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Biodiesel production using lipase immobilized on epoxychloropropane-modified Fe_3O_4 sub-microspheres

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ABSTRACT

Superparamagnetic Fe_3O_4 sub-microspheres with diameters of approximately 200 nm were prepared via a solvothermal method, and then modified with epoxychloropropane. Lipase was immobilized on the modified sub-microspheres. The immobilized lipase was used in the production of biodiesel fatty acid methyl esters (FAMEs) from acidified waste cooking oil (AWCO). The effects of the reaction conditions on the biodiesel yield were investigated using a combination of response surface methodology and three-level/three-factor Box–Behnken design (BBD). The optimum synthetic conditions, which were identified using Ridge max analysis, were as follows: immobilized lipase:AWCO mass ratio 0.02:1, fatty acid:methanol molar ratio 1:1.10, hexane:AWCO ratio 1.33:1 (mL/g), and temperature 40 °C. A 97.11% yield was obtained under these conditions. The BBD and experimental data showed that the immobilized lipase could generate biodiesel over a wide temperature range, from 0 to 40 °C. Consistently high FAME yields, in excess of 80%, were obtained when the immobilized lipase was reused in six replicate trials at 10 and 20 °C.

1. Introduction

Concerns regarding the depletion of petrochemical resources and increasingly serious environmental issues have resulted in a gradual trend toward the development of clean, renewable fuels. Biodiesel, which is a renewable, biodegradable, and non-toxic energy resource, has attracted considerable attention [1,2]. Biodiesel production using enzymatic catalysis has several advantages, including low energy consumption, mild reaction conditions, and low environmental impact, compared with traditional acidic or alkaline catalysis [3]. The main obstacles to the widespread use of biocatalysts in industrial production are the high cost and low stability of enzymes. Enzymes are typically difficult to separate from reaction products and are easily inactivated [4]. Immobilization can be used to improve the stability and recycling of enzymes, to reduce production costs [5]. Ideal immobilization supports are chemically stable and give good dispersion and high enzyme loading. Superparamagnetic particles have been widely used to immobilize enzymes because they can be readily separated from the reaction system using an external magnetic field and also have high specific surface areas and high diffusion values [6–10].

Recently, the modification of magnetic particles using polymers or macromolecules has been proposed. However, the synthetic processes are complex, and the magnetic responses and specific surface areas of the resulting magnetic composites are low [11–13]. The covalent immobilization of lipase on magnetic microspheres via active epoxy groups, resulting in magnetic composite microspheres, has been reported [3,14]. However, the process involves complicated suspension polymerization.

In the present work, Fe_3O_4 sub-microspheres were prepared via a solvothermal method and the surfaces of these spheres were then modified with epoxychloropropane (epichlorohydrin, EPI). Lipase from *Candida* sp. 99–125 was immobilized on these epoxy-modified superparamagnetic Fe_3O_4 sub-microspheres and used to produce biodiesel from acidified waste cooking oil (AWCO). The effects of the reaction conditions, namely temperature, amount of enzyme, free fatty acid (FFA) to methanol molar ratio, and solvent volume, were explored using a combination of Box–Behnken design (BBD) and response surface methodology.

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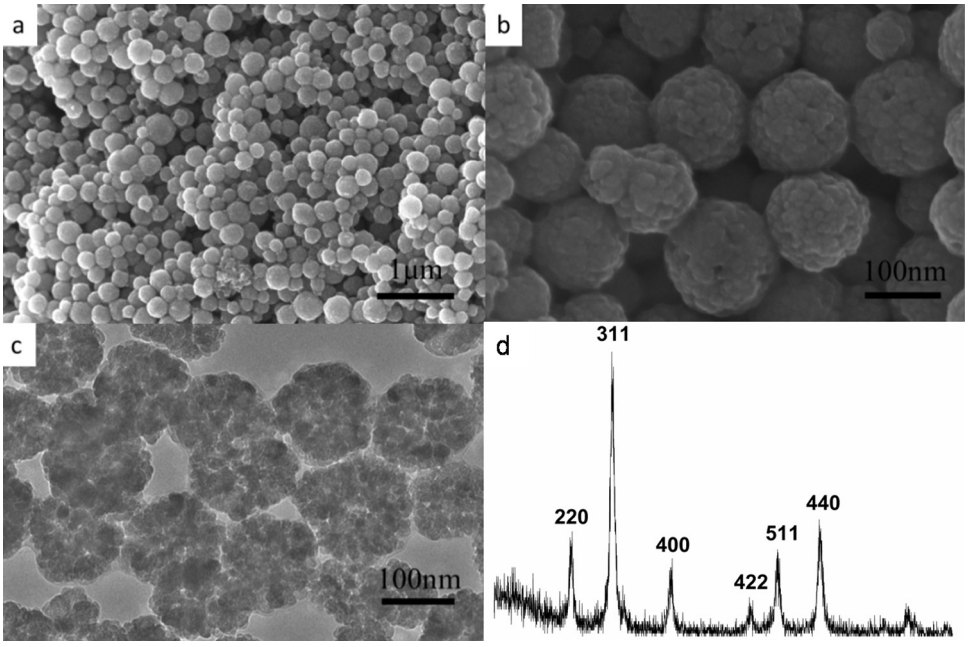

