Available online at www.sciencedirect.com
ScienceDirect
Journal of Molecular Catalysis B: Enzymatic 42 (2006) 14–19
JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC
www.elsevier.com/locate/molcatb

Hollow silica nanotubes for immobilization of penicillin G acylase enzyme

Qing-Gui Xiao [a], Xia Tao [b], Jie-Ping Zhang [a], Jian-Feng Chen [a,b,*]

[a] *Research Center of the Ministry of Education for High Gravity Engineering & Technology - Beijing, University of Chemical Technology, Bei San Huang Dong Road 15, Beijing 100029, China*
[b] *Key Lab for Nanomaterials of the Ministry of Education - Beijing, University of Chemical Technology, Beijing 100029, China*

Received 15 February 2006; received in revised form 17 May 2006; accepted 31 May 2006
Available online 18 July 2006

Abstract

In this study, porous hollow silica nanotubes (PHSNTs) synthesized via a sol–gel route using nano-sized needle-like $CaCO_3$ the inorganic templates were employed as a support for immobilization of penicillin G acylase (PGA) biocatalyst. The produced PHSNTs were characterized by BET and transmission electron microscopy (TEM). Effect of various factors such as loading temperature and ratio of carries to free PGA (g/mL) on the catalytic activity of the immobilized PGA was also investigated by unitary factor testing method. The results show that under optimized conditions the relative loading amount and the total activity yield of immobilized enzyme (IME) amounts to 97.20% and 88.80%, respectively. Several advantages, i.e. the rapid immobilization of PGA onto PHSNTs, the high tolerability to the pH, the less sensitivity to the temperature and the improved storage stability render PHSNTs potential support materials for enzyme immobilization.

Keywords: Enzyme immobilization; Hollow silica nanotubes; Needle-like $CaCO_3$; Penicillin G acylase

1. Introduction

Penicillin G acylase (PGA) with molecular dimensions of 70 Å × 50 Å × 55 Å can catalyze the cleavage of the amide bond in the benzylpenicillin (penicillin G) side-chain specifically and release phenylacetic acid and 6-aminopenicillianic acid (6-APA), among which 6-APA is an important precursor for the synthesis of many semi-synthetic penicillin antibiotics [1–4]. Nevertheless, isolated enzymes in biocatalytic processes suffer from some drawbacks, i.e. weak enzyme stability under operational conditions as well as difficult recovery and reuse cycles [5–7]. Therefore, more efforts have been devoted to the development of carriers to immobilize this kind of industrially valuable biocatalyst [8,13].

To improve catalytic efficiency of PGA, various matrices such as inorganic and organic materials have been studied for the immobilization of the enzyme catalyst [8–11]. Singh et al. used agar-polyacrylamide resins to immobilize PGA from *E. coli* NCIM 2563 [12]. Bianchi et al. described a new procedure for the immobilization of an industrial PGA by covalent coupling on the poly (enthacrylic ester) resin [13]. Danica Mislovičová et al. utilized concanavalin A-bead cellulose to immobilize of PGA [14]. Compared with organic supports, inorganic supports are more stable and against from organic solvents and microbial attacks [15]. However, limited pore size of the conventional inorganic materials, for example, Al_2O_3, SiO_2, microporous zeolites, and mesoporous molecular sieves of the MCM-41s family, makes the bulky enzymes and substrates difficult to diffuse into the narrow channel of supports [16–18]. In this paper, we choose porous hollow silica nanotubes (PHSNTs) synthesized via a sol–gel route using needle-like $CaCO_3$ nanoparticles as a support to immobilize the PGA biocatalyst. The structure of PHSNTs and the loading kinetics of PGA onto PHSNTs were investigated in more detail. Also, the activity and stability of immobilized PGA were discussed.

2. Experimental

2.1. Materials

Needle-like $CaCO_3$ templates were prepared by a unique high gravity reactive precipitation (HGRP) technology accord-

* Corresponding author. Fax: +86 10 64434784.
E-mail address: chenjf@mail.buct.edu.cn (J.-F. Chen).

1381-1177/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.molcatb.2006.05.011

ing to the method previously reported by us [19–21]. PGA was purchased from Hiader Company, Zhejiang, China. All chemicals used in the experiments were obtained from commercial sources as analytical reagents without further purification. Distilled water was used throughout the study.

2.2. Preparation of PHSNTs

PHSNTs were synthesized via a sol–gel route as follows: 10 g of needle-like $CaCO_3$ nanoparticles were added to 200 mL of diluted ethanol solution ($V_{ethanol}/V = 3:2$). The resulting suspension solution was dispersed by a KQ-100 ultrasonic device with a power of 100 W. Fifty milliliters of ammonia solution (25%) and 2.2 g of cetyltrimethylammonium bromide were added into the $CaCO_3$ suspension solution at room temperature under vigorous stirring, followed by the addition of tetraethylorthosilicate. Afterwards, the slurry was further stirred, filtered, rinsed, dried and calcinated to obtain a core-shell structured composite containing $CaCO_3$ and SiO_2. The composite was then dissolved into HCl (10 wt.%) to remove the $CaCO_3$ template completely. The resulting gel was again filtered, rinsed and dried to obtain PHSNTs.

