Immobilization and Characterization of Bacillus Thuringiensis HCB6 Amylase in Calcium Alginate Matrix

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Abstract	Free enzyme in solution react with substrates to result in products which cannot be recovered for reuse. TheseÃƒÂ,Ã‚Â problemsÃƒÂ,Ã‚Â canÃƒÂ,Ã‚Â beÃƒÂ,Ã‚Â overcomeÃƒÂ,Ã‚Â to a certain extent by the use of enzyme immobilization method. Immobilized enzymes are more robust and more resistant to condition changes. More importantly, the heterogeneous immobilized enzyme systems allow an easy recovery of both enzymes and products, multiple re-uses of enzymes, and continuous operation of enzymatic processes. Entrapment of enzymes in Ca-alginate is one of the simplest methods of immobilization. The aim of this research was to obtain the optimum condition of the making of immobilized amylase beads using a Ca-alginate bead and to determine its characteristics. The optimization of immobilized amylase beads includes variation of sodium alginates and variations of enzyme contact time with CaCl2. The characterization of immobilized amylase includes determination of optimum substrate concentration, optimum pH, and optimum incubation time as well as amylase stability test. Amylase activity was determined by using dinitro salicylic (DNS) method. The results showed that the optimum immobilized amylase obtained at alginate concentrations of 5% (w/v), contact time of 60 minutes and immobilization efficiency of 67.5%. Furthermore, immobilized amylase showed optimum substrate concentration of 1.5-2.5% (w/v), optimum pH of 6, an optimum incubation time of 20 minutes with the activity of 179.8 U/mL. The KM value for free amylase and immobilized amylases were 0.3 mM and 0.12 mM respectively. Vmax value for free amylase and immobilized amylases were 105.3 U/mL and 10.1 U/mL respectively. Immobilized Amylase can be used up to six times with the residual activity of 52.7%.
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