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Immobilization of lipase on chemically modified bimodal ceramic foams for olive oil hydrolysis

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ABSTRACT

The work reported in this paper is aimed at exploring the feasibility of immobilizing alkali lipase from *Penicillium expansum* on a bimodal ceramic foam, which has both macro- and micro-pore structures. After being chemically modified with a silane coupling agent, the ceramic foam was used as a support for lipase immobilization. To determine the preferable immobilization conditions, the effects of the amount of enzyme for loading, immobilization time, temperature, and pH on enzyme activity were investigated. The results showed that the chemically modified ceramic foam has a high loading capacity and a strong binding ability for the lipase. Thanks to the low internal mass transfer resistance, the ceramic foam has greatly enhanced the rate of immobilization. As a comparison, the immobilized-lipase activity was much higher than that on many frequently used porous materials like diatomite, alumina and activated carbon.

1. Introduction

Lipases (glycerol ester hydrolases E.C.3.1.1.3) are a family of enzymes that catalyze the hydrolysis of fats in their natural environment and constitute an important class of high selectivity and stereo-specificity industrial enzymes [1]. Lipases find useful applications in hydrolysis of fats and oils [2,3], synthesis of many organic intermediates for polymer synthesis [4–6], and preparation of esters for food, cosmetic and pharmaceutical industry [1,7] especially for diesel engine fuels (biodiesel ester) [8]. In many cases, the low catalytic efficiency and stability of native enzymes are considered as main barriers for the development of their large-scale applications [9]. Whereas immobilized lipase generally have good thermal stability and reusability. Moreover, the immobilization of enzyme minimizes the cost of product isolation and provides operational flexibility.

Many supports have been studied including polymers and resins [1,9,10], silica and silica–alumina composites [11–13], and carbonaceous materials [14–16]. These systems generally have a low mechanical strength and often exhibit severe diffusion limitations, leading to a relatively low enzymatic activity [17,18]. To minimize the internal diffusion limitation, porous supports are mostly used in particulate form [1,19]. The size of the carriers is even on the micron scale and cannot be used in fixed-bed reactors [20]. In viewpoint of industrial applications, novel monolithic supports in the form of foams, fibers, membranes and honeycombs have been developed to take the place of traditional granular ones [21,22]. Due to having high mechanical strength and good durability, porous ceramics are often used as supports for enzyme immobilization [22–26]. Macrostructured foam-like ceramics were more favorable primarily because of its low diffusion limitations [16]. In this work, a unique macrostructured ceramic foam with a bimodal pore size distribution was prepared using our patented methodology [27]. In industrial field, it can be potentially applied in monolithic fixed-bed reactors, which greatly facilitate continuous operation and product isolation [28].

For effective enzyme immobilization, the surface of porous ceramics often coated with a carbon layer [16,17,29]. It should be noted that the carbon layer may increase the preparation cost because of the complex procedures to produce the carbon layer [10]. On account of the relatively high surface hydrophobicity of lipases, simple adsorption of lipases on suitably hydrophobic supports has been the more popular strategy over covalent conjugation methods [30,31]. This method [32] has proven very useful to achieve hyperactivation of most lipases: the hydrophobic surface of the matrix induces the conformational change on lipases necessary to enable free access of substrates to their active centers. To obtain suitable hydrophobicity, the surface of inorganic supports may be modified with silane coupling agent [33]. Since the ceramic pore surface with a coating layer possessing methacryloyloxy group was able to immobilize lipase on successfully [34], our ceramic foams for lipase immobilization were first modified with a silane coupling agent also possessing methacryloyloxy group.

In oleochemical industry, the most important application of lipase is to produce fatty acids from oils by hydrolysis [2]. Partial

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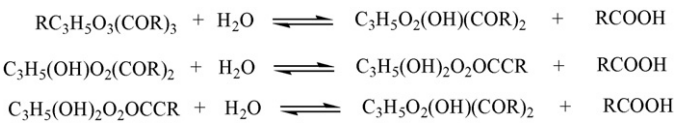

Fig. 1. Reactions of triglyceride hydrolysis catalyzed by lipases.

hydrolysis of triglycerides will yield mono- and diglycerides and fatty acids (Fig. 1). When the hydrolysis is carried to completion, the mono-, di- and triglycerides will hydrolyze to yield fatty acids and glycerol. The traditional method of hydrolysis involves the use of high temperature, pressure and chemical catalysts [35]. Thereupon, enzyme-catalyzed hydrolysis is recently attempted as an energy-saving method, especially for producing high value-added products or heat sensitive fatty acids [36,37]. The focus of this work is to investigate the optimal conditions for the preparation of immobilized lipase and its catalytic properties both in view of hydrolysis activity. An emulsifier was used to overcome the problem of intimate contact between substrate and enzyme [38,39]. On the base of this study, further work can be done in designing and developing a new monolithic fixed-bed reactor for the production of fatty acids.

