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Facile preparation of Fe_3O_4@MOF core-shell microspheres for lipase immobilization

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ABSTRACT

In this article, a Fe_3O_4@MOF core-shell microsphere has been successfully prepared by growing MIL-100(Fe), three dimensional (3D) metal–organic frameworks (MOFs) onto Fe_3O_4 nanoparticles. The resultant composite microsphere exhibited both magnetic characteristics and large specific surface area, making them excellent candidates for enzyme immobilization. *Candida rugosa* lipase was immobilized as a model enzyme on core-shell microspheres. The results showed that the immobilized enzyme for hydrolysis of olive oil retained >65% of its initial activity at 65 °C over 6 h; and the residual activity still remained about 60% of the initial activity after the 10th catalysis run. Therefore, such MOF based core-shell magnetic composite microspheres showed promising applications as an enzyme immobilization support.

1. Introduction

Enzymes are a particular nature's sustainable biocatalyst, which displays high chemical reactivity and selectivity while operating under ambient conditions [1]. Enzymatic processes are more green and cost-effective and therefore benefit particularly in the synthesis of pharmaceutics, fragrances, vitamins and other fine chemicals [2,3]. Lipases (E.C. 3.1.1.3) are ubiquitous enzymes with various biological activities such as enantioselectivity besides the high catalytic efficiency in the process of triacylglycerols hydrolysis, transesterification and synthesis of esters [4-6]. Notwithstanding all these advantages, the practical use of lipase in their native form is often hampered by a paucity of long-term operational stability, complex down-stream processing and difficult recovery and reuse [6,7]. One of the approaches to overcome these drawbacks is to immobilize enzymes on solid supports which can provides several advantages such as enhancement of stability, easy separation, convenience of reusability of enzyme, modification of substrate selectivity and enantioselectivity, and multi-enzyme reactions [8,9]. Moreover, the activity and stability of the enzymes are influenced by the immobilization protocol such as immobilization method, binding sites concentration on the support surface, and immobilization conditions [10-12].

Among immobilization methods, covalent binding of an enzyme to a carrier, where covalent bonds are formed with the enzyme, has the advantage that the enzyme is tightly fixed against leaching from the support surface in the reaction systems [1,13]. The metal–ion affinity interaction is an easy method of immobilization by invoking the zeta potential of the support and the opposite charges of enzymes, that can be varied to provide optimum condition for immobilization [14,15]. In addition to the normal benefits, immobilization can also lead to enhancement of the catalytic activity of the lipase, probably caused by conformational changes in the lipase molecules, opening of the lid to make the active site accessible, in analogy with the interfacial activation caused by a hydrophobic substrate phase [12,13,16,17].

Among a variety of promising supports, magnetic nanoparticles (MNPs), mostly magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3), offer many exciting opportunities for the easy separation and repeated use [17-19]. Fe_3O_4 nanoparticles are a promising candidate that the nanoparticles exhibit superparamagnetism with high saturation magnetic moment. However, bare Fe_3O_4 nanoparticles are very sensitive to oxidation and provide less active sites for the fixation of enzymes [20,21]. Metal–organic frameworks (MOFs) are highly ordered crystalline materials in which metallic canters are bridged via organic multitopic ligands to yield low density network structures of diverse topologies [22]. MOFs have attracted considerable attention in recent years due to their tunable pores and functionalities as well as high specific surface areas. These features are pretty attractive in the supports of immobilization of enzymes in

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that the functional sites coordinate enzymes and their pores accommodate enzymes for high immobilization efficiency. The building of a core-shell structure Fe_3O_4@MOF mircosphere is supposed to integrate their features into one system and allowed the elegant design to incorporate a bunch of functions, which will be beneficial for the convenient conjugation of lipase. Though varieties of materials with core-shell structure have been employed on the basis of zeolites [23], mesoporous oxides [24], polymers [10], and carbon materials [25] as biocatalyst, the structure of magnetic MOFs was rarely utilized as the support for enzyme immobilization. The present technique needs the surface modification with mercaptoacetic acid (MAA) [26], which is highly toxic and usually generates toxic gases when encountering some oxidants. It turns out to be that the present technique is inappropriate to be used as the support for the immobilization of enzyme.

So far, few investigations about the core-shell structure Fe_3O_4@MOF as the support of enzyme immobilization have been reported. Herein, we reported a facile, efficient and environmentally friendly approach to prepare the magnetic MOF composite microspheres, Fe_3O_4@MIL-100(Fe), by incorporation of carboxyl-functionalized Fe_3O_4 nanorods with nanocrystals of MIL-100(Fe), a three dimensional (3D) MOF with hierarchical pore size. To extend the potential applications, the pore walls were modified by post-synthetic modification. *Candida rugosa* lipase was immobilized via different routes to show the excellent immobilization capacities, easy separation from solutions by a magnetic field, high enzymatic catalytic activity, reuse, storage stability, tolerance to temperature and pH.

