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Preparation of a novel nanobiocatalyst by immobilizing penicillin acylase onto magnetic nanocrystalline cellulose and its use for efficient synthesis of cefaclor

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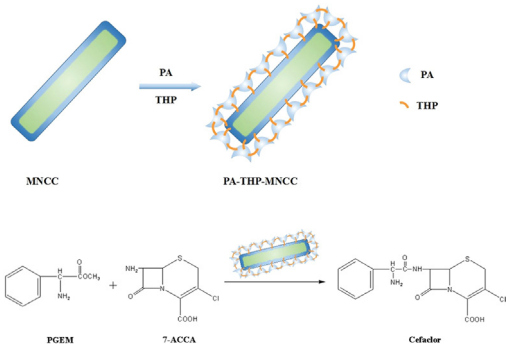
HIGHLIGHTS

- Magnetic nanocrystalline cellulose (MNCC) was prepared and used as enzyme support.
- The tri(hydroxymethyl)phosphine (THP) was using as a novel coupling agent.
- The PA-THP-MNCC achieved higher enzyme loading and better catalytic performance.
- The prepared biocatalyst exhibited good performance for the synthesis of cefaclor.

GRAPHICAL ABSTRACT



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ABSTRACT

Magnetic nanocrystalline cellulose (MNCC) was prepared and used as an enzyme support for the immobilization of penicillin acylase (PA). A novel coupling agent, tri(hydroxymethyl)phosphine(THP) instead of the conventional glutaraldehyde(GA), was used as a crosslinker in this study. The obtained results showed that the immobilized PA with THP (PA-THP-MNCC) had high enzyme loading (172.3 mg/g) and activity recovery (77.6%) in the optimal preparation conditions, which were remarkably superior to those of the counterpart using GA (PA-GA-MNCC, 148.4 mg/g and 48.7%, respectively). Compared with free PA and PA-GA-MNCC, PA-THP-MNCC displayed a higher optimum pH and temperature, and manifested relatively higher enzyme-substrate affinity and catalytic efficiency. In addition, PA-THP-MNCC exhibited significantly enhanced stability. Furthermore, PA-THP-MNCC was successfully employed for synthesis of cefaclor, an important second-generation cephalosporin antibiotic, affording a significantly higher yield of 84% than that reported previously.

Abbreviations: MNCC, magnetic nanocrystalline cellulose; PA, penicillin acylase; GA, glutaraldehyde; THP, tri(hydroxymethyl)phosphine; PA-THP-MNCC, immobilized PA with THP; PA-GA-MNCC, immobilized PA with GA; 6-APA, 6-aminopenicillanic acid; 7-ACCA, 7-amino-3-deacetoxycephalosporanic acid; S/H ratio, synthesis/hydrolysis ratio

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1. Introduction

Cefaclor is a second-generation semi-synthetic cephalosporin antibiotic. It has strong antimicrobial activity against both Gram-positive and Gram-negative bacteria [1]. The main methods of industrial preparation of cefaclor are established by Lilly company [2]. These synthesis processes synthetic processes are complicated and environmentally harmful. Enzymatic synthesis attracts much attention for its mild reaction condition, high specificity, environmental protection. But there exist many obstacles to apply free enzyme in industrial production due to its disadvantages such as high cost, poor operational stability, and difficulties in recovery and reuse [3]. In this case, the immobilized enzyme exhibits great potential in the industrialization synthesis reaction of cefaclor [4].

Penicillin acylase (EC 3.5.1.11, PA) was first found from *Penicillium Shrysogenum* in 1950 [5]. It can hydrolyze penicillin G and cephalosporin to β-lactam nuclei-6-aminopenicillanic acid (6-APA) and 7-amino-3-deacetoxycephalosporanic acid (7-ACCA), respectively. In addition, penicillin acylase can also catalyze the synthesis of semi-synthetic β-lactam antibiotics with a high catalytic efficiency and mild reaction conditions. PAs have been widely used in the industrial production of semi-synthetic β-lactam antibiotics [6,7]. However, further application of free enzymes is restricted heavily due to a number of disadvantages such as high cost, poor operational stability, and difficulties in recovery and reuse. Immobilization of enzymes can effectively solve these obstacles [8]. Choosing an appropriate immobilization carrier and method is the key issue of it.

Recently, our group used a simple coprecipitation-cross-link technique to prepare a novel biobased nanocomposite: magnetic nanocrystalline cellulose (MNCC) [9]. MNCC had high surface-to-volume ratio, aspect ratio, and satisfactory biocompatibility. Moreover, MNCC can easily be separated under a magnetic field. All of these properties make the MNCC be a suitable enzyme carrier, and it was successfully used as a carrier for lipase and papain [10,11]. However, using this novel nanomaterial as an enzyme carrier for Penicillin acylase immobilization requires further study. It is of great interest to explore the potential of MNCC to act as support for immobilization of penicillin acylase.

