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Evaluation of α-amylase enzyme immobilization upon chitosan polymer for the evaluation of biosensors synthesis

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Abstract

The application of enzymes in various fields such as medicine, the food industry, the environment, and biotechnology is very common. One of the important applications of enzymes is in enzymatic biosensors which can play a significant role in processes such as drug tracking and disease treatment. Enzyme immobilization is a method to increase their efficiency and facilitate their recovery. The aim of this study was to immobilized the α-amylase enzyme on a polymeric chitosan through covalent bonding using glutaraldehyde cross-linking agent. The results of this study showed that the enzyme activity in the immobilized state on the polymeric substrate was still within the normal range of enzyme activity, which was confirmed by XRD test.

Key words: Immobilization, Enzyme activity, α-amylase, Chitosan

1. Introduction

Enzymes are catalytic compounds that can increase the rate of chemical reactions. Amylases are classified under hydrolases and are used in processes that require partial or complete hydrolysis of starch, such as glucose production, and in the food and paper industries (1). Enzyme immobilization is a technique in which enzymes are fixed on a desired substrate before the reaction takes place. This technique offers advantages such as improving enzyme efficiency in various environmental conditions, enabling the reuse of the enzyme in continuous processes, and facilitating the separation of the enzyme from the reaction environment. Chitosan, as the second most common polysaccharide and one of the conventional carriers in the enzyme immobilization process, is widely used due to its low cost, availability, and non-toxicity. The presence of amino and hydroxyl groups in the chitosan structure can facilitate its interaction with enzymes, and thus, it has the ability to crosslink with glutaraldehyde, ultimately helping to immobilize the enzyme on this substrate (2, 3).

2. Methods

***Chitosan Preparation Prior to Enzyme Immobilization*:**

2.5 grams of chitosan were dissolved in 100 mL of 1% acetic acid solution and kept at room temperature. After a specified time, a specific amount of 2% NaOH solution was added to the sample to adjust the pH. To enhance crosslinking conditions, a specific amount of glutaraldehyde was added to a specific volume of distilled water and mixed well. Subsequently, the solutions prepared in the previous steps were added to this mixture and left at room temperature for a specific duration **(**4**).**

Enzyme Immobilization:

A specific amount of the enzyme was brought to the desired volume using a buffer and added to the chitosan mixture. This mixture was incubated in a shaker incubator for the required duration. After the specified time, the beads and mixture were centrifuged and washed, and finally stored at 4°C (4, 5).

Free and Immobilized α-Amylase activity determination:

Based on Miller's method, the amylolytic activity of free and immobilized alpha-amylase was determined by measuring the production of reducing sugars from a 1% starch solution as a substrate in a buffer, using dinitrosalicylic acid at a wavelength of 540 nm, at temperatures of 10°C and 37°C. One unit of α-amylase is defined as the amount of enzyme that produces 1 micromole of reducing sugar per minute (3).

3. Results and discussion

In this study, a chitosan composite was prepared as a carrier for enzyme immobilization.The results demonstrated that the chitosan polymeric matrix served as a suitable carrier for alpha-amylase. The surface modification of chitosan using glutaraldehyde and the immobilization of the enzyme were confirmed by XRD.The immobilized alpha-amylase retained 85% of its activity compared to the free enzyme and did not lose activity after three washes with buffer. Moreover, all wash liquids showed negative activity, indicating the retention of a significant amount of enzyme activity in the immobilized state. Subsequently, in another part of the research, the inhibitory effects of various plant extracts on the activity of both free and immobilized alpha-amylase were investigated.

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