

The Effect of Glutaraldehyde Cross-Linking on the Enzyme Activity of Immobilized β -Galactosidase on Chitosan Bead

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Abstract: The effect of glutaraldehyde solution concentration, cross-linking time, cross-linking pH and cross-linking temperature on the enzyme activity of the immobilized β -galactosidase on Chitosan beads were studied. The enzyme activity was measured after immobilized by detecting the absorbance value at 420 nm. The results were as follows: the immobilized enzyme activity reached the maximum when the concentration of glutaraldehyde solution was 0.3%. The immobilized enzyme had optimal cross-linking time of 3h, optimal cross-linking pH of 6.5, optimal cross-linking temperature of 25°C, under these conditions, the immobilized enzyme activity reached 101, 96, 90 U/g, respectively.

Keywords: β -galactosidase, chitosan, cross-linking, enzyme immobilized

INTRODUCTION

Immobilized β -galactosidase could save energy and resources, reduce environmental pollution and also improve food flavor, increase nutritional value, so the application of immobilized β -galactosidase is very wide in food industry, especially in dairy process.

From the studies of lactase preparations, hydrolyzed milk products can benefit the 'lactose-intolerance' people and be consumed by them (Quinn and Chen, 2001). However, because of the inability to digest lactose into glucose and galactose, lactose intolerance is a very common disease that cause lactose maldigestion, the symptom of the disease are diarrhea, pain, nausea and flatulence, this will lead to lots of people to avoid milk or milk products (Kocin, 1988).

The use of free enzymes has been limited, because they easily denatured and have a short lifetime and be unstable (Torchilin, 1987) the immobilization of enzymes can increase their stability and protect their chemical and biological from degradation.

Chitosan is one of the most abundant biopolymers in nature. Chitosan and its derivatives are known as ideal support materials for enzyme immobilization (Juang *et al.*, 2002; Muzzarelli, 1980) because of their many characteristics such as improving mechanical strength, avoiding the disturbance of metal ions to enzyme, resistance to chemical degradation and anti-bacterial property. Chitosan is an inexpensive, inert, hydrophilic, biocompatible support and is thus attractive for enzyme immobilization (Yazdani-Pedram *et al.*, 2000; Kumar, 2000). In addition, using chitosan

as immobilized carrier can decrease the contamination of impurity and simplify purification process (Orrego *et al.*, 2010). Using glutaraldehyde as cross-linking reagent, the adsorption of β -galactosidase on chitosan beads will avoid a direct contact of the enzyme with the surrounding medium, it also make the reagents to reach the catalytic site (Valentina *et al.*, 2011).

The purpose of this study is to obtain the optimization condition of a stable immobilized β -galactosidase. Effects of several operative parameters (glutaraldehyde solution concentration, cross-linking pH, time, temperature) on the enzyme activity have been studied.

MATERIALS AND METHODS

Materials: Glutaraldehyde was purchased from Tianjin Kemiou Chemical Reagent Co., Ltd. Chitosan, O-Nitrophenyl- β -D-Galactopyranoside (ONPG) and O-Nitrophenol (ONP) were obtained from X'A Luosenbo Technology Co., Ltd. The β -galactosidase was purchased from Harbin Meihua Biological Technology Co., Ltd.

Preparation of chitosan beads: With 20 g/L acetic acid solution dissolved 30 g/L chitosan. Using 1ml medical needle tube added chitosan into 1 mol/L NaOH solution, the chitosan beads were rinsed several times until neutral with distilled water after coagulating. Finally, the chitosan beads were filtered and air-dried (Wan Ngah and Fatinathan, 2008).

Preparation of immobilized lactase: The β -galactosidase was immobilized on chitosan beads by

using glutaraldehyde. Added 1g chitosan beads into 10 ml 0.5% glutaraldehyde and cross-linked at 25°C for 1h, then washed chitosan beads with distilled water until there was no residual glutaraldehyde solution. Chitosan beads were placed into 10 mL 1 g/mL lactase solution and soaked at 25°C for 2h, and then washed the immobilized enzyme with distilled water and filtered by suction until enzyme activity could not be detected in the distilled water, finally the immobilized lactase activity was measured.

Enzyme activity assays: Using ONPG as substrate, the activity of β -galactosidase could be assayed by colorimetric test. The β -galactosidase can catalyze ONPG to ONP and galactose, The ONP in alkaline medium was yellow, which has the absorbance value at the wavelength of 420 nm in the solution. A standard curve was constructed by using ONP at various concentrations (Cavaille and Combes, 1995).