2.3. Enzyme immobilization

PGA was immobilized on the PHSNTs as follows: 1 mL of enzyme solution (800 U/mL) and a certain weight (0.1–1.0 g) of support were immersed in 50 mL of distilled water. The mixture of solution was stirred and rinsed with distilled water. The wet immobilized enzyme (IME) was in stock for subsequent enzyme activity assay.

2.4. Enzyme activity assay

Enzymatic catalytic activity of the immobilized biocatalysts was determined by titrating phenylacetic acid. The process of enzyme activity assay was as follows: 100 mL of 2% (w/v) solution of penicillin G potassium was kept at 28 °C in the thermostatic bath and titrated with 0.25 mol/L NaOH solution to pH 8.0. Then wet IME (or free enzyme) was immersed in the solution above. The mixture of solution was automatically titrated with 0.25 mol/L NaOH solution at constant pH 8.0. The specific activity of enzyme was measured according to the following equation: IME $A\ (U/g) = V_{NaOH}C_{NaOH} \times 10^3/Wt$, in which V_{NaOH} (mL) is the volume of NaOH solution consumed, C_{NaOH} (mol/L) is the concentration of NaOH solution, W (g) is the weight of immobilized PGA, t (min) is the reaction time and the unit of U is μmol/min. Unless otherwise noted, the total relative activity is the ratio of the activity of immobilized PGA on PHSNTs to the total activity of initial PGA solution.

2.5. Measurement of enzyme loading kinetics of PGA on the supports

The loading kinetics of PGA on the supports was measured as follows: 1 mL of PGA solution was first diluted by 49 mL of distilled water and then a certain weight of support was added in the diluted solution under vigorous stirring. The 200 μL of the supernatant liquid was taken out at the given time intervals and then subjected to Bradford assay [22,23]. Spectrophotometer operated at a wavelength of 595 nm was used to determine the concentration of enzyme from the supernatant liquid.

Transmission electron microscopy (TEM) was performed by a JEM-2010F. The concentration of PGA solution was detected by a Shimadzu UV 2501 spectrometer at a wavelength of 595 nm. An ASAP 2010 surface area analyzer was used to determine BET surface area (S_{BET}) and pore size (D_{pore}) distribution of the PHSNTs samples.

3. Results and discussion

3.1. Characterization of the PHSNTs

Fig. 1 shows TEM images of $CaCO_3$ templates with needle-like structure and the PHSNTs prepared by using $CaCO_3$ as

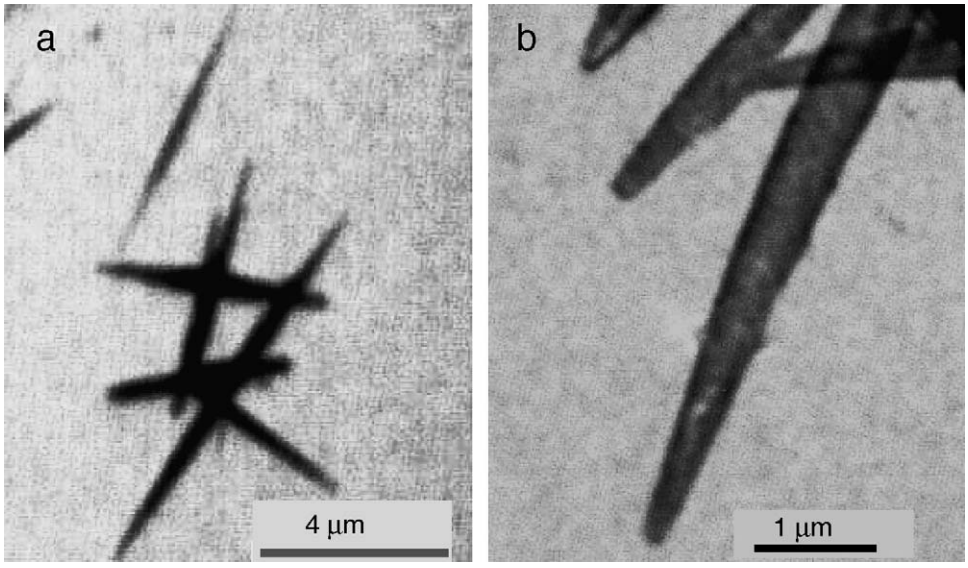

Fig. 1. TEM images of needle-like $CaCO_3$ templates (a) and porous hollow silica nanotubes (b).