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Short communication

Structured interlocked-microcapsules: A novel scaffold for enzyme immobilization

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ABSTRACT

A facile approach to prepare structured interlocked-microcapsules (SIMC) was developed, which combined the advantages of open mouthed structure, hierarchical porous nanostructure and interlocked architecture. The specific surface area of SIMC was 374.6 m^2/g and the diameter of the pores was 8.707 nm. Nitrile hydratase (NHase) was immobilized on SIMC via covalent bonding to realize the easy separation of the enzyme and improve properties of enzyme such as pH tolerance and heat stability. This work demonstrated that the enzyme-immobilized SIMC presented high catalytic performance and significantly improved stability.

1. Introduction

In recent years, mesoporous microcapsules have been widely concerned owing to their combination of hollow structures and mesoporous nanostructures [1–3]. Some of them like mesoporous silica nanoparticles included SBA, MCM and MSU have been widely used in preparing catalysts [4]. Due to their superior physicochemical properties, mesoporous microcapsules are particularly pursued as enzyme immobilization supports [5,6]. In general, both the microcapsule wall and the capsule lumen can be used for immobilizing enzyme. But up to now, most of the research encapsulated enzyme molecules in the capsule lumen, and the capsule wall only plays the role of preventing the leakage of enzyme molecules. What's more, the dense, thick capsule wall and the stagnant solution in the capsule lumen would cause the high transfer resistance between the capsule lumen and the bulk solution, and then the reaction rate would be influenced [7,8]. If the enzyme molecules were immobilized on the capsule wall, once the substrates come in contact with or diffuse through the capsule wall, the catalytic reaction would occur. Thus the catalytic efficiency will be significantly improved by increasing the contact probability of substrate and enzyme and shortening the mass transfer path. In addition, the special physical effects provided by the pores of capsule wall can endow the immobilized enzyme with improved chemical, biochemical, and mechanical properties. Therefore, it is urgent to design novel wall material according to the advanced concept of nanoarchitectonics, which was proposed for the assembly of structural materials at the nanoscale level [9]. Recently, a concept of open-mouthed microcapsules reactor was proposed for electrochemical and gaseous catalytic applications. Like the function of the cell membrane, the structure makes full use of the inner and outer wall of the microcapsule by forming open-mouth on the capsule wall. Benefiting from the unique structure, the capsule lumen is exposed and mass transfer resistance is also decreased [10]. This concept provides a new opportunity for the construction of robust enzyme catalysts. Parallel to the development of the new catalyst pellets with macropores, structuring of the catalyst is also an efficient way to enhance the effective mass diffusivity [11]. The structured catalysts are considered to be a promising research direction in the field of heterogeneous catalysis [12,13]. In our previous study, three-dimensional ordered macroporous biocatalysts were successfully prepared [14], and superior catalytic performance was obtained.

Hence, it can be conjectured that setting up a structured biocatalyst composed of open-mouthed microcapsules (or the structured interlocked-microcapsules, SIMC) could enhance the catalytic performance of the immobilized enzyme significantly. However, to the best of our knowledge, no investigation of preparing SIMC for enzyme immobilization has been previously reported. Thus, for the first time, we present a facile approach to prepare novel structured interlocked-microcapsules with interconnected ordered structure for enzyme immobilization. Subsequently, the SIMC was functionalized with amine groups and activated with glutaraldehyde (GA) which is one of the most widely used reagents in enzyme immobilization [15]. GA could reacted with amine groups(both in the carrier or enzyme molecules) rapidly at around neutral condition [16], on this account, the NHase could be

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Nomenclatures

SIMC	Structured interlocked-microcapsules
PSCCT	Polystyrene colloidal crystals templates
PBS	Phosphate buffer solution
NHase	Nitrile hydratases
SIMC-NH_2	Amino-functionalized SIMC
APTES	3-Aminopropyltriethoxysilane
GA	Glutaraldehyde

immobilized in SIMC. It is likely to be the most common form of the immobilized enzyme with GA [17,18].

2. Experimental

2.1. Preparation of SIMC

The preparation process of the structured interlocked-microcapsules (SIMC) was shown in Scheme 1 (SEM images of all the steps were provided in Supporting Information, Fig. S1). Firstly, polystyrene colloidal crystals templates (PSCCT) were soaked in concentrated sulfuric acid for 2 h at room temperature. The reaction of sulfonation was taken place at 40 °C under stirring for 12 h. After the reaction was completed, the PSCCT was washed completely with ultra-pure water.

