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Continuous degradation of maltose by enzyme entrapment technology using calcium alginate beads as a matrix

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ABSTRACT

Maltase from *Bacillus licheniformis* KIBGE-IB4 was immobilized within calcium alginate beads using entrapment technique. Immobilized maltase showed maximum immobilization yield with 4% sodium alginate and 0.2 M calcium chloride within 90.0 min of curing time. Entrapment increases the enzyme–substrate reaction time and temperature from 5.0 to 10.0 min and 45 °C to 50 °C, respectively as compared to its free counterpart. However, pH optima remained same for maltose hydrolysis. Diffusional limitation of substrate (maltose) caused a declined in V_{max} of immobilized enzyme from 8411.0 to 4919.0 U ml^{-1} min^{-1} whereas, K_m apparently increased from 1.71 to 3.17 mM ml^{-1}. Immobilization also increased the stability of free maltase against a broad temperature range and enzyme retained 45% and 32% activity at 55 °C and 60 °C, respectively after 90.0 min. Immobilized enzyme also exhibited recycling efficiency more than six cycles and retained 17% of its initial activity even after 6th cycles. Immobilized enzyme showed relatively better storage stability at 4 °C and 30 °C after 60.0 days as compared to free enzyme.

1. Introduction

Maltase [E.C 3.2.1.20] is an enzyme that catalyzes the hydrolytic process of α-1→4 glycosidic linkages and yields α-D-glucose as an end product. Maltase is used in different industries like food, brewing, distilling and pharmaceutical industry [1]. Catalytic property of an enzyme is one of the critical aspects for its commercialization and in some cases free enzymes are unstable to fulfill the requirement of industries due to low operational stability and recovery [2]. Various techniques such as protein engineering, chemical modification and immobilization have been investigated to overcome the limitation of enzymes for industrial bioreactors [3–5]. Immobilization is an encouraging approach that not only enhances the stability of enzymes but also ensures the reusability of enzymes for various industrial bioprocesses [6–9]. Different immobilization techniques including covalent binding, adsorption, crosslinking and entrapment are used for continuous use of different enzymes on industrial scale. Among them, entrapment confines the enzyme within the structured matrix space and create negligible impact on its catalytic properties [10,11]. There are various matrices such as alginate, polyacrylamide and agar–agar are employed for enzyme immobilization. Alginate is an anionic polysaccharide distributed widely in the cell walls of brown algae and commonly used for the formation of beads capable of entrapping different macromolecules in the form of calcium-alginate beads [12–14]. Calcium alginate is a cost effective and biocompatible matrix for the entrapment of different enzymes including α-amylase, protease and pectinase [15,16].

In the current study, maltase from *B. licheniformis* KIBGE-IB4 was immobilized using entrapment technique within calcium alginate beads.

2. Materials and methods

2.1. Induction of bacterial isolate for maltase production

Wheat starch was used for the induction of previously isolated *B. licheniformis* KIBGE-IB4 for extracellular maltase production. Culture was incubated in optimized fermentation medium: wheat starch (2.5%), peptone (1.0%), yeast extract (0.2%), meat extract (0.4%), K_2HPO_4 (0.3%) and KH_2PO_4 (0.1%) at 37 °C and pH-7.0 for 48 h [17]. The cells were harvested by centrifugation at 40,248 × *g* for 15.0 min at 4 °C. Cell free filtrate (CFF) was precipitated using 40% saturation of ammonium sulfate on ice bath and kept for 24 h at 4 °C. The obtained precipitates were dissolved in potassium phosphate buffer (100.0 mM, pH-6.5) and dialyzed against same

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buffer for 24 h at 4 °C and used for immobilization within calcium alginate beads.

2.2. Maltase immobilized calcium alginate beads formation

Partially purified maltase was mixed with equal volume (1:1) of sodium alginate (4.0%) and added drop wise into calcium chloride solution (0.2 M) with constant shaking on ice bath. Immobilized enzyme and control beads were hardened by storing them into fresh 0.2 M calcium chloride solution for 90.0 min at 4 °C. The beads were washed with deionized water and potassium phosphate buffer to remove any unbound enzyme molecules.

2.3. Enzyme assay for free and immobilized maltase

The enzyme activity of both free and immobilized maltase was determined by GOD–PAP method [18,19] using glucose and maltose as a standard and substrate respectively.

