Journal of Molecular Catalysis B: Enzymatic 126 (2016) 18–23

Contents lists available at ScienceDirect

Journal of Molecular Catalysis B: Enzymatic

journal homepage: www.elsevier.com/locate/molcatb

Enhancement of glucose isomerase activity by immobilizing on silica/chitosan hybrid microspheres

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ARTICLE INFO

Article history:
Received 21 December 2015
Received in revised form 16 January 2016
Accepted 26 January 2016
Available online 4 February 2016

Keywords:
Glucose isomerase
Immobilization
Silica/chitosan hybrid microspheres
Activity
Stability

ABSTRACT

Glucose isomerase (GI) plays a crucial role in the food industry as it serves as a catalyst for the conversion of glucose to fructose. Immobilized GI is often used due to increased stability as well as the expensive costs associated with free GI. In this study, GI was immobilized on silica/chitosan hybrid microspheres via simple process through in situ encapsulation. Enhanced rate of reaction was observed when the conversion of glucose to fructose was completed in 10 min catalyzed by immobilized GI because most GI was located on the shell of the support. Moreover, it was found that immobilized GI exhibited better pH, temperature, ions, storage and operation stability when compared to free GI. The relative enzyme activity was found to be above 90% with a wide pH range of 5.8–8.0, temperature range of 40–80 °C, storage range of 3 months and an increase in operation range of >15 times. Therefore, immobilized GI supported by silica/chitosan hybrid microspheres is an ideal candidate for biocatalysis.

1. Introduction

Glucose isomerase (GI), also known as D-xylose isomerase, is a very important water soluble enzyme commonly applied in drinks and various products in the food industry. GI is known to catalyze the conversion of many kinds of monosaccharides, such as D-glucose, D-xylose and D-ribose. Isomerization of glucose to fructose, is an indispensable process for the industrial production of high-fructose corn syrup (HFCS), main sweetener in many soft drinks and food [1,2]. The isomerization process should be performed in the pH range of 7.0–9.0 and temperature range of 70–80 °C in the presence of Mg^{2+} and Co^{2+} due to the reactive requirements of GI [3]. Additionally, the high price and difficulty in recycling of GI also lead to an increased cost associated with the production of HFCS. Comparatively, immobilized GI is usually used to increase the stability of GI against environmental changes and recycled to reduce the cost associated with the production process. Adsorption of enzyme on the carriers is usually the preferential immobilization method compared to other methods including embedding, co-valent binding and crosslinking mainly due to the simple process. However, some of the complications may arise with the method including a weak interaction between the carrier and the enzyme contributing to the loss of enzyme.

The stability of enzyme against environmental change is one of the most important properties for immobilized enzyme used in practical applications, resulting in more attention to improve or widen the stability range. So it is necessary to choose suitable carriers depending on the associated properties to make active sites of enzyme exposed and stabilized since it plays a crucial role in the catalytic performance and stability of immobilized enzyme. Various types of carrier materials have been reported and can mainly be divided into two kinds, polymeric materials (e.g., calcium alginate [4], GAMM [5], chitosan [6–8]) and inorganic materials (e.g., gold [9], TiO_2 [10], perovskite [11] and SiO_2 [12–14]). Even though the inorganic carriers are much more stable and of higher mechanical intensity, their interaction with enzyme through adsorption is weaker and usually results in the loss of enzyme. Chang et al. [14] prepared immobilized cellulase using mesoporous silica nanoparticles as carriers. Large loss percentage of cellulase from carriers was observed even though the yield of glucose was equal. Wu et al. [10] immobilized sucrose isomerase on mesoporous TiO_2 through adsorption to improve properties of enzyme. Better stability was obtained for immobilized sucrose isomerase compared to the free enzyme, however, the stability in reuse still needs to be improved. The immobilization of GI on the silica gel through adsorption has been previously studied in Song et al.'s work [15]. High catalytic activity was obtained in 45 min, however,

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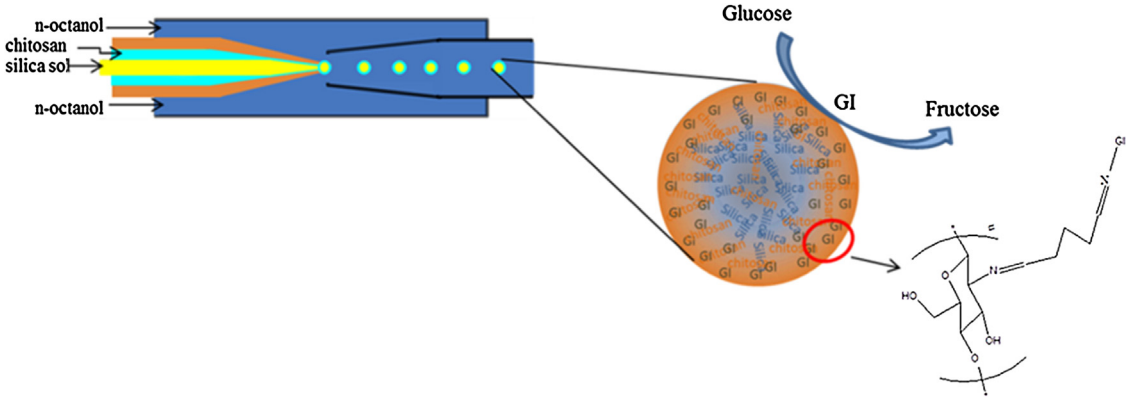
http://dx.doi.org/10.1016/j.molcatb.2016.01.013
1381-1177/



