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Efficient biodiesel production from phospholipids-containing oil: Synchronous catalysis with phospholipase and lipase

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ABSTRACT

Recently, the use of low quality oils as potential alternative oil feedstock for biodiesel production has become more and more attractive, which provides a feasible solution to reduce the cost of biodiesel production. However, in the low quality oils, expect triglycerides, other lipids represented by phospholipids also exist. It was found that the presence of phospholipids showed adverse effect on enzyme catalyzed biodiesel production. Therefore, it is necessary to develop an effective method for the conversion of phospholipids-containing oils. In recent years, the employment of free lipase in catalyzing biodiesel from refined oils has received great attentions due to its advantages of lower cost and faster reaction rate. To further promote its application in efficient conversion of phospholipids-containing oils, a synchronous catalysis together with phospholipase and lipase for biodiesel production from phospholipids-containing oils was developed. A high FAME yield of 94.9% can be obtained even with 10% phospholipids contained in the oil feedstock. Further study showed that the biodiesel product could also meet the phosphorus demand of EN14214 standard. Moreover, the good reuse stability of the two enzymes further indicates that the combination catalysis of phospholipase and lipase is very promising for biodiesel production especially with high phospholipids-containing oils as feedstock.

1. Introduction

Biodiesel, defined as alkyl esters of long chain fatty acids, is generally believed as a renewable and clean energy [1,2]. The development of biodiesel is favorable in dealing with the challenges caused by the global energy crisis and environmental concerns. In recent years, the employment of lipases as biocatalysts has received great considerations for its distinctive features such as lower oil quality requirement, mild reaction conditions and waste-free process [3,4].

Conventionally, biodiesel is mainly produced from refined edible vegetable oils including soybean, rapeseed, sunflower oils, and so on. In general, these refined raw materials are very expensive and can account for over 85% of the total cost of biodiesel production [5,6]. In addition, with the ever-growing biodiesel industry, the problem of edible oil feedstock shortage has become more and more serious. Therefore, other low-value, non-edible feedstocks need to be explored. Under such a background, many researchers have investigated the possibilities of crude vegetable oils and microbial oil as potential alternative oil sources for biodiesel [7,8]. However, these oils belong to low quality feedstock with some impurities, one important of which is phospholipids. For example, the extracted microbial oil is usually a mixture of neutral lipids and polar lipids. It was reported that relatively high amounts of phospholipids of more than 10% could exist in the microbial oils [9,10].

Phospholipids contained in these low quality oil feedstocks are usually glycerophospholipids, mainly distributed in cellular membranes of organisms [11]. The structure of a typical phospholipid consists of a glycerol backbone to which two fatty acid chains and a polar phosphorylated group are attached. Compared with refined oils, the conversion of the phospholipids-containing oils would be much more difficult. For immobilized lipase-catalyzed biodiesel production, the phospholipids contained in the oil are usually found as inhibitory substances by affecting the proper interaction between substrates and the active site of the enzyme [12–14]. Besides, the quality of biodiesel product could also be affected due to the presence of phosphorous. It was reported that excessive phosphorous could produce complex problems on the exhaust converter of diesel engines [15,16]. Furthermore, according to the ASTM D6751 and EN 142141 standard, the phosphorous content in the final biodiesel product is mandatory to be below 10 ppm and 4 ppm, respectively. Hence, there is an urgent need to

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develop a practical approach for the conversion of oil feedstocks with phospholipids, which can also guarantee the final product meet the phosphorus requirements of biodiesel standards.

In recent years, the utilization of free lipase for biodiesel production has attracted increasing interests due to its merits of lower preparation cost and faster reaction rate [17–19]. It has been found that free lipase NS81006 could efficiently catalyze the alcoholysis of refined soybean oils for biodiesel production with a final FAME (fatty acid methyl ester) yield of more than 90% [20,21]. To further realize the effective conversion of feedstock containing high content of phospholipids, phospholipase is introduced into the free lipase's reaction system in this work and it is supposed to catalyze the degradation of phospholipids simultaneously. Lecitase Ultra is one of the most commonly used phospholipases, which can catalyze the hydrolysis of the sn-1 ester bond of phospholipids and form the lysophospholipids and free fatty acids [22–24].

So in this work, the effect of phospholipids presented in the oil feedstock on the biodiesel production by free lipase NS81006 was first investigated. To improve the biodiesel quality, a synchronous catalysis with phospholipase Lecitase Ultra and free lipase NS81006 for biodiesel production was further studied. Then the catalytic performance of Lecitase Ultra during the synchronous catalysis and the reuse stability of the two enzymes were also discussed.

