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Synthesis, characterization and optimization of a two-step immobilized lipase

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ABSTRACT

Lipase (*Candida* sp. 99–125) was immobilized by a two-step process, adsorption and subsequent entrapment. The formation mechanism of immobilized lipase was investigated by SEM and EDS. With the support of diatomite, the internal network of immobilized lipase showed more porosity and was fully developed. The dimension of the network was more uniform in the present of gelatine. The maximum biodiesel yield was 92% at 40 °C in a 24 h reaction. The biodiesel yield was maintained at about 70% after 11 repeated batch reactions. This enzyme immobilization method has potential applications in biodiesel and other lipase catalyzed synthesis process.

1. Introduction

As biocatalysts, enzymes exhibit a number of advantages such as the high level of catalytic efficiency and high selectivity. However, there various practical problems exist in enzyme applications, such as high cost, instability and difficulty of enzyme recovery. These problems severely lower the practical application of enzyme [1–4]. Therefore, immobilization technology was often used not only for enhancing enzyme stability and activity, but also for simplifying the enzyme recovery process [5–13].

Among all the immobilization techniques available, adsorption is a simple and economic method in which high catalytic activity can be retained [14,15]. Generally speaking, the interaction in adsorption is not very strong, and some of the adsorbed enzyme will be desorbed during operation. Entrapment on the contrary can be defined as imposing physical restrictions to the enzyme within a confined space or network [16]. The lipase immobilized by entrapment is much more stable than physically adsorbed lipase. But the problems such as leakage of the enzymes during continuous use and insufficient substrate-enzyme interactions still have to be faced [17].

Immobilized lipase, combined with adsorption and entrapment could protect the lipase inside the pores and avoid desorption. Although the use of immobilized lipase is widespread, the formation and interactions between lipase and support is rarely reported upon. One of the major challenges is to understand the underlying mechanisms associated with two-step immobilization processes. In this work, the effect of immobilization conditions was investigated. Formation mechanism and internal structure of the immobilized lipase was explored by SEM. Biodiesel production using the immobilized lipase as a biocatalyst was conducted by transesterification. The thermal and operational stabilities of immobilized lipase were also observed.

2. Material and methods

2.1. Materials

Lipase from *Candida* sp. 99–125 was obtained from Kaitai Biochemical Technology Company, Beijing, China; The activity of the lipase was 30000 U/g. Diatomite (10 μm, BET surface area = 2.514 m^2/g, pore volume = 0.014 cc/g). Waste cooking oil

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(WCO) was obtained from Lvming Co. Ltd. Shanghai, China. All other chemicals were of analytical grade and used without further purification. Deionized water was used throughout the experiments.

2.2. Immobilization procedures

2.2.1. Adsorption

Sodium phosphate (150 mM, 30 mL) buffer solution containing lipase was mixed with diatomite in a 100 mL conical flask. The mixture was placed on a shaking table under different conditions.

2.2.2. Entrapment of lipase in alginate beads

The adsorbed lipase was mixed with 30 mL of sodium alginate solution. The mixed solution was dripped into 150 mL calcium chloride solution with a syringe, Ca-alginate beads were formed. After hardening for 1 h, the beads were separated from the $CaCl_2$ solution by vacuum filtration and dried at room temperature. Then the ultimately immobilized lipase was obtained (Scheme 1).

2.3. Activity assay of immobilized lipase

The esterification activity was measured by esterification of lauric acid with some modifications [18]. Immobilized lipase (150 mg) was added into a mixture of lauric acid (200 mg, 1 mmol), 1-octanol (316 μL, 2.0 mmol) and n-hexane (9.5 mL) in a 50 mL conical flask with cover. The reaction mixture was shaken at 160 rpm at 40 °C for 3 h. At last, the reaction mixture were sampled and analyzed by gas chromatography to determine the lipase activity.

2.4. Characterization of the formation of immobilized lipase

The surface and cross-section of immobilized lipase was studied using scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS). All samples were sputter-coated with platinum prior to observation.

