Biochemical Engineering Journal 49 (2010) 207–212

Contents lists available at ScienceDirect

Biochemical Engineering Journal

journal homepage: www.elsevier.com/locate/bej

Two-step lipase catalysis for production of biodiesel

Md. Mahabubur Rahman Talukder*, Jin Chuan Wu, Ng Mei Fen, Yeo Li Shi Melissa

Institute of Chemical and Engineering Sciences, 1 Pesek Road, Jurong Island, Singapore 627833, Singapore

ARTICLE INFO

Article history:
Received 3 July 2009
Received in revised form 27 October 2009
Accepted 23 December 2009

Keywords:
Biodiesel
Lipase
Two-step catalysis
Crude palm oil
Methanolysis

ABSTRACT

Lipase-catalyzed methanolysis of vegetable oils has attracted considerable interests for the production of biodiesel (BD). However, the activity of lipase such as Novozym 435 (immobilized *Candida antarctica* lipase B) is negatively affected by methanol. To minimize this problem, two-step lipase catalysis was investigated. Crude palm oil (CPO), which is relatively cheaper because of avoiding refining cost, was used as the source of BD. CPO was first hydrolysed to fatty acids (FA), which was then esterified to BD. *Candida rugosa* and Novozym 435 lipases were used as biocatalysts for the hydrolysis of CPO and the esterification of FA, respectively. The complete conversion of CPO to FA was achieved under an optimal condition of buffer to CPO ratio 1:1 (v/v), buffer pH 7.0, lipase 0.1 wt.% of CPO, isooctane to CPO ratio 1:1 (v/v), temperature 30 °C, shaking speed 250 rpm and time 4 h. The methyl esterification of FA with 1.2-fold stoichiometric excess of methanol reached the equilibrium after 2 h at which BD yield was 98%. *C. rugosa* and Novozym 435 lipases were repeatedly used for 10 and 50 cycles, respectively without significant loss of their activities. The developed two-step process is very promising because of its feedstock flexibility: it can be used for production of BD and FA from crude, refined and waste oils.

1. Introduction

Alkali-catalyzed methanolysis is usually adopted in conventional chemical process for the production of fatty acid methyl esters, which are collectively called BD [1–3]. However, the conventional alkali process produces salt containing glycerol, and also suffers from complicated downstream processes. The alkali process also requires feedstock (e.g. vegetable oils), which must comply with rigorous specifications [4]. For example, it must be essentially anhydrous and the free FA content must not exceed 0.5 wt.%. The presence of these impurities generates soap, which reduces BD yield. To conform to such demanding feedstock specifications necessitates the use of highly refined but costly vegetable oils, which accounts for 70–80% of the total production cost of BD. Therefore, the exploration of low cost feedstock generally associated with high content of free FA and water, is of interest in recent BD research [5–8].

The aforementioned drawbacks of alkali process have led researchers to seek catalytic and processing alternatives that could ease the technical difficulties of utilizing low cost feedstocks such as crude vegetable oil, used frying oil, trap grease, soapstock and acid oil. Methodologies based on acid-catalyzed reactions have the potential to convert the feedstock with free FA to BD since acid catalyst can simultaneously catalyze esterification of FA and methanolysis of triglycerides [8,9]. However, acid-catalyzed methanolysis is relatively slow and the side reactions such as methanol etherification [4] cause difficulties in products purification. Lipase-catalyzed methanolysis has attracted considerable attention for the production of BD [10–14] as it is considered to be an effective mean of circumventing drawbacks involved in the chemical processes. Unfortunately, lipase such as Novozym 435 is deactivated when more than one-third stoichiometric amount of methanol present in the reaction [10–14]. This deactivation was caused by the contact between Novozym 435 and insoluble methanol, which exists as droplets in the oil. Different techniques have been employed to overcome the methanol deactivation of lipases. Organic solvents have been used to improve the solubility of methanol [12,15,16]. A very low concentration of methanol during the reaction was maintained by stepwise addition of methanol [10–14]. Methanol has been replaced by methyl acetate [17]. Salts saturated solutions [18] and silica-gel [19] based controlled release system for methanol have also been applied. However, these techniques have some inherent problems. The methanolysis in organic solvent such as hexane is relatively slower. Although a noticeable increase in reaction rate using *t*-butanol has been reported, a large molar excess of methanol (methanol to oil ratio 6:1) is required to obtain a higher BD yield. Maintaining methanol concentration at a very low level by stepwise addition is not an appropriate approach for the large-scale production of BD. When methanol is replaced by methyl acetate, the byproduct triacetylglycerol causes difficulties in product purification. Furthermore, methyl acetate is more expensive than methanol. The presence of salt saturated

* Corresponding author. Tel.: +65 67963826; fax: +65 63166182.
E-mail address: talukder@ices.a-star.edu.sg (Md.M.R. Talukder).