2. Materials and methods

2.1. Materials

Free lipase NS81006 from the genetically modified *Aspergillus niger* (lipase activity 3300 LU/ml), and free phospholipase A1 Lecitase Ultra from the genetically modified *Aspergillus oryzae* (phospholipase activity 12,000 LU/ml) were both kindly donated by Novozymes (Denmark). One unit of lipase/phospholipase activity (LU) is defined as the amount of lipase/phospholipase that produces 1 μmol free fatty acid per minute. Heptadecanoic acid methyl ester as GC standard and phosphatidyl choline (PC), phosphatidyl ethanolamine (PE), lysophosphatidylcholine (LPC) as HPLC standards were chromatographically pure and purchased from Sigma–Aldrich (St. Louis, MO). Soybean phospholipids (containing 18.3% PE, 21.5% PC and 4% LPC, 20.7% other phospholipids components) were provided by a local company. All other chemicals and solvents were obtained commercially of analytical grade.

2.2. Biodiesel production catalyzed by phospholipase and lipase

The enzymatic process was performed in a 500 ml three-neck round-bottom flask equipped with mechanical stirrer in a water bath of 45 °C. The reaction mixture contained 100 g soybean oil, different amount of phospholipids, water (3%, based on oil weight), 20 ml methanol (the mole ratio of methanol/oil is 4.4:1), free lipase NS81006 (1.5%, based on oil weight) and free phospholipase A1 Lecitase Ultra (4%., based on phospholipids weight). The reaction was conducted under stirring speed of 1200 rpm. Methanol was added stepwise and the addition strategy was as follows: 20%, 15%, 20%, 15%, 15% and 15% of the total methanol was added into the reaction mixture at 1 h interval from 0 h to 5 h. Samples were taken from the reaction mixture at specified times and then centrifuged to get the upper layer for gas chromatography analysis. In addition, a water washing treatment (water:oil = 2:1, 65 °C) of the final biodiesel product was carried out before the phosphorus analysis.

For the reuse of enzymes, phospholipids were repeatedly added, since the whole reaction system would be totally emulsified with 3% water and such high phospholipids content, 10% water content was used to conduct the reuse experiments.

2.3. GC analysis of FAME

The fatty acid methyl esters (FAMEs) contained in the reaction mixture was detected by Agilent 7890A GC system (Agilent Technologies, Santa Clara, USA) equipped with a CP-FFAP CB capillary column (25 m × 0.32 mm × 0.30 μm, Agilent Technologies, USA). Heptadecanoic acid methyl ester was served as the internal standard. 50 mg of the upper layer and 0.6 ml of 0.7 mg/ml heptadecanoic acid methyl ester (ethanol as the solvent) were mixed thoroughly. The resultant mixture of 1 μl was injected for analysis. The initial column temperature was set at 180 °C and held for 0.5 min, then heated to 250 °C at the rate of 10 °C/min and maintained for 6 min. Injector and detector temperatures were set at 245 °C and 250 °C, respectively. FAME yield was calculated as the percentage of the actual amount of methyl esters detected in the reaction process divided by the theoretical quantity of methyl esters from soybean oil.

2.4. HPLC analysis of phospholipid composition

The phospholipids components of PC and PE in the reaction mixture were analyzed by a Shimadzu 20 A HPLC system (Shimadzu Corp., Kyoto, Japan) fitted with an ELSD-LTII low temperature-evaporative light scattering detector. Agilent Prep Sil Scalar column (4.6 mm × 150 mm, 10 μm) (Agilent Technology, USA) was used for the separation and controlled at 37 °C. The mobile phase consisted of solvent A (n-hexane/isopropanol, 3:4, v/v) and solvent B (n-hexane/isopropanol/water, 3:4:0.75, v/v/v), and it was pumped with a gradient elution program at the rate of 1.2 ml/min. The drift pipe temperature was controlled at 40 °C and the nitrogen pressure was controlled at 340 kPa, respectively.

In order to determine the phospholipids content over time, parallel experiments were carried out and stopped at different times, respectively. After the completion of reaction, all the reaction mixture was heated at 80 °C for 10 min, then transferred into a tap funnel and left to settle to separate the biodiesel phase, middle emulsion layer and water phase. Before HPLC analysis, the biodiesel layer, emulsion layer and water layer were diluted to a proper concentration with n-hexane, solvent B and isopropanol, respectively. After mixing thoroughly, the resultant mixture of 20 μl was injected for related analysis.

2.5. Phosphorus content analysis

1–15 g of test sample was weighted into a crucible with 0.5 g of zinc oxide, followed by charring with an electric furnace and then heated in a muffle furnace to afford a white ash. Then, phosphorus content analysis was conducted according to AOCS Official Method Ca 12-55 standard (AOCS, 2009). The final phosphorus content can be calculated according to the following equation:

$$\text{Phosphorus content (ppm, mg/kg)} = \frac{P}{m} \times N \times 1000$$

where P is the phosphorus amount calculated based on the standard curve (mg); m is the mass of test portion (g); N is the dilution ratio during the colorimetric process. All assays were carried out twice for the calculation of the mean value.

3. Results and discussion

3.1. Free lipase catalyzed biodiesel production from phospholipids containing oil

Phospholipids and soybean oil were mixed in different proportions as oil feedstock. After free lipase NS81006-mediated