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Improved lipase-catalyzed methanolysis for biodiesel production by combining in-situ removal of by-product glycerol

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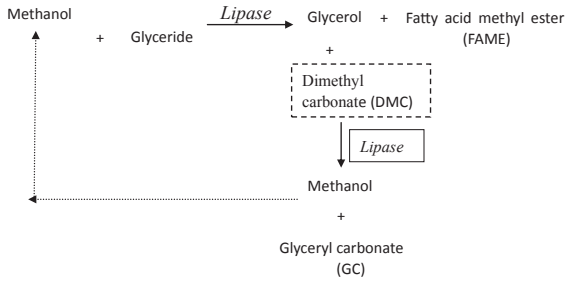
GRAPHICAL ABSTRACT



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ABSTRACT

During lipase-catalyzed biodiesel production, it has been demonstrated that by-product glycerol had negative effect on the catalytic performance of lipase especially in the repeated uses. This work presents a novel process by introducing dimethyl carbonate (DMC) into lipase-mediated methanolysis in due time, where DMC reacts with by-product glycerol and release methanol simultaneously, which not only realizes the in-situ removal of glycerol, but also achieves higher biodiesel yield at shorter reaction time compared to only use DMC as the acyl acceptor. At the optimized condition a biodiesel yield of 95.3% could be achieved with glycerol concentration only 0.049%. Improved reusability of lipase was given and there was a negligible loss in lipase's activity after its being consecutively used for 6 batches. This process for in-situ removal of glycerol during lipase-catalyzed methanolysis was prospective for practical application in biodiesel industry.

1. Introduction

Due to the exhaustion of remaining petroleum energy by the industrial development and the environmental problems associated with greenhouse gas emission, more and more countries have focused on the search for new alternative energies [1,2]. Biodiesel, as a renewable fuel, is regarded as promising substitute for fossil diesel, which has attracted considerable attention in recent years [3]. Biodiesel is mainly produced by transesterification between glyceride and short chain alcohols, either with chemical catalyst or biocatalyst. Although biodiesel has been commercially produced by chemically catalyzed processes, especially with alkaline as the catalyst, problems such as high requirements on oil feedstock, complicated downstream process for the separation of catalysts and saponified products have caused increasing attention. Alternatively, immobilized lipase has been explored extensively due to its well-recognized advantages, such as good thermal stability and the easiness of lipase's reuse [4]. However, it has been demonstrated that during immobilized lipase-catalyzed biodiesel production process, by-

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product glycerol has a poor solubility in renewable oils, and subsequently causes negative effects on lipase's catalytic performance especially during the repeated uses [5–8].

To eliminate the above-mentioned negative effect caused by by-product glycerol, some researchers proposed to explore the potential of using dimethyl carbonate (DMC) instead of short chain alcohols as the acyl acceptor for biodiesel production [6–15]. Seong [7] studied lipase-mediated biodiesel preparation with DMC as the acyl acceptor in organic solvent, with a biodiesel yield of 84.9% achieved. A-Ra [8] studied lipase-catalyzed biodiesel production with DMC as the acyl acceptor in solvent-free system and 96.4% biodiesel yield obtained. It was also observed that much better operational stability of lipase could be achieved with DMC as the acyl acceptor for biodiesel production since no glycerol was produced in the process [9]. In Jo's work [13], DMC was used as lipid extraction agent and acyl acceptor for transesterification of the extracted triglycerides. Min reported that biodiesel was synthesized from corn oil and dimethyl carbonate (DMC) via transesterification using lipase (Novozyme 435) in solvent-free reaction in which excess DMC was used as the substrate and reaction medium [14]. Su studied lipase-catalyzed transesterification of vegetable oils for biodiesel production with dimethyl carbonate as the acyl acceptor [15]. Generally speaking, longer reaction time is usually needed with DMC for biodiesel production, compared to that with traditional short chain alcohols such as methanol or ethanol [10,11]. But better lipase's operational stability could be obtained with DMC as the acyl acceptor for biodiesel preparation. How to combine the advantage of methanol and DMC together is extraordinary important for lipase-mediated biodiesel production.

In our previous study [16], we demonstrated that during a two-step methanolysis, after the reaction catalyzed by free lipase NS81006 and immobilized lipase Novozym435, DMC introduced into the system was effective for the removal of trace amount of glycerol. Nevertheless, both free lipase and immobilized lipase were adopted in this two-step process and the recovery of free lipase required extra centrifugation as well as membrane recovery process, leading to complicated downstream unites and higher operation cost. Herein, we focused on one-step reaction process where only immobilized lipase was adopted for biodiesel preparation in this paper, and during the process, we explored the possibility of removing by-product glycerol in-situ by introducing DMC into the methanolysis system in due time. This process was supposed to be faster compared to that only using DMC as the acyl acceptor, but also better lipase's reusability could be obtained compared to that only adopting methanol as the acyl acceptor.

