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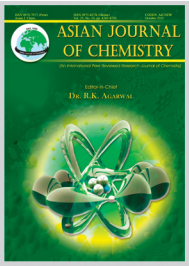

NOTE

Study on the Cellulase-Immobilized Efficiency of Polyethylene-Glycol-Modified Chitosan as a Carrier

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To study the cellulase-immobilized efficiency of polyethylene glycol (PEG)-modified chitosan as a carrier, we evaluated the quantity, optimum temperature, optimum pH and Michaelis constant of immobilized enzymes that used such a carrier. The results showed that the immobilized enzymes which used the polyethylene glycol-modified chitosan as a carrier increased in quantity and showed improvements in optimum temperature from 5 to 10 ºC ($P < 0.05$) and optimum pH ($P > 0.05$). The Michaelis constants were 0.4693 g/L (Km_M) and 0.4848 g/L (Km_O), corresponding to y(M) = 1.0076x + 2.1472 and y(O) = 1.1086x + 2.287, respectively. Thus, utilizing polyethylene glycol-modified chitosan as a carrier can improve the efficiency of cellulase immobilization.

Key Words: Polyethylene glycol modification, Chitosan, Cellulose, Immobilized.

Cellulose, the major polysaccharide in plant photosynthesis, is the earth's most abundant renewable resource[1,2]. Hydrolysis is an efficient, pollution-free and ideal method of breaking down cellulose. Many researchers use hydrolysis to degrade plant cell wall polysaccharides[3,4]. Cellulase is composed of numerous highly synergistic hydrolytic enzymes, which can be divided into three main components and can hydrolyze cellulose. In recent years, to improve enzyme properties, more and more studies based on chitosan as a carrier for the immobilization of free enzymes have been conducted[1-3] particularly because chitosan is safe, non-toxic and has good mechanical properties, heat resistance and high biocompatibility. The amino group of chitosan facilitates covalent binding with enzymes and its molecular moieties can form complexes with metal ions, thereby avoiding enzyme inhibition by metal ions. Thus, chitosan is a good carrier of immobilized enzymes[1-4]. The purpose of this study is to evaluate the cellulase immobilization efficiency of modified chitosan as a carrier.

Cellulase was purchased from Qing Dao World Biotechnology Co. Ltd., chitosan was manufactured by Chengdu Kelon Biological Reagent Co., Ltd., glutaraldehyde was obtained from Fluka and glucose was purchased from Sigma.

Modification with chitosan: Modification with chitosan was performed as described in Zhang *et al.*[5] a slight improvement in the carrier after modification with polyethylene glycol was observed. Approximately 250 mL isopropanol and water were combined as a mixed solved at room temperature, with swelling overnight. The modified chitosan samples were then dried and weighed.

Cellulase immobilization: A certain amount of chitosan was accurately weighed, passed through a 40-mesh sieve and mixed with a certain concentration of glutaraldehyde. Stirring was performed for 2 h at 25 ºC, after which the mixture was placed overnight in a static refrigerator at 4 ºC. Repeated filtration was performed with distilled water. A certain concentration of cellulase was added to the mixture, which was then stirred for 2 h at 25 ºC and placed in a refrigerator overnight at 4 ºC. Afterwards, adequate washing and filtering of the samples was performed to yield immobilized enzymes.

Measurement of CMC activity: Acetate buffer was added to a beaker and a certain amount of immobilized enzyme was added at 50 ºC for 0.5 h. DNS reagent was added to a metering density of 540 nm. CMC was measured according to international standards, which is defined as 1 g enzyme per min solution that yields 1 μmol of glucose hydrolysis, that is, one enzyme activity unit in IU (μmol/min/mL)[6].

Quantity of immobilized cellulase: Six modified chitosan and six common chitosan samples, all of which measured 0.2 g,

were put in a small beaker and combined with 10 mL glutaraldehyde (6 %). A constant stirring for 2 h was performed on magnetic stirrer. Afterwards, repeated filtration and washing were done. Then, 10 mL liquid cellulase was added to all beakers under the same conditions and stirring was continued for 2 h. Overnight refrigeration was performed at 4 ºC and filtration and washing were done three times to obtain immobilized enzymes. Detection of the amount of immobilized enzymes followed (Fig. 1). The results showed that with 0.2 g of chitosan, the active moiety is certain. In contrast, the modified chitosan, due to changes in its functional groups, could adsorb larger amounts of cellulase.

