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Macroporous Poly(Glycidyl Methacrylate-co-Divinylbenzene) Polymer Particles for the Immobilization of β-galactosidase from *Aspergillus Oryzae*

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ABSTRACT

Macroporous polymer particles containing surface epoxy groups were synthesized using glycidyl methacrylate(GMA) as monomer, divinylbenzene(DVB) as crosslinking agent, and dodecyl alcohol and cyclohexanol as porogenic agents by suspension copolymerization for immobilization of β-galactosidase.The Scanning electron microscopy (SEM) micrographs were done to characterize their surface structure. Under the optimum conditions, β-galactosidase was immobilized on the carrier obtained above, and satisfactory results were obtained in enzyme activity, pH stability, thermal stability, and operational stability. The conclusion obtained indicated that the ploy(GMA-co-DVB) was suitable to immobilize β-galactosidase from aspergillus oryzae.

Keywords:glycidyl methacrylate, divinylbenzene, β-galactosidase; immobilization; poly(GMA-co- DVB).

INTRODUCTION

β-D-galactosidase galactohydrolase(EC 3.2.1.23)from *Aspergillus oryzae*, which efficiently catalyzes not only the hydrolysis of the β-galactoside linkages of lactose to glucose and galactose but also the transgalactosylation reaction to produce galactooligosaccharides, could be immobilized on various supports by different methods. The most common methods including: adsorption, entrapping, cross-linking and covalent-blinding[1-4]. The immobilized enzyme made by adsorption was relatively little in the loss of enzyme activity, but was not sold in the combination and the practical value was small; The immobilized enzyme made by entrapping had higher activity recovery, but was easy to lose activity during the reaction time. The cross-linking was difficult to control reaction conditions, though it had good stability and reusability. The high-level structure of enzyme protein was changed when the reaction was intense[5]; currently, the covalent-blinding is the most popular method of enzyme immobilization. The immobilized enzyme made by ovalent-blinding had solid connection with

carrier, good stability and repeatability, which could prevent the activity of enzyme degeneration[6].

Among all the materials used to immobilize enzyme, epoxy activated carriers seemed to be almost ideal systems to develop very easy protocols for enzyme immobilization, because epoxy group could exhibit good reactivity under mild conditions and would be very stable at neutral pH values even in wet conditions and it's also easy to be modified because of its active epoxy group. In this study, the novel macroporous poly(GMA-co-DVB) copolymer beads was synthesized firstly as enzymeimmobilization matrix with a mixture of dodecyl aleohol and cyclohexanol as pore-forming agent and azobisisobutyronitrile(AIBN) as initiator by inverse suspension polymerization technique, ant the surface structure of it were characterized by Scanning electron microscopy (SEM),under the optimum conditions, the copolymer was used to immobilize β-galactosidase *Aspergillus oryzae,* the enzyme activity was determined and the characteristics of the immobilized enzyme including pH stability, thermal stability, operational stability was also determined and compared with those of the free enzyme in order to examine the suitability of the supporter acquired from liquid and solid porogen to immobilize enzyme.

EXPERIMENTAL SECTION

Reagents and Apparatus

Glycidyl methacrylate(GMA) was acquired from Shanghai Jinchao Chemical Co. Ltd. Divinyl benzene(DVB) (45%) was acquired fromTianjin Guangfu Fine Chemical Industry Research Institute. β-galactosidase from Aspergillus oryzae (11.2U/mg) and o-nitrophenyl-β-D-galactopyranoside (ONPG) were acquired from Sigma.Azo-bis-isobutyronitrile(AIBN) and other reagents were all analytical grades.

Ultraviolet Spectrotometer (T6 New Century), Vacuum Pump with Circulated Water Syst-em(SHZ-D(^{0}C),Vacuum Desiccator (DZ-6020), Digital pH Meter (PHS-3C) and Water Co-nstant Temperature Oscillator (SHA-B) used for the study.

Preparation of Enzyme and Substrate Solution

0.0400g of β-galactosidase was immersed in10mL 0.1M citric acid buffer (pH 4.0), then the enzyme solution was kept in the refrigerator for use. The substrate solution was acquired by dissolving 0.0150g ONPG in 10mL distilled water.

Preparation of poly(GMA-co-DVB)

The macroporous copolymer of GMA/DVB was synthesized by inverse suspension copolymerization technique. Typical procedure: 3.5mL of porogenic agent was prepared by mixing 1.5mL dodecyl aleohol and 2mL cyclohexanol. The porogenic agent was added to a mixture of solution (4.5mL GMA as monomer and 2.3mL DVB as agent) in which the free radical initiator AIBN (0.0395g) was dissolved. The mixture was degassed and homogenized by ultrasonication for 20 min and then was placed in a 40^{0}C water bath temperature oscillator to prepolymerize for 24 hours. A mixture of 55mL PVA(2%) and 55mL glutin(0.1%) was added into a four-necked flask equipped with a thermometer, reflux condenser, stirrer and nitrogen inlet bute. The prepolymer was added into the mixture solution which was stirred to 55^{0}C under nitrogen. Then the reaction mixture was allowed to proceed at 65^{0}C for 3h and at 85^{0}C for 2h. The beads formed were filtrated and washed with water completely, and they were soaked in ethanol for 24h to remove the porogenic agent, and then dried in vacuum for 5h.