Cross-link is a frequently-used method of enzyme immobilization owing to its high enzyme loading and strong binding force between enzyme and carrier, and the most commonly used coupling reagent is glutaraldehyde(GA)[12]. However, as the excessive crosslinking of the glutaraldehyde upon enzyme and the reversibility of the Schiff base linkage, it creates many inherent difficulties. This leads to a reduced activity recovery of immobilized enzyme [13]. Tri(hydroxymethyl) phosphine (THP) has become a novel coupling reagent for enzyme immobilization in recent years [14]. THP containing > P-CH_2-OH groups are well known to undergo Mannich-type condensation reactions at room temperature with N-H group-containing compounds, giving aminomethyl phosphines (> P-CH_2-N). This P-CH_2-N linkage is very stable towards hydrolysis [15], and this coupling reagents can preferentially react with nonessential NH_2 groups on the enzyme to minimize enzyme inactivation. Furthermore, the P-CH_2-N linkage is more flexible than the Schiff base linkage, and it has been suggested for the maintenance of higher levels of catalytic activity [16].

In the present study, the synthesis of cefaclor by an immobilized biocatalyst was investigated (Scheme 1). Firstly, PA was successfully immobilized onto MNCC by using a new coupling agent THP. Secondly, a comparative study of free PA, PA-GA-MNCC and PA-THP-MNCC was performed. Moreover, the effects of several key factors on the biocatalytic process were explored systematically.

2. Experimental section

2.1. Materials

Cellulose microcrystalline was purchased from Sinopharm Chemical Reagent Co., Ltd. (China). Ferric chloride hexahydrate ($FeCl_3{\cdot}6H_2O$) and ferrous chloride tetrahydrate ($FeCl_2{\cdot}4H_2O$) were obtained from Guangzhou Chemical Reagent Co., Ltd. Penicillin acylase was purchased from Zhejiang Shunfeng Haider Co., Ltd.

2.2. Preparation of magnetic nanocrystalline cellulose (MNCC)

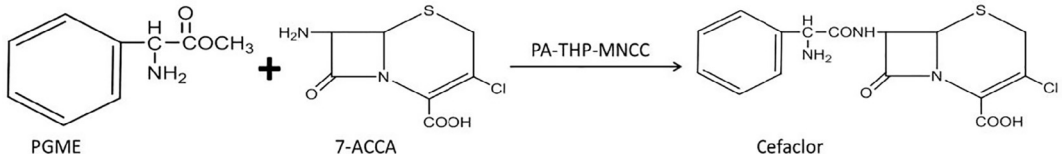
The preparation of MNCC was based on the method reported previously with some modifications [9]. In a typical experiment, 10 g of microcrystalline cellulose was mixed with 250 mL of 6 M HCl solution and then stirred at 90 °C. After continuous hydrolysis for 90 min, the compound was cooled at 4 °C to stop the reaction and washed with deionized water (centrifuged at 4000 rpm for 5 min) for 5 cycles later. After centrifugation, the product was dialyzed against deionized water until neutral pH was reached. So far, the cellulose nanocrystalline (CNC) was well prepared. 6*g* of CNCs dispersed in 100 mL distilled water and mix with 100 mL aqueous solution including 5. 36*g* of $FeCl_2{\cdot}4H_2O$ and 13. 6*g* $FeCl_3{\cdot}6H_2O$. 30 mL acetic acid buffer solution (1%) and chitosan (0.15*g*) were added to the suspension with mechanical agitation for 60 min. Then, a suspension of sodium tripolyphosphate (TPP, 0.3*g*) and 20 mL NH4OH solution (28%) was added to the solution with slow stirring for 40 min at 80 °C. Subsequently, the MNCC were washed followed by centrifugation five times and then stored in buffer solution.

2.3. Immobilization of penicillin acylase onto MNCC

The immobilization of PA on MNCC using glutaraldehyde and THP was carried out by two similar procedures.

For immobilization coupling with glutaraldehyde, 0.1 g MNCC was stirred with 50 mL of glutaraldehyde of a given concentration at room temperature for 1 h. after incubation, the MNCC was then washed to remove excess glutaraldehyde using distilled water for three times. The activated MNCC were incubated with given concentrations of penicillin acylase after washing with deionized water three times. The mixture was subsequently agitated at room temperature for 4 h and then stayed at 4 °C overnight. The uncross-linked enzyme was removed by washing with distilled water until no protein was detected by the Bradford method. The washing solutions were collected to detect the amount of uncross-linked penicillin acylase. The amount of immobilized penicillin acylase loaded on the MNCC was calculated as the difference between the initial and the un-crosslinked penicillin acylase.

Different with using glutaraldehyde as a coupling agent, THP needs to be synthesized prior to coupling. THP solution was synthesized from tetrakis(hydroxymethyl)phosphonium chloride(THPC) and a 0.995 M equivalent of KOH. For instance, in order to get a 3.75 mg/ml solution of THP, 0.465 g of 80% THPC was added to 95 mL of water. KOH



Scheme 1. Enzymatic synthesis of Cefaclor.