2. Materials and methods

2.1. Materials

Alkaline lipase from *Penicillium expansum* (2950 U/g lipase) was purchased from Shenzhen Leveking Bio-engineering Co. Ltd., China. Coomassie brilliant blue G250, γ-(methacryloxy)propyhrimethoxy silane (A-174), oxalic acid, ethyl alcohol, 95% ethyl alcohol, sodium hydrogen phosphate, monopotassium phosphate, sodium hydroxide, crystal bovine serum albumin, and sodium chloride were all of analytical grade. Polyethylene glycol (PEG) (WM 1750 ± 50) and olive oil were of chemical grade. The concentration of phosphoric acid was ≥85%. The ceramic foam is prepared by the following method: First, the ceramic raw materials (20 g alumina, 8 g kaolin and 22 g feldspar all in the form of powder) were put into 100 g water, and then 5 g ethanol (95%) was added. After stirring, the suspension of the raw materials was obtained. The flocculating agent, 100 g polyacrylamide (0.3% mass concentration) was dropped into the suspension under agitation. The raw particles in suspension gradually became floccules with water in between them. The floccules were then added into a matrix in the shape of the desired ceramic foam to prepare the earthen ceramic base. By the process of drying and sintering, the water in the ceramic base was removed and cellular structure of the ceramic foam was formed in situ. More detailed procedures are from related literatures [27,40]. In this work, a ceramic cylinder of 100 mm in diameter and 10 mm in thickness was fabricated as shown in Fig. 2. The ceramic foam was treated subsequently by hot concentrated hydrochloric acid. After this treatment, the nanopores can be enlarged to some degree. The pore size distribution of the ceramic foam was measured with the mercury porosimeter, Autopore IV 9500 (Micromeritics Intrement Corp., USA).

2.2. Chemical modification of ceramic foam

The ceramic foam cylinder was cut into cubic forms of 5–10 mm in each edge length as the enzyme supports. The weight of each support was controlled at 200–250 mg. An aqueous alcohol solution was prepared at 1:1 (v/v), and oxalic acid was added into the solution to adjust its pH to 3.5–4.0. A-174 was then put into it to obtain a modification solution at 0.2% (wt%). The supports (total mass ≤5 g) was put into the modification solution (40 ml). In each run, the total amount of A-174 was not less than 1% (wt%) of supports. The mixture was shaken in a 30 °C water bath at 100 rpm for 5 h. After that, the support was washed for several times with alcohol and deionized water separately, and then dried at 110 °C for at least 8 h in an oven. Finally, the modified ceramic foam was cooled to room temperature in a silica gel drier for experimental use.

2.3. Immobilization of lipase

First the amount of crude lipase powder was determined by the total mass of the supports to be used and the enzyme amount per gram of support, generally 12.5 g lipase/g support. Then so much lipase powder was put into a 0.025 M and pH 8.0 phosphate buffer (PBS), to make the enzyme suspension of 0.333 g lipase powder/ml PBS. The enzyme suspension was stirred every 10 min for 30 min, and then centrifuged at 1000 rpm. The resultant supernatant was filtrated with filter paper for further purification. Subsequently, the modified ceramic foams were impregnated in the lipase filtrate at 20 °C and 150 rpm for 4 h. Finally, the ceramic foams with adsorbed lipase were thoroughly rinsed with PBS for three to five times and dried at room temperature for at least 24 h.

2.4. Determination of lipase activity

The enzyme activity of free and immobilized lipase was measured by the classical olive oil emulsion method [41]. Emulsion of olive oil was produced by emulsifying 50 ml olive oil and 100 ml 4% PEG solution (40 g PEG/1000 ml water) at 17,000 rpm.

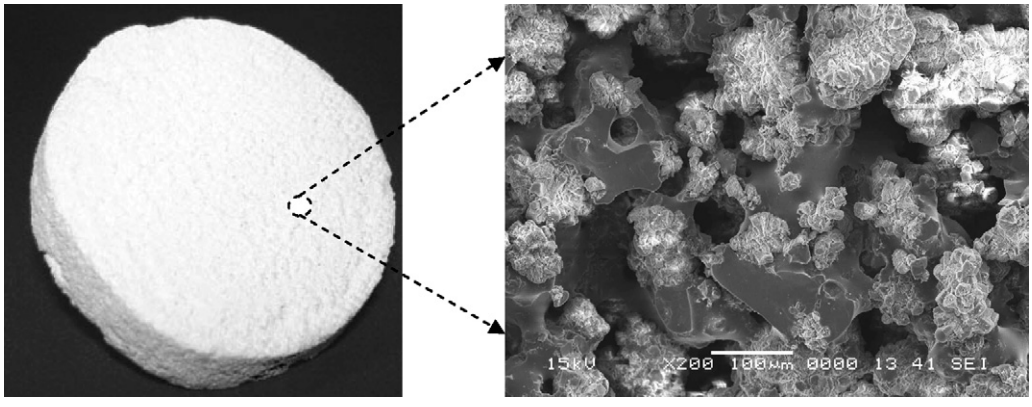

Fig. 2. Photograph of ceramic foam in the shape of cylinder with 100 mm diameter and 10 mm thickness (left) and scanning electronics microphotograph (SEM) of ceramic foam treated by HCl (right).