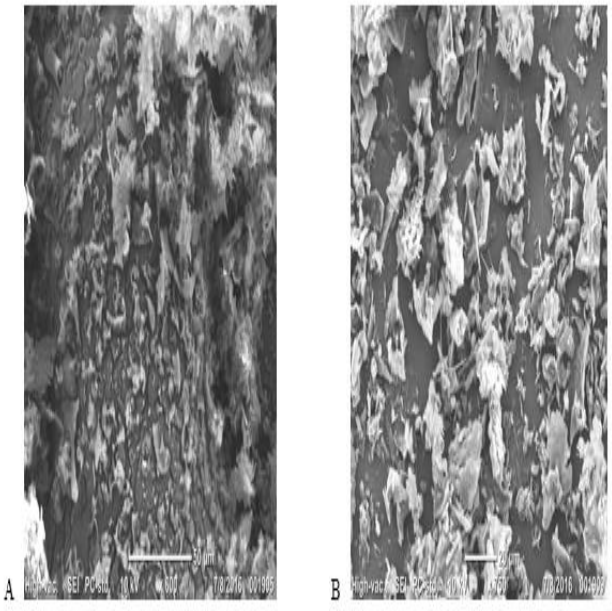

Fig. 5. Scanning electron microscopy of dual matrix beads. A, Unloaded and B, Loaded beads with enzyme

Results of scanning electron microscopy revealed that mixing of dual matrix resulted in uneven and irregular binding pattern of matrix in the beads (Figure 5 A). However when enzyme immobilized beads were prepared there was better distribution and binding of enzyme as shown in figure 5 B.

In the present study the characterization and potential of dual matrix beads loaded with alpha amylase at different temperatures i.e. 28°C, 37°C, 50°C and pH i.e. 3.0-7.0 was evaluated, as many authors have described that maximum activity of amylase was observed under conditions of the above mentioned range.

Amino acids like aspartate and glutamate can lose their charge at low pH that can lead to denaturation of enzyme. It was observed in the study that dual matrix beads were found to be stable at pH 3, and enzyme was able to retain 75% of its activity at 28°C, 37°C and 50°C. This method also prevents enzyme losses and also maintains the activity of biocatalyst at high concentration [9]. These results indicate that dual matrix beads can protect enzymes from low pH. This could be due to new binding site available for entrapment of protons in the matrix when sodium alginate and i-carrageenan are mixed together. One other factor, which may have contributed to high activity, could be the transport of substrate within the matrix. i-carrageenan has properties suitable for making soft gels, allowing for more porosity. However preparation of spherical beads using only i-carrageenan, as matrix is not suitable as the gel deforms and does not have stability to withstand high temperature. However, mixing of sodium alginate with i carrageenan may have provided stability and improved transport of substrate like starch molecules across matrix. Binding pattern of unloaded beads can be improved further by changing ratio of matrix or mixing of matrix at different temperature range. To our best knowledge this is the first report in which dual matrix has been used to retain amylase activity under low pH or acidic conditions.

IV. CONCLUSION

Method developed in the present study for immobilization of alpha amylase using dual matrix beads has been found to be reliable and reproducible in retaining enzyme activity particularly at low pH. Combination of dual matrix (sodium alginate: i-carrageenan) can be further applied in immobilizing other enzymes of commercial use.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest in the publication.

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