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ORIGINAL CONTRIBUTION

A novel alginate–CMC gel beads for efficient covalent inulinase immobilization

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Abstract Covalent immobilization of inulinase, produced by the marine-derived fungus *Aspergillus terreus*, on novel gel beads was made by the combination of alginate and carboxymethyl cellulose. Optimization of the loading time and loading units was done by response surface methodology. The bound enzyme displayed a change in optimum operating pH from 5.0 to 5.5 while the optimum operating temperature increased from 50 to 55 °C. K_m value has been increased (from 3.6 to 7.1 mg/ml) in comparison with the free enzyme. However, the V_{max} was lowered (from 145 to 77.5 U/g carrier) after immobilization. The immobilized inulinase showed enhancement in thermal stability against high temperature. There was an observed increase in half-lives and *D* values which revealed the improvement in the enzyme thermal stability. Thermodynamically, after immobilization, a remarkable increase in enthalpy and free energy was observed due to the enhancement of enzyme stability. Immobilized inulinase showed retention of 60% of its original activity after 10 successive cycles. Stability and reusability of immobilized inulinase on alginate–CMC enable the enzyme to be more convenient for industrial application.

Keywords Inulinase · Covalent immobilization · Response surface methodology · Alginate · Carboxymethyl cellulose

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Introduction

Inulinases catalyze inulin hydrolysis to produce inulo-oligosaccharides, fructose, and glucose. These can be classified into two types: endoinulinase (E.C. 3.2.1.7) that produces inulo-oligosaccharides) IOS) and exoinulinase (E.C. 3.8.1.80) which splits the terminal units of fructose. Exoinulinase is used for the production of high-fructose syrup (HFS) from inulin, while endoinulinase can be used for production of inulo-oligosaccharides of different lengths [1]. Fructose has a higher sweetening power compared to sucrose (about 70% higher), and it is used as a sweetener in food and beverage industries. It is well tolerated by diabetics, improves iron absorption by children, and helps in the removal of ethanol from the blood of alcoholics [2]. Fructo-oligosaccharides (FOS) increase the population of the gut bifidobacteria and improve the absorption of minerals [3].

The use of immobilized enzymes in industrial processes is more advantageous than the free forms as they help in the separation of reactants and products, allow the recovery of the enzyme for reuse, increase the enzyme stability, and reduce the costs of the process. Additionally, immobilization increases the selectivity of enzymes and reduces its inhibition by the products [4–7].

Many immobilization techniques were used previously for enzyme immobilization, such as gel entrapment [8], cross-linking [9, 10], physical adsorption [11], and covalent binding. Some authors have worked for many years on enzyme covalent binding immobilization using natural hydrogels and performing different modifications on gel formation to increase the enzyme resistance at high temperature and also to improve the enzyme reusability and the shelf stability of the enzymes [12–16].

The response surface method (RSM) is a combination of mathematical and statistical techniques for experimental