Optimization of process parameters for the preparation of alkaline protease immobilized by sodium alginate/arabic gum

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Sodium alginate (SA) is a natural polysaccharide carbohydrate that is soluble in water and combines readily with some divalent cations to form gels. Arabic gum (AG) is the most widely used type of gum, which has good acid stability[1]. Both are food-grade natural colloids, safe, non-toxic, cheap and easy to obtain, and have a wide range of applications in nutrition, medicine, food industry and other aspects[2]. At present, there are more studies on the preparation of immobilized carrier materials using SA. In this experiment, the immobilized carrier of enzyme was prepared by composite of SA and AG, and the immobilized alkaline protease was obtained by adsorption method. The effects of composite gel concentration, calcium chloride concentration, pH, and enzyme addition on the specific activity of immobilized enzyme were investigated, and the enzymatic properties of the prepared immobilized alkaline protease were studied to provide theoretical reference for the practical application of immobilized alkaline protease.

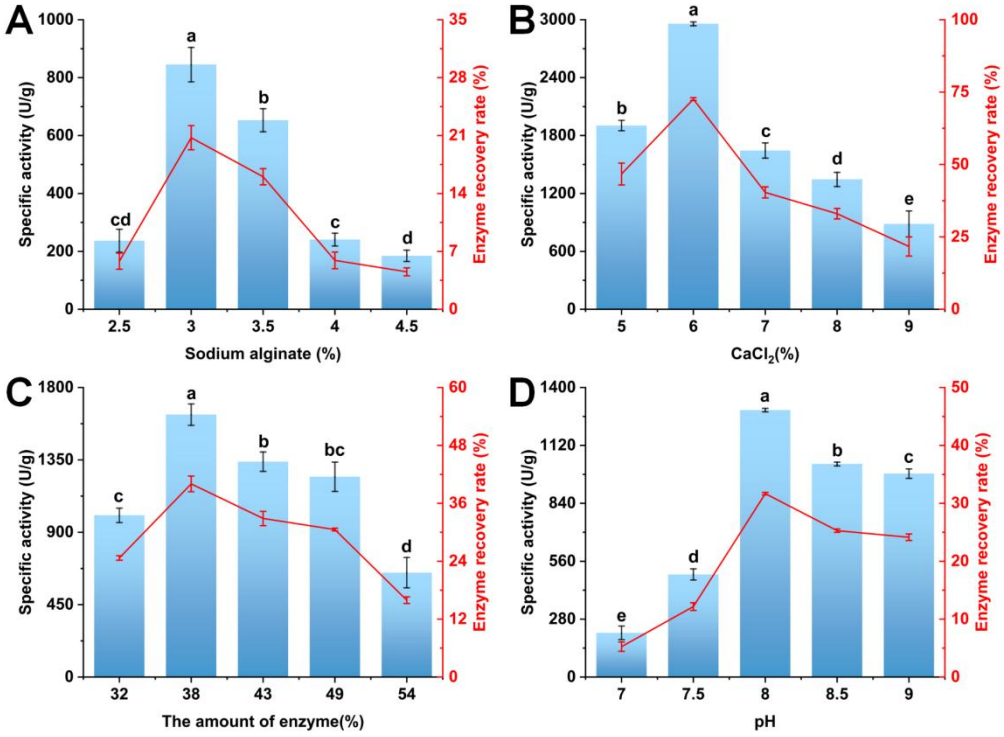

Figure 1 Effect of different preparation parameters on the specific activity of immobilized alkaline protease (A) concentration of sodium alginate; (B) concentration of $CaCl_2$; (C) the amount of enzyme; (D) pH

