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Lipase immobilized on novel ceramic supporter with Ni activation for efficient cinnamyl acetate synthesis

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ABSTRACT

The metal ceramic powder (MCP) was used for lipase immobilization. The MCP containing Ni^{2+} (Ni-MCP) exhibited the best affinity to lipase. The effects of heating rate, lipase concentration and immobilization time on immobilized lipase (Ni-MCP-lipase) activity were measured. Under the optimal preparation conditions (heating rate: 1 °C/min, lipase concentration: 9 mg/mL, immobilization time: 8 h), Ni-MCP-lipase could obtain 1.4 U/g and 216% of activity yield. The Ni-MCP-lipase, with improved thermal stability and storage stability, had optimal pH value of 6.0 and optimal temperature of 40 °C. The characterizations clarified the effect of heating rate on Ni-MCP-lipase activity, and confirmed that lipase had been efficiently immobilized on Ni-MCP surface. Finally, cinnamyl acetate synthesis demonstrated that Ni-MCP-lipase had improved efficiency compared with free lipase. Under the optimal reaction conditions (Ni-MCP-lipase loading: 3 g; reaction temperature: 35 °C; acetic acid/cinnamyl alcohol: 2:1 in 15.0 mL reaction system), the cinnamyl acetate yield would reach 62.56%.

1. Introduction

Lipase (triacylglycerol acylhydrolase, EC 3.1.1.3) has aroused significant attention in the fields of biotechnology, food industry, pharmaceutical chemistry due to its application in organic solvent and broad specific catalysis function [1,2]. Just as other enzymes, immobilized lipase would obtain improved stability and reusability. The solid carriers with appropriate hardness, density and specific surface area would make the immobilized lipase appropriate for the application. Many solid materials, such as silicon particle [3], smectite [4] and Fe_3O_4-chitosan particles [5] have been used as carriers for lipase immobilization. Physical adsorption and covalent cross-linking are the main methods for lipase immobilization. However, the physical adsorption only provided weak affinity between the carrier and lipase molecular, which could not supply excellent reusability. The cross-linking agents, such as glutaraldehyde might result in enzyme activity loss. In another way, enzymes modified by some small molecules could obtain higher activity, better catalytic properties [6], such as amino acid, anhydride and polyalcohol. However, the modified enzyme needed to be immobilized for further practical application.

Recently, due to the high immobilization efficiency, simple immobilization process and improved catalytic properties, enzyme immobilization by metal affinity method has attracted significant attention [7]. Moreover, some kinds of metal ion could improve enzyme activity and catalytic properties [8,9]. However, the carriers for enzyme immobilization through metal affinity, usually lacked mechanical strength, density or hardness and leaded to some disadvantages in practical application. Fortunately, metal ceramic could be used as the ideal material, not only the significant mechanical strength, excellent hardness and abrasive resistance, but also mass of metal active sites on the surface for enzyme immobilization. However, the study on metal ceramic applied for enzyme immobilization has been barely reported.

Therefore, in this paper, the metal ceramic powder was used as carrier for lipase immobilization integrated the advantages of metal affinity immobilization method, metal activation effects and metal ceramic. Lipase immobilized on the metal ceramic powder would obtain high immobilization efficiency, better catalytic properties and excellent reusability. In addition, the disadvantages of physical adsorption and covalent cross-linking method would be overcome. Firstly, metal ceramic powder (MCP) would be prepared and used

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as carrier. Secondly, lipase was immobilized on MCP through a simple process. Then, the optimal conditions were investigated to obtain the MCP-lipase with the highest activity. At the same time, the catalytic property and stability of MCP-lipase were measured. Furthermore, FT-IR spectra, SEM and BET-N_2 method were used for characterization. At last, to measure the practical application of MCP-lipase, synthesis of cinnamyl acetate, a kind of important spice widely used in daily detergents [10], was selected as model reaction to evaluate the efficiency of enzyme. Considering the improved properties, the MCP-lipase would have wide prospects for hydrolysis, degradation and synthesis applications.

2. Materials and methods

2.1. Material

Lipase from procine pancreas, Type II (PPL) (CAS 9001-62-1) was purchased from Sigma Chemical Company (USA); cinnamyl alcohol (E1226034) was purchased from Aladdin Company; glyceryl triacetate, ethyl acetate, silica, calcium oxide, magnesium oxide were purchased from Guangfu Company (Tianjin, China). Other chemicals were analytical grade and obtained from common commercial sources without further purification.

2.2. Carrier preparation

The carrier preparation process had been mentioned in our previous work [11], briefly, metal hydroxide precipitation was prepared by dissolution of metal chloride in deionized water and precipitated by NaOH solution. The precipitation was filtered and washed for 3 times. Then, the obtained metal hydroxide precipitation was dried at 70 °C for 24 h.