2.5. Thermal stability of immobilized lipase

The thermal stabilities of the free and immobilized lipase were determined by incubation at different temperatures for 2 h. The remaining activities of the free and immobilized lipases were measured as described in Section 2.4.

2.6. Operational stability of immobilized lipase

In order to evaluate the stability of immobilized lipase in repeated use, batch transesterification of WCO and methanol was conducted by using the immobilized lipase. The immobilized lipase was reused with fresh substrates for each cycle. The reaction conditions were the same as described before [19].

3. Results and discussion

3.1. Effects of immobilization parameters

3.1.1. Immobilization parameters in the adsorption process

Towards immobilization parameters in the adsorption process, the enzyme activity reached its highest value at the optimized conditions, being a mass ratio 1:3 of the lipase and diatomite, immobilization duration 3 h, temperature 25 °C and pH 7.5.

3.1.2. Alginate and $CaCl_2$ concentration

Upon addition of alginate solution to $CaCl_2$ solution, cross-linking between alginate and Ca^{2+} ions lead to gelation with precipitation of Ca-alginate. The concentration of alginate and $CaCl_2$ were major variable parameters for enzyme entrapment (Fig. 1). The highest immobilized lipase activities were obtained at the conditions of 3, 6, 8 and 9 (1.5% alginate and 40 mM $CaCl_2$). With a decrease of the concentration of alginate and $CaCl_2$ (below the data of number 9), there was inadequate or incomplete gelation of alginate beads. At a higher concentration of alginate and $CaCl_2$, the beads rigidity was improved, but the immobilized lipase activity decreased. This perhaps due to the limitation of substrate transfer from the bulk phase into the alginate bead to access the lipase [20]. Thus the alginate and $CaCl_2$ at the optimum level 1.5% and 40 mM were chosen. These concentrations both ensured that the gelation of alginate beads avoiding the leakage of the lipase from the beads and maintained the immobilized lipase activity at a higher level.

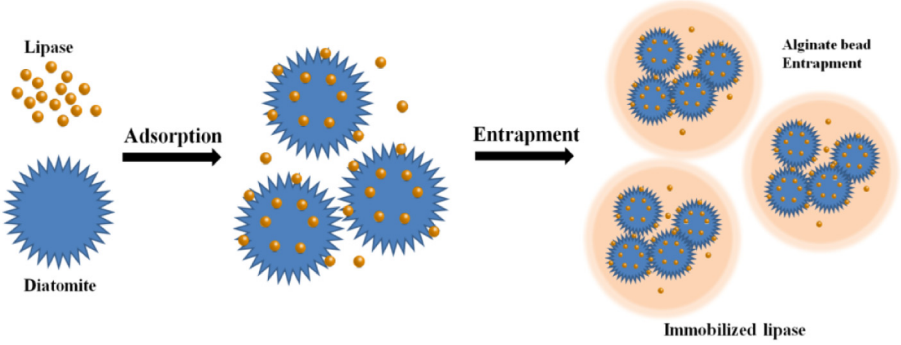
3.1.3. The addition of gelatine

Gelatine, a cheap chemical, is available for enzyme immobilization. In order to find out whether the gelatine had a supplementary function, 1% gelatine was added into sodium alginate solution and the activity of entrapped enzyme was assayed for five cycles. It indicated that the immobilized enzyme with gelatine was able to maintain good activity (above 80%) after five subsequent uses, but without gelatine the activity came down to zero after three cycles (Fig. 2). Therefore, gelatine played a protective role during the catalytic reaction and the enzyme entrapped in alginate with 1% gelatine showed higher activity and stability.

3.2. Surface morphology and internal network formation mechanism of the immobilized lipase

3.2.1. Surface morphology of the immobilized lipase

The dependence of bead morphology on $CaCl_2$ and alginate



Scheme 1. Synthesis of immobilized lipase by two-step processes.