2. Material and methods

2.1. Materials

Novozym 435 (from *Candida antarctica*, activity 10,000 PLU/g) was provided by Novo Industries (Bagsvaerd, Denmark). The methyl esters of palmitic acid, stearic acid, oleic acid, linolenic acid and heptadecanoic acid were purchased from Sigma-Aldrich (St. Louis, MO) and they were chromatographically pure. DMC and methanol of analytical grade was obtained from Beijing Lanyi Chemical Products Co., Ltd (Beijing, China). Other reagents were obtained locally with analytical grade.

2.2. Transesterification of oil with methanol or dimethyl carbonate respectively

Transesterification of oil with methanol or dimethyl carbonate (DMC) was carried out in a 50 mL shaking flask. The reaction mixture consisted of 10 g soybean oil, a certain amount of methanol or DMC, and some amount of immobilized lipase. DMC was added into the system at the beginning of reaction, while methanol was stepwise added into the system with the adding strategy specified in each reaction condition. The reaction was conducted at 180 rpm, 60 °C and samples were taken at appropriate intervals for further analysis.

2.3. Introducing DMC during lipase-mediated methanolysis

Lipase-mediated methanolysis was carried out first at the reaction condition specified in 2.2. When the methanolysis proceeded at 4 h, various amount of DMC was added into the system for initiating the in-situ removal of by-product glycerol with the catalysis of lipase and the reaction was conducted at 180 rpm, 60 °C.

2.4. Reuse of lipase

Reuse of lipase was manipulated in a 50 mL shaking flask at the reaction condition as described in part 2.2 and 2.3. After the reaction was completed, lipase was removed from the reaction mixture through filtration. The recovered lipase was then remixed with fresh reactants to initiate a new reaction. In each reaction cycle, the sample was withdrawn for further analysis.

2.5. Glycerol concentration analysis

The glycerol concentration in the reaction mixture was detected by a 10AVP HPLC system (Shimadzu, Kyoto, Japan), equipped with an Aminex HPX-87H Column using 0.005 M H_2SO_4 as the mobile phase with a flow rate of 0.6 mL/min, and detection via refractive index. The column temperature was set at 65 °C.

2.6. Fatty acid methyl ester (FAME) analysis

The FAME yield is defined as follows:

$$\text{FAME yield (\%)} = \text{FAME content of reaction mixture} / \text{the fixed lipid FAME content} \times 100\%.$$

The fixed lipid FAME content was measured according to the standard AOAC 991.39 (Association of Analytical Communities) procedure, which was given as follows: 25 mg crude lipid, 2 mg Heptadecanoic acid methyl ester (internal standard) and 1.5 mL NaOH (0.5 mol/L) in methanol were put into a glass tube for shaking well, and the mixture was then heated at 100 °C for 15 min. After cooling down, 2 mL 14% BF_3 (*w/v*) in methanol was added and the mixture was then heated at 100 °C for 30 min. Being cooling down to room temperature, then 5 mL saturated NaCl solution and 1 mL hexane were added into the mixture, agitating thoroughly and then layered. Subsequently the upper hexane layer of 1 µl was injected for further GC analysis.

The FAME content of reaction system was measured as follows: 6 µl sample obtained from 2.2, 2.3, and 2.4 was mixed with 0.6 mL solution of methyl heptadecanoate (0.6 mg/ml) in ethanol. Then 1 µl sample of the above resultant was taken and injected for further GC analysis.

GC analysis was carried out by Agilent 7890A GC system (Agilent Technologies, Santa Clara, USA) and a CP-FFAP CB capillary column (25 m × 0.32 mm × 0.30 µm, Agilent Technologies, USA). The column temperature was initially set at 180 °C and maintained for 0.5 min, then heated to 250 °C at the rate of 10 °C/min and then held at 250 °C for 6 min. Detector and injector were set at 250 °C and 245 °C, respectively.

2.7. GC–MS analysis

The sample was analyzed using a GC-2014 gas chromatography (Shimadzu, Kyoto, Japan) equipped with DB-5HT capillary column supplied by Agilent. The Same operation and sample treatment conditions as described in part 2.6 were adopted for GC–MS analysis. The column temperature was kept at 70 °C for 1 min, heated to 190 °C at 15 °C/min, then heated to 260 °C at 7 °C/min, then heated to 300 °C at 20 °C/min and maintained for 14 min. Electron bombardment ion source was adopted in MS analysis.