Method of immobilization

The immobilization of β-galactosidase on poly(GMA-co-DVB) copolymer was carried out by adding an amount of polymer particles (0.0500g) to 0.5mL 0.1M citric acid buffer (pH 4.0) containing enzyme (4mg/mL). The reaction was undergone in ultrasonic cleaning machine at 25^0C for 3 hours. Then the immobilized enzyme was filtered and washed with distilled water and 0.1M citric acid buffer (pH 5.0) until no protein was detected. The enzyme bound on the carrier was called the immobilized enzyme.

Testing of enzyme activity

Activities of free and immobilized β-galactosidase were tested according to the references[7,8]: the citric acid buffer (pH 5.0) was added into 0.1mL of free enzyme or 0.0500g of immobilized β-galactosid-ase for 2 min, then added 0.2mL of ONPG (1.5mg/mL) as the substrate at 55^0C. After exactly 15 min of incubation, the reaction was stopped by addition of 2mL 1M Na_2CO_3 solution, and the absorbance was measured directly at 420nm. The activity yield was calculated as the ratio of immobilized enzyme to enzyme subjected to immobilization. One unit of β-galactosidase activity is defined as the amount of enzyme that liberated 1μmol of product per minute under fixing condition.

Discussion about the carrier obtained

According to the methods described above, the carrier was got and the scanning electron micrographs of the dried polymer were obtained using KYKY-2800B scanning electron microscope. According Fig.1.SEM micrographs, the apparent morphology of the carrier with macroporous surface was exhibited. The carrier was used to immobilize β-galactosidase under its optimum conditions, the activity of the immobilized enzyme reached a maximum of 247.2U/g dry carrier.

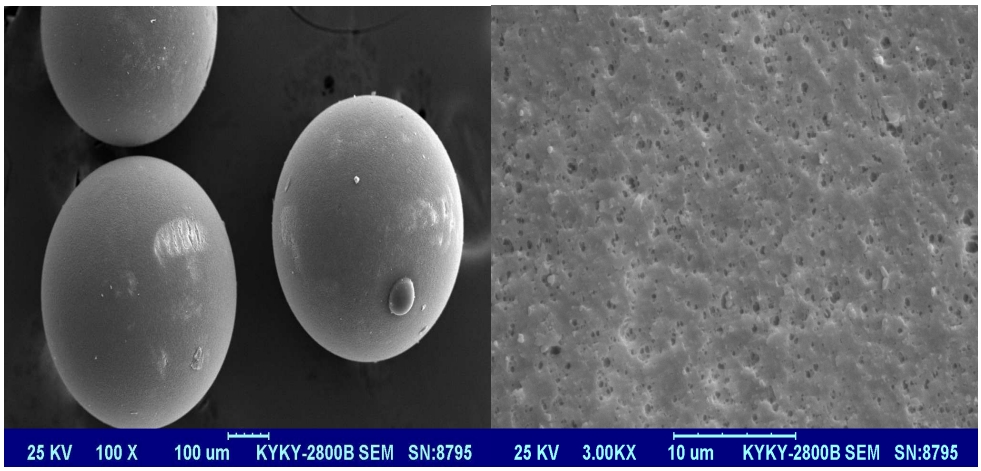

Fig.1 Scanning electron micrographs of the support

Properties of the immobilized enzyme

Optimum Temperature

The free enzyme and immobilized enzyme activities were determined by ONPG as substrate at various temperatures (40-65^0C), the result was exhibited in Fig.2. The optimum temperature of the free and the immobilized enzymes were both 55^0C.

