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Short Communication

Immobilization of α- Amylase (1, 4-α-D-Glucanglucanohydralase) by calcium alginate encapsulation

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Abstract

α-Amylase (EC No 3.2.1.1) is a hydrolytic enzyme which hydrolyses long chain carbohydrates. It hydrolyses starch into maltotriose and maltose from amylose; maltose, glucose and limit dextrin from amylopectin. Amylase has wide industrial applications like production of high fructose syrup, ethanol, detergents and so on. Immobilization is a method to enhance the stability and reusability of enzymes. In this study, we have immobilized pancreatic amylase by encapsulation using calcium alginate beads. Immobilized enzyme showed starch hydrolyzing activity similar to free enzyme and it was stable. The activity was found to be 0.2 IU/5 beads and 0.4 IU/10 beads. The activity was determined repeatedly for 5 times and the activity was almost unaltered. Hence, we propose that, this method can be used to produce immobilized amylase which can be used in various areas such as diagnostics, food, medicine and cosmetics.

Introduction

The hydrolysis of α-1, 4 linkages in polysaccharide like starch and glycogen to produce glucose and maltose is catalyzed by the enzyme α-Amylase (EC 3.2.1.1) (Pugh *et al*., 2000). It is the major form of amylase found in humans and other mammals. α-amylase tends to be faster-acting than β-amylase. In animals, it is a major digestive enzyme and its optimum pH is 6.7-7.0 (Pfeiffer *et al*., 1954). Although found in many tissues, amylase is most prominent in pancreatic juice and saliva, each of which has its own isoform of human α-amylase. They behave differently during isoelectric focusing and can also be separated by using specific monoclonal antibodies. It is one of the most important enzymes used in many industries like food, paper and textile (Nigam and Singh, 1995; Crabb and Mitchinson,1997; Marshal *et al*., 1999; Pandey and Nigam, 2000). α-amylase is used in ethanol production to break starch in grains into fermentable sugars. A α-amylase called "Termamyl", sourced from *Bacillus licheniformis*, is also used in some detergents, especially in dishwashing and starch-removing detergents (Chaplin *et al*., 2004).

An immobilized enzyme is an enzyme that is attached to an inert, insoluble material such as calcium alginate. This can provide increased resistance to changes in conditions such as pH or temperature. It also allows enzymes to be held in place throughout the reaction, following which they are easily separated from the products and may be used again. Enzyme immobilization can be achieved using several methods. In entrapment, the enzyme is trapped in insoluble beads or microspheres, such as calcium alginate beads. Encapsulation using calcium alginate beads offers many advantages due to its simplicity and non-toxic character (Gombotz and Wee, 1998; Goksungur and Zorlu, 2001). This immobilization technique consists of enclosing the enzyme in a semipermiable membrane capsule. There are mainly two advantages of this method, firstly, capsule structure allows interaction between substrate and enzyme, in addition these capsules can be reused several times (Roy and Gupta 2004). Encapsulation in calcium alginate beads occurs under very mild conditions and it is produced by low cost and easy to use (Longo *et al.*, 1992; Gombotz and Wee 1998; Bladino *et al*., 2001; Dey *et al*., 2003). By changing the gel conditions it is possible to easily control some of the capsule characters like thickness or permeability. The main objective of this work was to study the immobilization of α-amylase in calcium alginate gel capsules and to check its repeated usability. The application of immobilized amylase include areas such as diagnostics, food, medicine and cosmetics. These immobilized enzymes are used industrially when extremely specific catalytic conditions are required such as production of high-fructose corn syrup (Ellis *et al.*, 2001).

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Materials and Methods

Commercially available α-amylase (Diastase), soluble starch, maltose, 3, 5-dinitrosalicylic acid, sodium alginate and calcium chloride were purchased from Hi-Media laboratories private limited, India.

Enzyme immobilization

Entrapment of α-amylase in calcium alginate beads was carried out by extruding through a pipette, mixture of aqueous sodium alginate (1% w/v) and α-amylase into CaCl2 solution. The beads formed were recovered by filtration using Whatman grade 1filter paper and washed with distilled water to remove excess of $CaCl_2$ and non trapped enzyme. The capsules were dried between two sheets of filter paper and then in open air for 2 hrs before use (Figure 1).