Fig. 1. (a) SEM, (b) enlarged SEM, and (c) TEM images, and (d) XRD pattern of Fe_3O_4 sub-microspheres.

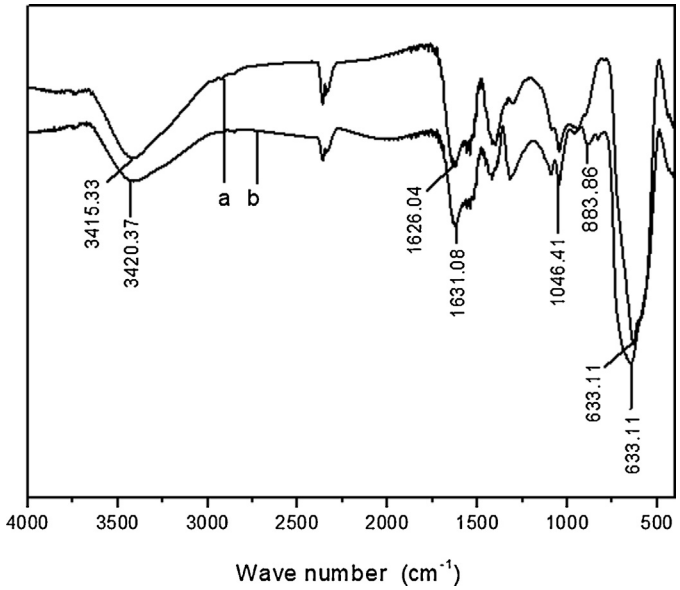

Fig. 2. (a) FTIR spectra of unmodified and (b) epoxy-modified Fe_3O_4 sub-microspheres.

2. Materials and methods

2.1. Materials

The AWCO used in this work was a gift from the Shanghai Luming Environmental Science Co., Ltd. The main components of the AWCO were FFAs (96.23%), diglycerides (1.23%), and triglycerides (2.53%). Lipase was prepared in our laboratory from *Candida* sp. 99–125 [15]. All chemical reagents were analytical grade and were obtained from major chemical supply companies.

2.2. Preparation of hollow Fe_3O_4 sub-microspheres

Fe_3O_4 sub-microspheres were synthesized via a modified solvothermal method. Briefly, $FeCl_3{\cdot}6H_2O$ (1.35 g) was dissolved in ethylene glycol (40 mL), giving a clear solution, followed by addition of sodium glutamate (2 g), sodium acetate (1.5 g), and polyethylene glycol (1.5 g). The mixture was stirred vigorously, and then transferred to a Teflon-lined stainless-steel autoclave (100 mL capacity) and held at 200 °C for 8 h. The autoclave was allowed to cool to room temperature and the products were collected by magnetic separation, washed five times each with deionized water and ethanol, and then dried under vacuum at 60 °C for 6 h to give Fe_3O_4 sub-microspheres.

2.3. Activation of Fe_3O_4 sub-microspheres by EPI

NaOH (0.6 g) was dissolved in deionized water (18 mL). 1,4-Dioxane (1 mL) and EPI (2 mL) were added to the solution. Fe_3O_4 sub-microspheres (0.5 g) were dispersed in the mixture, and the