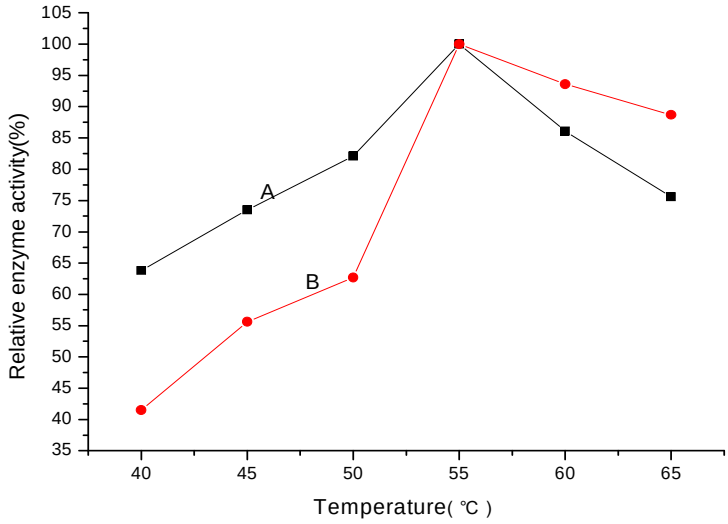

Fig. 2. Effect of temperature on the activity of the free and immobilized enzymes
(A: Free enzyme; B: Immobilized enzyme)

Thermostability

According Fig.3 and Fig.4 ,we could see that the immobilized enzyme was more stable than the free enzyme. At 50^0C, more than 71.3% of immobilized β-galactosidase remained active after 8h, while the activity of the free enzyme was 50.9%. As the same, over a period of the same time, the residual activity of the free enzyme was 48.2% at 60^0C, whereas that of the immobilized enzyme was 58.1%.The experiment result proved that the immobilization remarkably enhances the heat resistance of β-galactosidase.

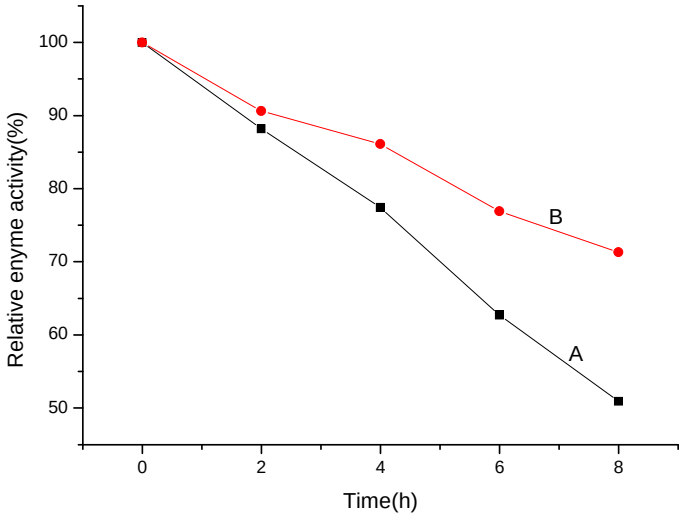

Fig.3 thermostability of different enzyme at 50^0C
(A: Free enzyme; B: Immobilized enzyme)

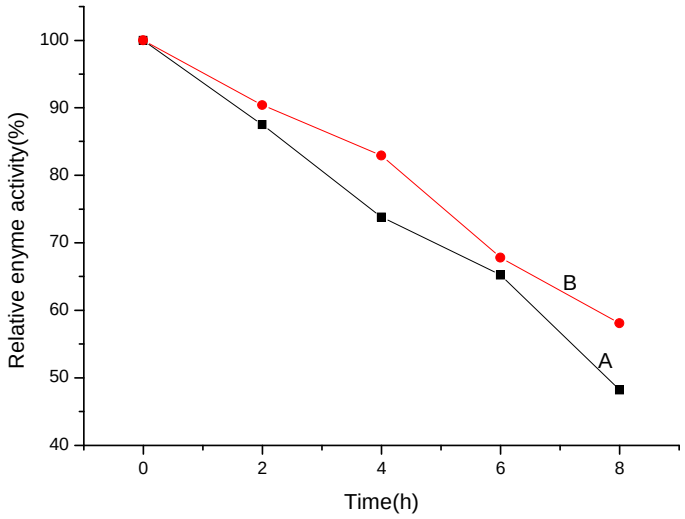

Fig.4 thermostability of different enzyme at 60^0C
(A: Free enzyme; B: Immobilized enzyme)

pH Optima

In Fig.5 the effect of pH values of free and immobilized enzymes were both determined in 3.0-10.0 pH range and the result showed that the pH profile of the free enzyme peaked at pH 5.0. Similar pH was also found for the immobilized enzyme.

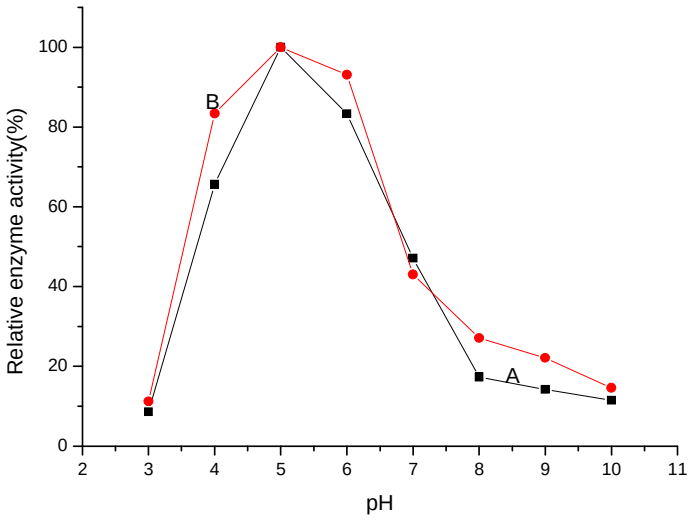

Fig. 5. Effect of pH on the activity of free and immobilized enzymes
(A: Free enzyme; B: Immobilized enzyme)