2. Materials and methods

2.1. Materials

Ferric chloride ($FeCl_3{\bullet}6H_2O$), sodium acetate (NaAc), ethylene glycol (EG) and ethylenediaminetetraacetic acid disodium salt (EDTA-2Na) were purchased from Tianjin Guangfu Fine Chemical Industry Research Institute (China); Benzene-1,3,5-tricarboxylic acid (H_3btc), *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (EDC•HCl, 99%), *N*-hydroxysuccinimide (NHS, 97%), *Candida rugosa* lipase (CRL, Type VII, 1180 units/mg solid) and bovine serum albumin (BSA) were supplied from Sigma Chemical Co.; Other chemicals obtained from Tianjin Chemical Reagent Company (China).

2.2. Preparation of mesoporous carboxyl-functionalized Fe_3O_4 nanoparticles

The mesoporous carboxyl-functionalized Fe_3O_4 nanoparticles were synthesized and characterized as described in our previous report [27]. Firstly, 0.8 g of $FeCl_3{\bullet}6H_2O$ was dissolved in 40 mL ethylene glycol to form a clear solution. Then, 2.0 g of anhydrous NH_4OAc and 0.5 g of EDTA-2Na were added to the resulting dispersion. The mixture was stirred vigorously until it became homogeneous and then transferred into a Teflon-lined stainless-steel autoclave (100 mL capacity). The autoclave was heated to 200 °C and maintained for 12 h. Then it was cooled to room temperature. The obtained black products were washed repeatedly with ethanol and deionized water and dried in a vacuum oven at 60 °C for 12 h.

2.3. Preparation of magnetic Fe_3O_4@MIL-100(Fe) microspheres

The magnetic Fe_3O_4@MIL-100(Fe) microspheres were synthesized by a modified one-pot assembly procedure [28,29]. In terms of the following procedure: 0.05 g carboxyl-functionalized Fe_3O_4 (prepared as above) was dispersed in 4 mL of $FeCl_3{\bullet}6H_2O$ ethanol solution (10 mM), and then 4 mL of H_3btc ethanol solution (10 mM) was added with constant stirring for 10 min. After stirring at 70 °C for 2 h, the product was collected by magnetic separation and washed with ethanol three times. Then the product was reintroduced into a fresh $FeCl_3{\bullet}6H_2O$ ethanol solution and H_3btc ethanol solution for further growth reaction. This process was repeated five times to increase the thickness of the MIL-100(Fe) shell, and the samples were recovered using a permanent magnet and washed with ethanol, and dried in a vacuum oven at 60 °C for 12 h.

2.4. Lipase immobilization

The magnetic Fe_3O_4@MIL-100(Fe) microspheres were first activated by EDC/NHS mixture or Zn^{2+} solution, respectively, according to the method reported in our previous study [27,30]. These activated supports were used to immobilize *Candida rugosa* lipase by covalent bonding (strategy I) and metal–ion affinity interactions (strategy II), respectively (Fig. 1). The obtained magnetic supports (50 mg) were dispersed in 5 mL phosphate buffer solution (50 mM, pH 6.0) by sonication. After separation, the supports were added into 5 mL lipase solution, and then the mixed solution was incubated at 30 °C in a shaker with continuous shaking rate (120 rpm) for 6 h. The immobilized lipase were separated by magnetic separation and washed five times with 0.1 M phosphate buffer solution. The collected reaction solution and washing solution were collected to assay the amount of residual enzyme. The immobilized lipase was stored at 4 °C for activity tests and noted as C-ICRL (immobilized by covalent bonding) and M-ICRL (immobilized by metal-ion affinity interactions), respectively.

2.5. Lipase activity assay

The amount of protein in the enzyme solution and the protein concentration were determined by the Bradford method, using BSA as a standard [31]. The catalytic activities of free and immobilized lipase were assayed by the titration of the fatty acid, which results from the hydrolysis of olive oil and reverse titration method under the action of lipase [32]. One unit of lipase activity is defined as the amount of enzyme that released 1 μmoL of fatty acid per minute under assay conditions. The maximum activity is defined as 100%, and the relative activity refers to the percentage that an enzyme activity accounts for the highest one. Immobilized yield is calculated as the ratio between the enzymatic units expressed by the immobilized enzymes *versus* the enzymatic units loaded on the carrier [6]. The expressed activity (%) was the ratio between the activity of immobilized lipase and the activity of free lipase. All the activity measurement experiments are carried out in triple.

2.6. Characterization

Powder X-ray diffraction (XRD) patterns were collected using an X-ray diffractometer (Rigaku D/MAX-2400 X-ray diffractometer with Ni-filtered Cu Kα radiation). The morphologies were investigated by transmission electron microscopy (TEM, FEI Tecnai G20) and scanning electron microscopy (SEM, Hitachi S-4800). Fourier transform infrared spectroscopy (FTIR) spectra were recorded by a Fourier-transform infrared spectrophotometer (American Nicolet Corp. Model 170-SX) using the KBr pellet technique. The magnetic properties of the products were studied with a vibrating sample magnetometer (LAKESHORE-7304, USA) at 300 K. The Brunauer-Emmett-Teller (BET) specific surface areas were measured with a Micromeritics ASAP 2010 M instrument. Thermogravimetric analysis (TGA) was observed by a TG-DSC apparatus (NETZSCH STA 449C) by heating the samples from room temperature to 800 °C under N_2 atmosphere at a heating rate of 20.0 K min^{-1}. All the characterization of the carrier was measured under dry conditions.

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