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Novel enzyme/exfoliated bentonite nanohybrids as highly efficient and recyclable biocatalysts in hydrolytic reaction

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ABSTRACT

Bentonite exfoliation liberated the interlayer surface with large area for immobilization of enzyme at high enzyme loading. Upon the electrostatic attraction between positively charged bovine pancreatic lipase (BPL), *Yarrawia lipolytica* lipase (YLL), trypsin and negatively charged surface of unilaminar bentonite, three nanohybrids with a stably card-like structure named as BPL-B, YLL-B and TB were facilely produced. These nanohybrids showed higher activities in hydrolysis as 115.2% (BPL-B), 154.8% (YLL-B) and 138.2% (TB) of that of the correspondingly free enzyme. Furthermore, BPL-B, YLL-B and TB retained 80%, 90% and 82% of each original activity after repeated use for 10, 12 and 8 times, respectively. The recyclable and active nanohybrids are expected to be widely applied as industrial catalysts in the future.

1. Introduction

Nowadays, enzymatic catalysis has been regarded as a green and sustainable technology in fine chemistry [1], pharmaceuticals [2], energy [3] and environmental technology [4]. However, the disadvantages of non-recycle, instability and prone to deactivation hampers the application of enzymes [5]. These drawbacks can be overcome through enzyme immobilization [1]. Nonetheless, enzyme immobilization generally leads to the decline of enzymatic activity arising from steric hindrance, enzyme structure distortion and mass transfer resistance on the support [1,6,7]. Thus, how to keep or improve the activity of immobilized enzymes has always been a difficult task for enzyme immobilization technology. During the past decades, nanobiocatalysts based on integration of enzymes on nanomaterials, such as silica nanoparticles [8,9], polymer biomimetic materials [10], nano-inorganic minerals [11] and self-assembled nanomaterials [12], have provided new options for enhancing the performance of immobilized enzymes in different applications, but their preparative processes are always difficult and time-consuming.

Bentonite, a natural and biocompatible mineral with a lamellar structure [13], is also a good candidate for enzyme immobilization relying on the advantages of large specific surface area, strong mechanical strength and adsorptive capacity, thermal stability and chemical inertness [14–16]. However, due to the larger dimension of enzyme than the interlayer spacings of original and modified bentonites (about 1.2–2.2 nm), previous studies mainly focused on the adsorption of enzymes onto the external surfaces of bentonites [17–19], which only occupied a small part of their total surface areas, causing low enzyme loading and activity. Furthermore, the weakly adsorbed enzymes were easily dissociated from bentonite's external surface under stirring, resulting in the decrease of catalytic stability. Although the intercalation of excessive polymers could enlarge the interlayer spacing of layered clays for accommodation of protein molecules [20,21], the resulting steric hindrance and diffusion limitation would easily reduce enzymatic performance of immobilized enzymes. Therefore, preparing an efficient and recyclable immobilized enzyme on bentonite is too difficult to be accomplished.

In this study, by using a green and facile method constituting of bentonite exfoliation and subsequent enzyme adsorption, three highly efficient nanohybrids of enzyme/exfoliated bentonite with a stably card-like structure were produced. The enzyme loading efficiencies, catalytic activities and stabilities were measured. The mechanisms for the high enzyme loading and superiorly catalytic performance of these immobilized enzymes were also clarified.

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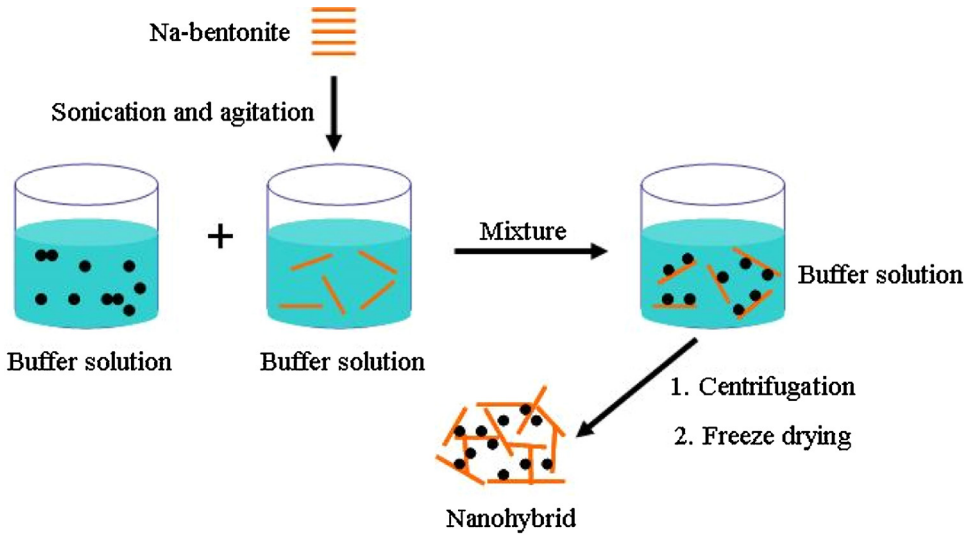

Scheme 1. Schematic illustration for preparation of the enzyme/exfoliated bentonite nanohybrid.