One unit of maltase was defined as the "*amount of enzyme required to release 1.0 μmol of glucose per minute under the standard assay conditions* ".

2.4. Effect of sodium alginate and calcium chloride concentration on beads formation

Different concentrations of sodium alginate (1.0–6.0%) and calcium chloride (0.1–0.5 M) were used to attain the maximum immobilization yield of maltase with stable beads structure.

2.5. Effect of curing time on percent immobilization yield of maltase

The influence of curing time on percent immobilization of enzyme was examined by keeping beads in 0.2 M calcium chloride solution for different time intervals ranging from 30.0 to 180.0 min. Beads were washed with double deionized water and potassium phosphate buffer (pH-6.5).

2.6. Effect of bead size on the activity of immobilized maltase

The effect of beads size on the catalytic activity of immobilized maltase was determined by preparing beads having different diameters ranging from 1 to 5 mm.

2.7. Effect of reaction time on the catalytic activity of free and immobilized enzyme

The effect of reaction time on the catalytic activity of free and immobilized maltase was studied by performing the enzyme assay at different time intervals keeping the reaction temperature, pH and substrate constant.

2.8. Effect of temperature on the catalytic activity of free and immobilized enzyme

The effect of temperature on the catalytic activity of free and immobilized maltase was studied by varying the enzyme–substrate reaction temperature ranging from 30 °C to 60 °C, keeping other parameters constant.

2.9. Effect of pH on the catalytic activity of free and immobilized enzyme

The influence of pH on the catalytic activity of free and immobilized maltase was investigated by performing the enzyme assay at different pH ranging from 5.0 to 8.0. Different buffers such as acetate buffer (pH: 5.0–6.0), citrate buffer (pH: 5.0–6.0) and potassium phosphate buffer (pH: 7.0–8.0) with same ionic strength (100.0 mM) were used.

2.10. Substrate kinetics of free and immobilized enzyme

The kinetic parameters such as K_m and V_{max} of free and immobilized maltase were determined using Lineweaver–Burk plot by performing the enzyme assay at different substrate concentrations (2.5–50 mM).

2.11. Thermal stability of free and immobilized enzyme

Thermal stability of free and immobilized maltase were examined by keeping them at different temperatures such as 40 °C, 45 °C, 50 °C, 55 °C and 60 °C for different time intervals (30.0–180.0 min). Sample aliquots were retrieved after every 30.0 min and enzyme assay was performed at 50 °C for 10 min.

2.12. Storage stability of free and immobilized enzyme

Storage stability of free and immobilized maltase was determined by storing them at different temperatures (4 °C and 30 °C) for 60 days. Control and immobilized enzyme beads were placed in 0.1 M potassium phosphate buffer (pH-6.5) and stored at aforementioned temperatures. Percent residual activity was calculated by considering activity of control as 100%.

2.13. Recycling efficiency of immobilized enzyme

Reusability of immobilized maltase was investigated by using 0.5 g of beads for several reaction cycles. Beads were washed with phosphate buffer (pH-7.0) after each reaction cycle and fresh substrate was mixed for next reaction. Percent residual activity was calculated by considering the activity of first run as 100%.

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

Scanning electron microscopy was used to study the morphological changes of beads with and without immobilized maltase. For this purpose, beads were allowed to dry at 37 °C for 24 h and coated with gold using auto coater (Jeol Japan, Model JFC-1500). These gold coated samples were subjected to scanning electron microscope (SEM) (Jeol Japan, Model JSM 6380 A) for morphological analysis at different magnification power range (2500 × and 5000 ×).

3. Results and discussion

Bacterial isolates are preferred to use at commercial level due to short fermentation time and selective product formation but free enzyme always faces problem due to difficult recovery from reaction mixture which also decreases the product purity. Immobilization is a supportive technique which not only permits easy recovery of product and enzyme from reaction mixture but also improves the thermal and operational stability of an enzyme. Effect of sodium alginate concentration on entrapment of maltase was studied different concentrations of sodium alginate. It was observed that the percent immobilization yield of maltase was found maximum at 4.0% sodium alginate (Fig. 1A) and as the concentration of sodium alginate increased from maximum level, immobilization efficiency decreased which might be due to the formation of beads having small pore size that causes hindrance to enter the substrate in calcium alginate beads and to react with immobilized enzyme. However, low concentration of sodium alginate resulted in the formation of soft and fragile beads with