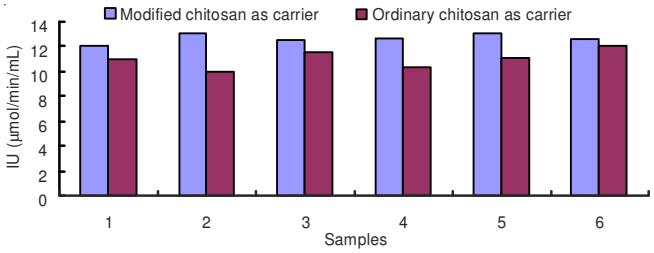

Fig. 1. Amount of cellulase adsorbed after enzyme immobilization

Optimum temperature of immobilized cellulase: We obtained immobilized enzymes and subjected them to a temperature range of 50 to 80 ºC, with 5 ºC as a spacer and each spaced set composed of 6 parallel samples. The results of optimum temperature determination are shown in Fig. 2. The optimum temperature of the immobilized cellulase that used the modified chitosan as a carrier ranged from 60 to 65 ºC. Immobilized cellulase that used ordinary chitosan as a carrier showed an optimum temperature of 55 ºC. The properties of modified chitosan as a carrier for cellulase immobilization can improve the optimum temperature of cellulase. These results can stimulate the development of immobilized enzymes in the industrial scale. Statistical analyses showed two groups of data with significant differences ($P < 0.05$).

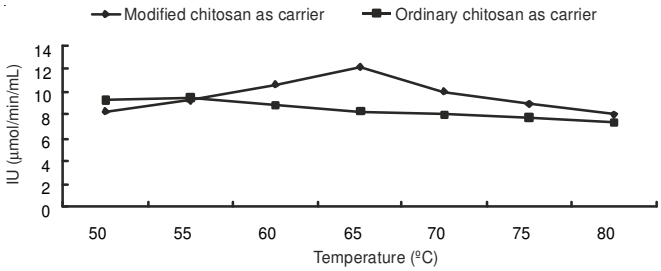

Fig. 2. Optimal temperature of immobilized cellulase

Optimum pH of immobilized cellulase: We obtained immobilized enzymes and subjected them to a pH range of 3 to 9, with 1 as an interval and each interval utilizing a spaced set of six parallel samples. The results of optimum pH determination are shown in Fig. 3. Compared with the immobilized cellulase that used ordinary chitosan as a carrier, the optimum pH of immobilized cellulase using modified chitosan showed minimal amplitude changes and greater enzyme activity between pH 5 and 7. A separate test showed that the use of modified chitosan as a carrier for immobilized enzymes can cause them to adapt better to acidic and alkaline environments, but statistical data on the optimum pH showed no significant difference ($P > 0.05$) between modified and ordinary chitosan as carriers.

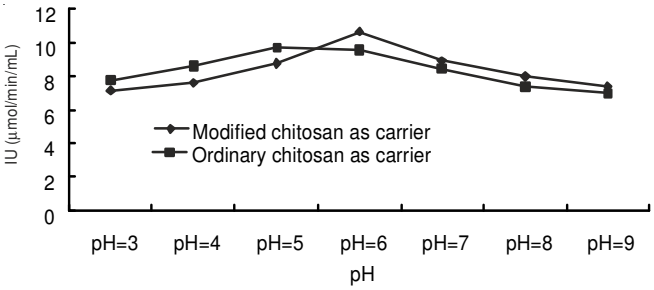

Fig. 3. Optimal pH of immobilized cellulase

Michaelis-Menten constant of immobilized cellulase: Six individual immobilized enzyme samples that used modified and ordinary chitosan as carriers were accurately weighed. The K_m of the immobilized enzyme was detected using the Lineweaver-Burk plot method, wherein the substrate is a different concentration of CMC. The results showed that Km_M was 0.4693 g/L and Km_O was 0.4848 g/L, corresponding to $y(M) = 1.0076x + 2.1472$ and $y(O) = 1.1086x + 2.287$, respectively. The double reciprocal curve is shown in Fig. 4. The results showed that Km_M is less than Km_O, similar to a previous study by Pei *et al.*[7]. This indicates that the substrate affinity of immobilized enzymes with PEG-modified chitosan as a carrier is larger than of enzymes with ordinary chitosan as a carrier.

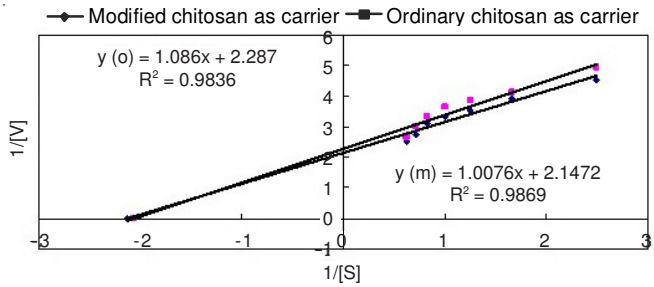

Fig. 4. Michaelis-Menten constant of immobilized cellulase

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