Subsequently, metal ceramic powder (MCP) was prepared by mixing of 22 g matrix (containing 10 g SiO_2, 5 g Al_2O_3, 5 g Na_2SiO_3, 1 g MgO, 1 g CaO), 5 g metal hydroxide precipitation and 10 g deionized water. Then the MCP was obtained by drying in muffle at 150 °C for 2 h and calcining through 1 °C/min, 3 °C/min, 5 °C/min, 7 °C/min to 850 °C, respectively, and the temperature was preserved for 4 h. Meanwhile, the ceramic powder (CP) was also prepared with the same process as the control without mixing metal hydroxide precipitation.

2.3. Lipase immobilization

Surface activation: MCP surface was activated by 5% (w/w) sulfuric acid for 1 h under vigorous stirring at room temperature. Then, the activated MCP was washed with deionized water for 6 times.

Immobilization process: 5.0 g of activated MCP was added into 10.0 mL lipase solution with certain concentration and stirred at 4 °C for different time, then the immobilized lipase (MCP-lipase) was washed by deionized water for 3 times to remove the unimmobilized lipase.

2.4. Enzyme activity assay

The activities of free lipase and MCP-lipase were determined by hydrolysis of glyceryl triacetate [12]. The enzyme samples were added into 30.0 mL saturated glyceryl triacetate solution (pH 6.3) and the NaOH solution (0.05 mol/L) was added into the mixture to maintain the solution with pH 6.3. Meanwhile, the reaction time was set as 30 min. One unit activity (U) of free lipase or MCP-lipase was defined as the amount of enzyme needed to liberate 1.0 μmol of acetic acid in 1 min at 35 °C and pH 6.3.

The activity and activity yield of lipase were calculated based on the following equations:

$$\text{Lipase activity (U)} = \frac{(V_{sample} - V_{blank}) \times 50}{M_e \times 30} \quad (1)$$

$$\text{Activity yield (\%)} = \frac{E_{immobilized}}{E_{free}} \times 100 \quad (2)$$

where V_{sample} (mL) is the volume of NaOH (0.05 mol/L) solution used to neutralize the acetic acid liberated by lipase hydrolysis; V_{blank} (mL) is the volume of NaOH (0.05 mol/L) solution consumed by glyceryl triacetate solution, while in this situation, the lipase was heated at 90 °C for 24 h. M_e is the lipase mass added into the glyceryl triacetate solution. 50 is the conversion factor of NaOH to acetic acid. 30 (min) is the reaction time. $E_{immobilized}$ is the activity of all MCP-lipase obtained from the original lipase solution after immobilization, and E_{free} is the activity of all free lipase before immobilization.

2.5. Catalytic properties of lipase or MCP-lipase

Reaction temperature: The activities of free lipase and Ni-MCP-lipase were determined by adding the enzyme samples (30 mg of free lipase or 1 g of Ni-MCP-lipase) into 30.0 mL substrate solution (saturated glyceryl triacetate solution) at different temperatures (25–55 °C) for 30 min, and pH value was maintained 6.3.

Reaction pH: The activities of free lipase and Ni-MCP-lipase were determined by adding the enzyme samples (30 mg of free lipase or 1 g of Ni-MCP-lipase) into 30.0 mL substrate solution (saturated glyceryl triacetate solution) under different pH (5–10) for 30 min, and the temperature was maintained 35 °C.

2.6. Stabilities of lipase or MCP-lipase

The thermal stabilities of free lipase (1 mg/mL) and Ni-MCP-lipase (50 mg/mL) were determined by measuring the residual activities of enzyme samples incubated in phosphate buffer at pH 6.3 and 70 °C. The incubating time was from 1 h to 5 h and the time interval was 1 h. The storage stabilities of free lipase (1 mg/mL) and Ni-MCP-lipase (50 mg/mL) were determined by measuring the residual activities of enzyme samples in phosphate buffer at pH 6.3 and 4 °C. The storage time was set from 1 to 7 days.

2.7. Characterizations

The XRD (X'Pert Pro, 3.0 kV, Cobalt bomb), SEM (S4800, 5 kV, high power mode), BET-N_2 adsorption (F-sorp 2400, liquid nitrogen temperature), EDS (S-4800, 15 kV, 15,000× magnification) and IR spectrum (Bio-Rad FTS 6000, FTIR, KBr disk method) were performed to characterize the properties of Ni-MCP and Ni-MCP-lipase.

2.8. Enzymatic synthesis of cinnamyl acetate

Synthesis reactions were carried out in 50 mL shaking flask at 35 °C under 150 rpm for 10 h. The reaction mixture contained 0.12 g acetic acid, 0.268 g cinnamyl alcohol, 15.0 mL *n*-hexane and certain amount of free lipase or Ni-MCP-lipase.

The cinnamyl acetate (CA) yield was determined by measuring the acetic acid content in the reaction mixture through titration with NaOH solution (0.1 mol/L) and calculated based on the following equation:

$$\text{CA yield (\%)} = \frac{C_0 - C_s}{C_c} \times 100 \quad (3)$$