Then the sulfonated PSCCT (1.5 g) was immersed in an excess amount of TEOS for 5 h to allow the saturated absorption of TEOS. Subsequently, a mixture of ethanol/water (1:1 v/v) was added to form silica-based capsule wall through sol-gel process. After being aged and dried at 60 °C for 12 h, the composites were calcined to remove the template, and finally the SIMC was obtained.

2.2. Preparation of immobilized NHase and stability measurement

Briefly, SIMC was dispersed in ethanol, and then APTES was added slowly. The reaction was performed under reflux overnight. Thereafter, the solid was washed three times and then dried at 60 °C under vacuum for 10 h. The obtained solid was named SIMC-NH_2. Subsequently, SIMC-NH_2 was activated with GA (100 mg/mL) for 1.5 h and immersed in NHase solution for 4 h.

To assess the pH stability of free and immobilized NHase, their activities were measured after incubating in pH 4.0 and pH 10.0 sodium phosphate buffer solution (PBS) for different times at 30 °C. To determine thermal stability, both the free and immobilized NHase were immersed in 40 and 50 °C PBS and the activity was assayed at regular time intervals.

Storage stability of free and immobilized NHase was also tested by immersing them in pH 7.0 PBS at 4 °C. At regular time intervals, the samples were taken out to measure the residual activities.

2.3. Determination of kinetic parameters

Taking equal activity (1.441 U) of free and immobilized NHase reacted with different concentration of substrate range from 2 to 15 mM in pH 7.0 at 30 °C and the reaction system was maintained 2 mL. After 2 min, the reaction rate was determined by HPLC. The Km and Vmax values were calculated by the plotting method of Lineweaver-Burk, and Kcat was obtained from the formula: Kcat = Vmax/[E].

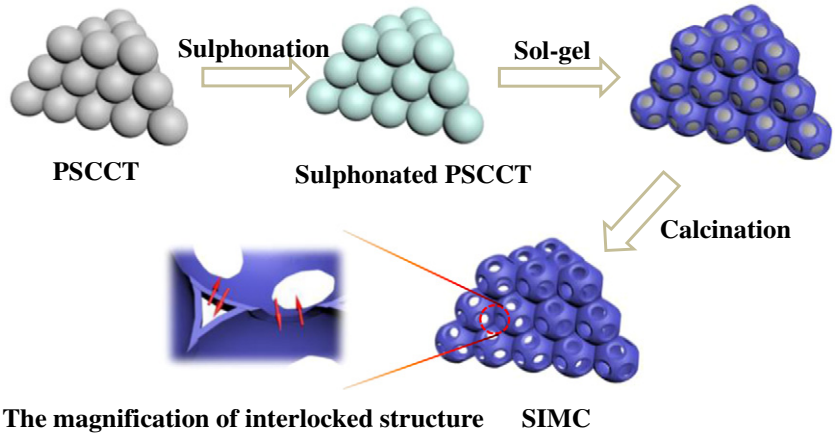
3. Results and discussion

3.1. Characterization of the SIMC

Fig. 1a shows that the SIMC is composed of vast regularly shaped microcapsules which have unique particle size of 400 nm. The macropores (open mouths) can be clearly observed on the capsule wall which stemmed from the point of contact between template microspheres. Owing to the SIMC is fabricated by replicating PSCCT which has a hexagonal close-packed structure [19], twelve macropores could be obtained on each microcapsule wall in theory. The microcapsule can highly coalescent through the edge of macropores (marked with red arrow) and further present a grape-like network which is internal interconnected. TEM image demonstrated the hollow structure and interlocked architecture of the SIMC as expected (Fig. 1b).

3.2. The pH and thermal stability of free and immobilized NHase

For enzyme immobilization, the amino-functionalized SIMC(SIMC-NH_2) was prepared by post grafting of 3-aminopropyltriethoxysilane (APTES) on SIMC. The SIMC-NH_2 was activated via GA and then the GA-activated SIMC-NH_2 was used to immobilize NHase through covalent bonding approach (the detailed immobilization process can be found in ESI)·The pH stability of free and immobilized NHase were shown in Fig. 2a, the immobilized NHase was inactivated at a slower rate than the free NHase. After 3 h of incubation in pH 4.0, the immobilized NHase maintained 93.85% of its initial activity. In contrast, free NHase only retained 11.74% of its initial activity. Similar behavior was also observed at pH 10.0. This can be attributed to the intense multipoint covalent attachment between enzyme molecule and SIMC via a short spacer arm, which will lead to significantly improved



Scheme 1. Schematic synthesis process of the structured interlocked-microcapsules.