Scheme 1. The generation process of microspheres.

the catalytic activity was found to decrease largely in the recycle use. Another group of researchers were trying to address this problem by using surface-modified calcium alginate to adsorb GI to get much more stable recycle use, however, the stability range of pH and temperature was narrow [4]. High enzyme activity could be obtained only under the pH of 7.5 and temperature of 60 °C. Moreover, chitosan–polyacrylic acid hybrid microspheres were prepared as the GI carriers to improve the stability of pH, temperature and operation [16]. Although it was found that the immobilized GI had better operation stability due to crosslinking after GI adsorption, the process was complicated and dependent on the presence of ions with low enzyme activity. Therefore, it is necessary to develop a simple approach to prepare immobilized GI with higher enzyme activity and wider stability range against the environmental changes.

Silica/chitosan core–shell hybrid microspheres with good mechanical intensity and strong interaction with metal ions were prepared using microfluidic technology in our previous work [17]. The silica/chitosan supported catalyst can decrease mass-transfer resistance effectively while increasing reaction velocity of the mass-transfer limited reaction process due to most catalyst combined with chitosan was located on the shell of microspheres. Moreover, chitosan has outstanding properties of biocompatibility, adsorption and being environment-friendly, it is beneficial for the exposure of active sites of enzyme and interaction with enzyme strongly [18]. Hence, silica/chitosan core–shell hybrid microspheres can be an ideal carrier candidate to immobilize GI. Therefore, the immobilized GI was prepared simply by in situ encapsulation of GI in the silica/chitosan hybrid mic rospheres in this work. High yield was obtained in 10 min using immobilized GI as catalyst and the stability range of pH, temperature and storage was expanded to a large extent without dependence on metal ions. Furthermore, the immobilized GI can be recycled for many times with high catalytic activity.

2. Experimental

2.1. Materials and chemicals

Aqueous solution (10.0 g) of chitosan(0.20 g) with degree of deacelation below 95% (Sinopharm Chemical Reagent Co., Ltd., Beijing, PR China) dissolving in acetic acid (0.20 g, VAS Chemical Co., Ltd., Tianjin, PR China) was served as the middle fluid. Polymer aqueous solution (10.0 g) with tetraethoxysilane (TEOS) (0.20 g) dissolving in acetic acid (0.20 g) was used as the inner fluid whereas *n*-octanol (VAS Chemical Co., Ltd., Tianjin, PR China) as the continuous phase. *n*-Octane (10.0 g) with glutaraldehyde (0.040 g) and Span 80 (0.20 g, VAS Chemical Co., Ltd., Tianjin, PR China) was used as the solidification bath in which glutaraldehyde was served as the cross-linking reagent. Glucose isomerase from *Streptomyces rubiginosus* (Zhengzhou Zhongxin Chemical Reagent Co., Ltd.) and D-glucose (Beijing Chemical Works) were used as purchased and all of the reagents were analytically or chemically pure.

2.2. Preparation of silica/chitosan hybrid microspheres supported GI

The preparation procedure of silica/chitosan hybrid microspheres was described in detail in our previous work [17]. Dual co-axial microfluidic device was used, as shown in Scheme 1. 2.0 g of glucose isomerase powders were dissolved in 100 mL of deionized water and then centrifuged to get the supernatant as GI solution. 1.80 g of aqueous solution with 2.0 wt.% chitosan and 2.0 wt.% acetic acid was mixed with 0.20 g GI solution, which was used as middle fluid. Silica sol, the inner fluid, was obtained by stirring the aqueous solution with 2.0 wt.% acetic acid and 2.0 wt.% TEOS for 12 h at room temperature. They were dispersed into droplets by the shearing force of continuous fluid consisting of *n*-octane at the intersection of the microchannel. The droplets were collected out of the microchannel in the solidification bath consisting of *n*-octanol with 0.5 wt.% glutaraldehyde and 2.0 wt.% Span 80. The droplets were pre-solidified into microspheres through the Schiff's base reaction between —CHO from glutaraldehyde and $—NH_2$ from chitosan and GI and the extraction of water out of droplets by octanol. The microspheres were pre-solidified for a certain time (15–45 min) in the solidification bath and then washed with *n*-octane followed by a submersion for 24 h to gelate the silica sol. The silica/chitosan supported GI was then obtained after freeze-drying.

The immobilized GI prepared by adsorption of GI onto the microspheres were also prepared for comparison in this work. First, silica/chitosan hybrid microspheres were prepared according to the procedure mentioned above, with the exception that no GI solution was added in the middle fluid. Then silica/chitosan hybrid microspheres were incubated in the GI solution placed in a water-bath shaker under 25 °C, 130 rpm for 24 h for GI adsorption. The microspheres were then separated and washed with deionized water to remove the remnant GI followed by immersion in glutaraldehyde solution (0.2 wt.%) under 25 °C for 30 min enabling crosslinking between chitosan and GI for further immobilization. Lastly, the microspheres were washed with buffer solution to remove the residue glutaraldehyde and stored in fridge under 4 °C after freeze-drying.