The immobilized alkaline protease was prepared by setting the mass concentration of the composite gel at 2.0 g/100 mL in terms of the total volume of 30 mL of the carrier solution and the mass ratio of SA to AG at 1:1, weighing the colloid and dissolving it in distilled water at 50~60 ℃, and ultrasonically defoaming it rapidly until there were no air bubbles in the composite gel. Using a 10 mL medical syringe at a speed of about 1 drop/s, the composite gel was dripped into a calcium chloride solution with a concentration of 5.0 g/100 mL, cured in a refrigerator at 4 ℃ for 1.0 h, and rinsed with purified water for 3~5 times to remove calcium chloride from the surface. The immobilized enzyme was put into the enzyme

solution with pH 8.0 and 1,760 U/g for 1.0 h. The immobilized particles were filtered out with gauze, washed and the water on the surface was sucked out with filter paper, and the spherical particles were made to obtain the immobilized enzyme. The optimization of alkaline protease immobilization conditions was carried out in a one-factor test to investigate the quality concentration of SA (2.0 g/100 mL, 2.5 g/100 mL, 3.0 g/100 mL, 3.5 g/100 mL, 4.0 g/100 mL, 4.5 g/100 mL), the quality concentration of $CaCl_2$ (5.0 g/100 mL, 6.0 g/100 mL, 7.0 g /100 mL, 8.0 g/100 mL, 9.0 g/100 mL), enzyme addition (32%, 38%, 43%, 49%, 54%), and pH of the enzyme solution (7.0, 7.5, 8.0, 8.5, 9.0) on enzyme recovery.

Figure 1 shows the results of the one-factor test, which showed that when the mass concentration of the composite gel was 3%, the highest enzyme recovery was 20.74±1.46%, and the results were significantly different ($p<0.05$) compared with the results of other concentrations. When the mass concentration of the SA was too high, the enzyme protein would again diffuse unevenly, resulting in the formation of particles of different sizes, affecting the contact between alkaline protease and substrate[3]. The highest enzyme recovery of alkaline protease was 72.58 ± 0.49 % when the $CaCl_2$ concentration was 6%. The enzyme activity showed a tendency of increasing first and then decreasing during the $CaCl_2$ concentration from 5% to 9%. The reason is that the $CaCl_2$ concentration affects the mechanical strength of the gel particles, when the Ca^{2+} concentration is too low, the cross-linking degree of SA and $CaCl_2$ is low, and the alkaline protease's calcium alginate immobilized enzyme is not easy to be formed, and thus the enzyme molecules are easy to be lost into solution. When the concentration of Ca^{2+} is too high, the cross-linking of sodium alginate and calcium chloride is large, the gel particles formed are poorly permeable, the surface of calcium alginate immobilized enzyme is covered with Ca^{2+}, and the pore size of the immobilized enzyme mesh structure formed is small[4]. Therefore, too high or too low concentration of $CaCl_2$ will lead to inadequate reaction, and 6% $CaCl_2$ concentration is the best concentration for the test. When the enzyme addition was 38%, the enzyme recovery was 40.03 ± 1.63%, which was significantly higher than the corresponding 24.68 ± 0.46% at 32% ($p < 0.05$). However, when the enzyme addition was in the range of 43% to 54%, the efficiency of immobilization decreased with the increase of enzyme addition, and the corresponding enzyme recovery was 32.86 ± 1.48% when the enzyme addition was 43%. The reason for this is that when the amount of enzyme added increased to a certain level, the binding site of the protein on the carrier was saturated, but the continued addition of enzyme made the enzyme molecules continue to increase, allowing the enzyme molecules to aggregate with each other, which ultimately led to changes in the structure of the enzyme active center, and consequently, to a decrease in enzyme activity[5]. The highest enzyme recovery of 31.67±0.21% was obtained when the pH of the enzyme solution was 8. The results were significantly different ($p<0.05$) when compared to the results of other concentrations.

In summary, this experiment screened the composite preparation of enzyme immobilization carrier with sodium alginate and gum arabic, and used the adsorption method to obtain the immobilized alkaline protease, and optimized the process parameters of the immobilized enzyme through the one-factor experiment, in order to efficiently prepare the composite gel beads, to improve the recovery of enzyme activity, which has the ecological and environmental effects of saving resources and energy, reducing or preventing pollution, and is in line with the strategic requirements of the contemporary sustainable development.

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