pH stability

The free and immobilized enzymes were both exposed to different pH (2.0-9.0) at room temperature for 1h, and then the enzyme activities were determined with ONPG as substrate. The curve showed in Fig. 6 fully illustrated that the immobilized enzymes hold better adaptability than free enzyme at 3.0-9.0 pH region.

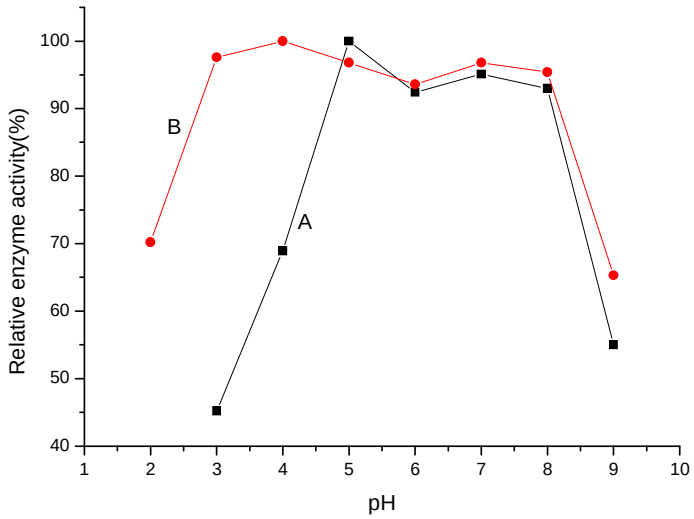

Fig. 6. Effect of pH on the stability of free and immobilized enzymes
(A: Free enzyme; B: Immobilized enzyme)

Operational stability of immobilized enzyme

The experiment was repeated 7 times at the same initial concentration of ONPG by using the procedures mentioned with the same immobilized enzyme .The experiment results illustrated that the immobilized β-galactosidase was still retained above 95% of the original activity without significant loss in activity, which were summarized in Fig.7. It meant that, in the course of the reaction, almost no enzyme was dissociated from the surface of the Poly(GMA-DVB) carrier , which indicate that the immobilized enzyme obtained was very stable.

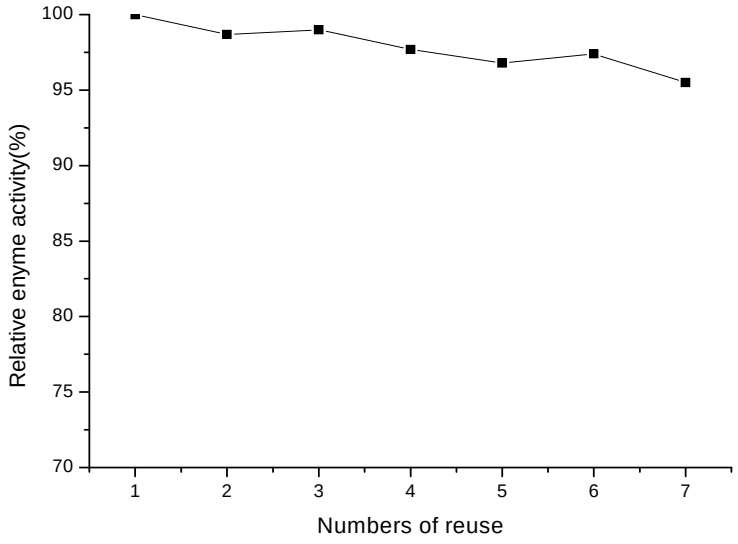

Fig. 7. Operational stability

CONCLUSION

In this paper, the reactive, macroporous poly(GMA-co-DVB) was obtained using glycidyl methacrylate(GMA) as monomer, divinylbenzene(DVB) as crosslinking agent via suspension

copolymerization. Under the optimum conditions, β-galactosidase from *Aspergillus Oryzae* was immobilized on the carrier described above and the basic properties of the immobilized enzymes were determined and compared with those of the free enzymes, the results of the immobilized enzyme which were obtained in pH stability, thermal stability and operational stability were satisfactory. All the achievements described above illustrated that the poly (GMA-co-DVB) obtained here was valuable as enzyme immobilization carrier for industrial application.

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