Dissolved 100mg β -galactosidase into phosphate buffer (pH 6.5) and diluted to 100ml with distilled water to prepare enzyme solution. Then 1ml the enzyme solution was diluted by using 100ml phosphate buffer (pH 6.5). The 3mL ONPG solution prepared with phosphate buffer (4 mg/mL) was added to test tube and set at 38°C for 7 min, the ONPG solution was well mixed with the 1ml diluted enzyme solution and kepted at 38°C for 10 min. Added 2 mL 1 mol/ L Na_2CO_3 solution to terminate the reaction, then the absorbance value was measured at 420 nm.

Under the measurement conditions (38°C, pH6.5, for 10 min), an enzyme activity Unit (U) is the amount of enzyme that catalyses to generate 1 μmol ONP per min under standard assay conditions. The immobilized enzyme activity was measured by the same method, However, we have to consider the immobilized protein content.

RESULTS AND DISCUSSION

Effect of glutaraldehyde concentration on the immobilized enzyme activity: The 1 g chitosan beads were added into 10 mL glutaraldehyde solution at different concentrations of 0.3, 0.5, 0.7, 1, 3%, respectively, washed the immobilized enzyme with distilled water and filtered by suction after cross-linked 12 h at 25°C, then added 10 mL pH6.5, 8 mg/mL lactase solution, respectively and immobilized at 25°C for 6h. Finally measured the enzymatic activity. The effect of glutaraldehyde concentration on the immobilized enzyme activity was showed in Fig. 1.

With the concentration of glutaraldehyde solution increasing, the enzyme activity of the immobilized β -galactosidase was also increased. When glutaraldehyde solution was 0.3%, immobilized effect was best and the activity reached the maximum. Subsequently, as the glutaraldehyde solution concentration increased, the immobilized enzyme activity decreased gradually. The

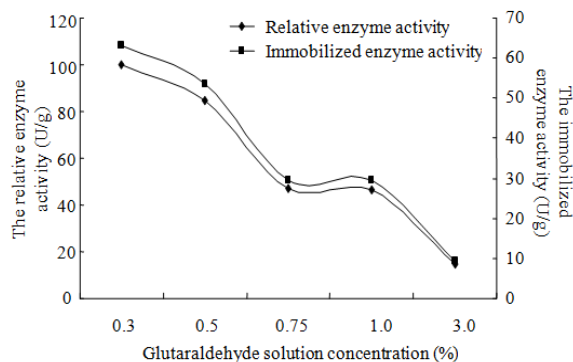


Fig. 1: Effect of glutaraldehyde concentration on the immobilized enzyme activity

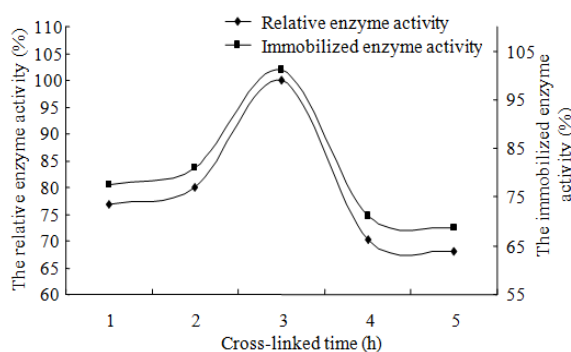


Fig. 2: Effect of cross-linked time on the immobilized enzyme activity

reasons were that Chitosan is a product of chitin by deacetylation, which has many of the free amino groups. When the concentration of glutaraldehyde solution was lower, active groups in the chitosan were less, the immobilized amount of the enzyme was also less, the immobilized enzyme activity was lower. With the concentration of glutaraldehyde solution increasing, the amino groups of chitosan were activated, the immobilized amount of the enzyme also increased. However, when the concentration of glutaraldehyde solution was too much, the chitosan bound excessive active aldehyde, leading to the enzyme molecules formed a multi-point binding with the carrier, thus existed a spatial structural obstacles to inactivate the enzyme. Meanwhile, the increasing amount of the enzyme bound to the active aldehyde may change the spatial structure of the active center of the enzyme, so that the enzyme activity decreased.

Effect of cross-linked time on the immobilized enzyme activity: Five g chitosan beads, each 1 g were added into 10 mL pH6.5, 0.3% glutaraldehyde solution, cross-linked at 25°C for 1, 2, 3, 4, 5h, respectively, then added 10 mL pH6.5, 8 mg/mL lactase solution, respectively and immobilized at 25°C for 6h. Measured the enzyme activity and the effect of cross-linked time on the immobilized enzyme activity was demonstrated in Fig. 2.