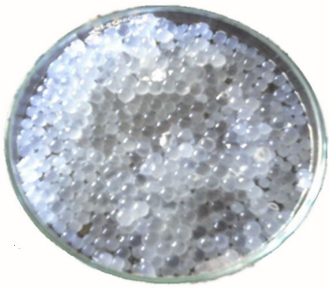

Figure 1. Immobilized gel beads with α-amylase

Enzyme activity

The enzyme activity was determined using 3, 5- dinitro salicylic acid method (Miller et al., 1959). The starch solution (1% w/v) was prepared by boiling in phosphate buffer, pH 6.9 (0.2M). The immobilized enzyme beads were taken in two different test tubes having 5 and 10 beads, respectively. The beads were then allowed to act on starch solution to produce reducing sugars, which in turn reduce 3,5 - dinitro salicylic acid into 3- amino, 5- nitro salicylic acid the intensity of which is measured colorimetrically at 545 nm.

Reusability

After each hydrolysis process, the beads were removed and washed with distilled water and stored at 4°C until next use. The same process was repeated until 5th use. The catalytic ability of immobilized enzyme was determined for 5 days with a time interval of 24 hours.

Results and discussion

The immobilized α-amylase hydrolyses starch into reducing sugars. By measuring the amount

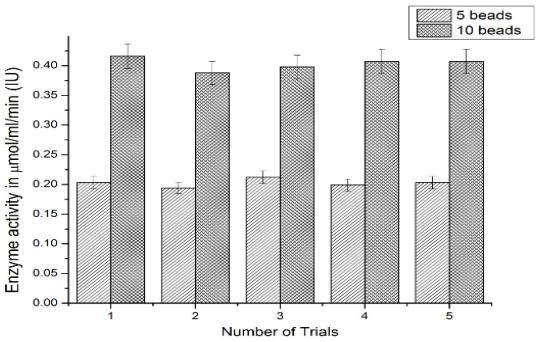

Figure 2. α-amylase activity after immobilization

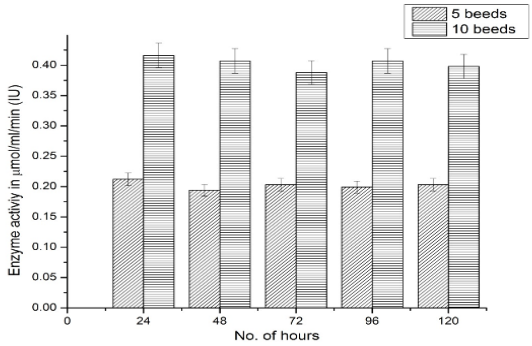

Figure 3. Activity of immobilized α-amylase at different time intervals

of reducing sugars released, enzyme activity was determined. The enzyme activity was found to be 0.2 IU/5 beads and 0.4 IU/10 beads, which remained unaltered even after 5 repeated uses (Figure 2). This shows that the activity remains the same with repeated use of immobilized enzyme, indicating the stability of the encapsulated enzyme. The reusability was checked at different time intervals of 24 hours for up to 120 hours which indicated that there was no significant loss of activity even after 120 hours (Figure 3). This data indicates that these immobilized enzymes can be stored and be used even after several hours after immobilization. This is an important requisite for the industrial applications as these immobilized enzymes are used for several times repetitively for long duration of time.

Conclusion

In this study, the enzyme α-amylase was immobilized by calcium alginate gel bead encapsulation. The Immobilized α-amylase showed a significant activity even after several repetitive use. The use of calcium alginate beads for encapsulation is safe, simple and inexpensive. The immobilization also enables the repeated use of the enzyme for long duration of time. Hence, we propose that the present method of immobilization can be adapted for large

scale enzyme utilization in various industries as it is an industrially important enzyme. However, the parameters such as thickness and temperature resistance of the bead needs to be studied further in detail.

Acknowledgment

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