2. Experimental

2.1. Materials

Bovine pancreatic lipase (BPL), *Yarrawia lipolytica* lipase (YLL), trypsin and *N*-benzoyl-L-arginine ethyl ether (BAEE) were purchased from J&K chemicals in China. Bovine serum albumin, *p*-nitrophenyl palmitate (*p*-NPP) were purchased form Sigma-Aldrich. Sodium bentonite was obtained from Sanding technology Co. Ltd. in Zhejiang province. All other chemicals were of analytical grade.

2.2. Exfoliation of bentonite

A certain amount of sodium bentonite was added into deionized water at concentration of 20 mg mL^{-1}. A 250 mL conical flask containing 100 mL of this suspension was placed in an untrasonic apparatus at room temperature, then the suspension was stirred at 2000 rpm and treated by ultrasonication at 40 KHz simultaneously. Therein, each run of ultrasonication treatment was conducted for 0.5 h and paused for 10 min subsequently. After 5 cycles, the whole exfoliation process was over, and the obtained exfoliated suspension was diluted into 1 mg mL^{-1} of colloidal solution with addition of acetate buffer (pH 4.0, 10 mmol L^{-1}) for further use.

2.3. Preparation of nanohybrids on bentonite

Three enzymes including BPL, YLL and trypsin were separately dissolved in acetate buffer (pH 4.0) at concentration of 1 mg enzyme mL^{-1}, and 50 mL of each enzyme solution was mixed with the exfoliated bentonite suspension (1 mg mL^{-1} in acetate buffer, pH 4.0) at the same volume. Every mixture was agitated at 200 rpm for 12 h under ambient condition, and three nanohybrids including BPL-B, YLL-B and TB were subsequently prepared after centrifugation (12,000 rpm, 10 min), washing and freeze drying.

2.4. Zeta potential measurement

BPL, YLL and trypsin were respectively dissolved into acetate buffer (pH 4.0, 10 mmmol L^{-1} in ultrapure water) at concentration of 1 mg mL^{-1}, and the suspension of exfoliated bentonite at the same concentration was also prepared in pH 4.0 acetate buffer (10 mmmol L^{-1} in ultrapure water). The sample cell was washed by acetate buffer for three times before sample injection. The measurements for every sample were conducted in triplicate by using a Nano ZS90 particle-sizer (Malvern, Worcestershire, UK).

2.5. Enzymatic activity assays

The hydrolysis of *p*-NPP by BPL and YLL, and the hydrolysis of BAEE by trypsin were used as the model reactions to determine the activities of these free and immobilized enzymes. The details were shown in Supplementary material.

2.6. Reusabilities of nanohybrids

The reusabilities of these nanohybrids of BPL-B, YLL-B and TB were investigated by repetition of the corresponding activity assay as described in Supplementary material (Section 1). Between two consecutive assays, the nanohybrids were collected by centrifugation (10,000 rpm, 5 min) and washed with the buffer used in the activity assay twice. The residual activity was determined by comparison with the first running (activity defined as 100%).

2.7. Catalytic kinetics of nanohybrids and free enzymes

The kinetic parameters, K_m (mmol L^{-1}) and V_{max} (U g^{-1} enzyme min^{-1}), of free and immobilized BPL and YLL were calculated from the Michaelis-Menten models via Lineweaver-Burk using varying concentrations of *p*-NPP from 0.01–0.2 mmol L^{-1} in the aqueous medium. By the same method, K_m (mmol L^{-1}) and V_{max} (U mg^{-1} enzyme min^{-1}) of free and immobilized trypsin were determined using varying concentrations of BAEE (0.05–0.3 mmol L^{-1}).

3. Results and discussion

3.1. Preparation of enzyme/exfoliated bentonite nanohybrids

The preparative process for enzyme/exfoliated bentonite nanohybrids were composed of the exfoliation of layered bentonite and the subsequent adsorption of enzyme on the unilaminar bentonite, as shown in Scheme 1. The unilaminar layers of bentonite with the length between 20 and 200 nm were obtained after exfoliation, which were explicitly shown in Fig. 1(A). The layered bentonite showed the diffraction peak ($2\theta = 7.30^\circ$) referring to its