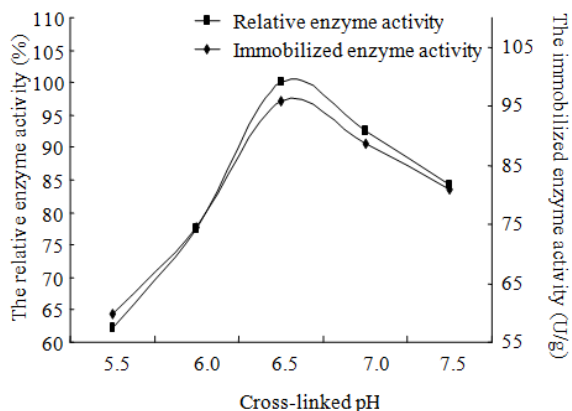


Fig. 3: Effect of cross-linked pH on the immobilized enzyme activity

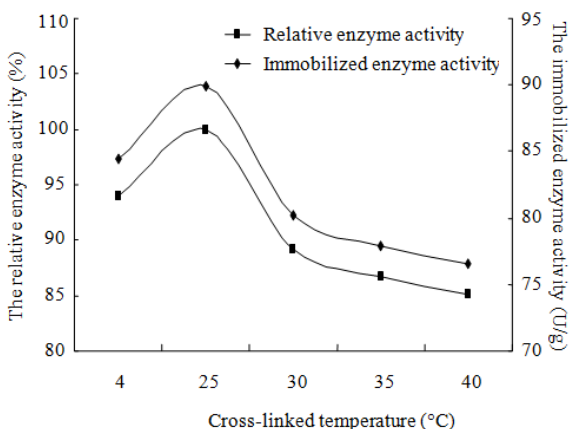


Fig. 4: Effect of cross-linked temperature on the immobilized enzyme activity

With the cross-linked time rising, the enzyme activity increased during 1-3h and reached maximum when cross-linked for 3h, during 3-4h, the enzyme activity decreased rapidly, over 4h there was no change in enzyme activity. The reasons were that the cross-linked time was too short, the degree of crosslinking was not enough, the cross-linked enzyme of carrier was less and leading to the immobilized enzyme activity was low. With the extension of the cross linking time, the reaction was carried out gradually complete, thus enzyme activity increased. When the cross-linking time was too long, the configuration of the immobilized enzyme may changed significantly due to excessive cross linking, caused the denaturation of enzyme protein.

Effect of cross-linked pH on the immobilized enzyme activity: One g chitosan beads were added into 10mL, 0.3% glutaraldehyde solution at pH 5.5, pH 6.0, pH6.5, pH7.0, pH7.5, cross-linked at 25°C for 3h, washed with distilled water and filtered by suction, then added 10 mL pH6.5, 8 mg/mL lactase solution.

respectively and immobilized at 25°C for 6h. Measured the enzyme activity according to the method mentioned above and the results were presented in Fig. 3.

With the pH rising, the enzyme activity also increased during pH5.5-pH6.5, over the pH6.5, the enzyme activity gradually decreased. When pH was 6.5, immobilized effect was best and the activity reached the maximum. So the optimal pH of cross-linked was 6.5. Because too acid or too alkaline will cause enzyme denaturation which lead to the enzyme activity reducing. The finding was consistent with the results of Zupei Liang's research, which urease was immobilized on chitosan beads by using glutaraldehyde and optimal cross-linking pH was found to be 6.5 (Liang *et al.*, 2006).

Effect of cross-linked temperature on the immobilized enzyme activity: Five g chitosan beads, each 1 g were added into 10mL pH6.5, 0.3% glutaraldehyde solution, cross-linked at different temperature of 4, 25, 30, 35, 40°C, respectively for 3h, Washed with distilled water and filtered by suction, then added 10 mL pH6.5, 8 mg/mL lactase solution, respectively and immobilized at 25°C for 6h. Measured the enzyme activity and the effect of cross-linked temperature on the immobilized enzyme activity was showed in Fig. 4.

With the temperature rising, the enzyme activity also increased during 4°C-25°C, during 25-30°C the enzyme activity decreased rapidly, over the 30°C, the enzyme activity slowly decreased. The optimal temperature of cross-linked was 25°C. The reasons were that when the temperature is too high, the aldehyde group in the glutaraldehyde and lactase amino bound too fast, leading to the increasing of enzyme steric hindrance, so the enzyme activity decreased. The finding was different to the report of S. Akkus Çetinus who immobilized catalase on chitosan beads. The research of S. Akkus Çetinus showed that optimum temperature was at about 35°C for free and immobilized catalase (Senay *et al.*, 2003); this indicated that different enzymes had different optimum immobilized temperatures.

CONCLUSION

Different glutaraldehyde solution concentrations, cross-linking time, cross-linking pH and cross-linking temperature had a significant effect on the enzyme activity of the immobilized β -galactosidase on Chitosan beads. In the experiments, the immobilized enzyme activity reached the maximum when the concentration of glutaraldehyde solution was 0.3%. The immobilized enzyme had optimal cross-linking time of 3h, optimal cross-linking pH of 6.5, optimal cross-linking temperature of 25°C, under these conditions, the immobilized enzyme activity reached 101 U/g, 96 U/g, 90 U/g, respectively.

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