1369-703X/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.bej.2009.12.015

Table 1
Specifications of CPO.

Parameters	Composition (wt.%)
Free fatty acids (as palmitic)	5.97
Moisture	0.25
Iodine value	52.4
Gum (as phospholipids)	0.05
Carotene	0.04
Vitamin E	0.06

solution or silica-gel causes difficulties in downstream separation.

In this study, the production of BD from CPO via *Candida rugosa* lipase-catalyzed hydrolysis followed by Novozym 435-catalyzed esterification was investigated. The effect of different parameters on the hydrolysis of CPO and the esterification of FA were examined. The results showed that the developed two-step process is more efficient than the single-step Novozym 435-catalyzed methanolysis, and produces a higher BD yield. Since methanol solubility in FA is higher than that in oil, the methanol deactivation of Novozym 435 can be easily minimized in the two-step process.

2. Materials and methods

2.1. Materials

Novozym 435 (*Candida antarctica* lipase B immobilized on acrylic resin), *t*-butanol (GC) and standard methyl esters (98–99%) were from Sigma. *C. rugosa* lipase was from Meito Sangyo Co. Ltd., Osaka, Japan. CPO was purchased from Wawsan Tebrau SDN BHD, Johor, Malaysia. The saponification value of CPO was 195 mg KOH/g. The saponification value was determined by a method reported previously [20]. The other specifications of the CPO provided by the suppliers are shown in Table 1. HPLC grade methanol, isopropanol, isooctane and hexane were from J.T. Baker, USA. All chemicals unless mentioned otherwise were of analytical grade and used as-received.

2.2. Hydrolysis of CPO

2 g CPO with or without isooctane was preheated in a glass bottle (80 ml size) at a reaction temperature of 30–40 °C and 250 rpm for about 30 min by a shaker incubator. The reaction was initiated by adding an appropriate amount of lipase solution. This lipase solution was prepared by dissolving *C. rugosa* lipase in 0.1 M sodium phosphate buffer (pH 7.0) and directly used. The lipase concentration was varied according to 0.01–0.1 wt.% of CPO. The buffer to CPO ratio was varied in the range of 0.25:1 to 2:1 (v/v). The isooctane to CPO ratio was varied in the range of 0.05:1 to 5:1 (v/v).

2.3. Separation of FA after hydrolysis step

20 ml isooctane was added to the reaction mixture and mixed at 30 °C and 250 rpm for 15 min. The mixture was centrifuged at 4000 rpm and 25 °C for 10 min. The upper layer containing FA in isooctane was separated and used as feedstock for Novozym 435-catalyzed esterification. To measure FA concentration, 5 ml of upper layer was mixed with 50 ml ethanol–acetone (50/50, v/v) solution and titrated against 0.2N NaOH using phenolphthalein (1% in 95% ethanol) as an indicator. To determine the average molecular weight of FA, isooctane was completely evaporated from FA solution (0.35–0.36 M) and gravimetric analysis was done. The average molecular weight of FA was 270.8. FA yield is calculated as the amount of FA produced (actual value in mole) divided by theoretical value in mole.

2.4. Esterification of FA or methanolysis of CPO in the presence of isooctane

10 ml FA solution (0.35–0.36 M) and an appropriate amount of methanol according to methanol to FA molar ratio of 1:1 to 2:1 were mixed in a glass bottle (80 ml size) at a reaction temperature of 30–60 °C for about 15 min by a shaker incubator. The reaction was initiated by adding 0.04 g Novozym 435. Methanolysis of CPO was performed under the same conditions described above except that molar concentration of CPO was 0.12 M, 3-fold lower than that of FA as 1 mol of CPO gives 3 mol of FA or BD.

2.5. Esterification of FA or methanolysis of CPO in the absence of isooctane

Isooctane was completely removed from stock solution of FA by a rotary evaporator. 5 g FA was preheated in a glass bottle (80 ml size) at 60 °C and 250 rpm for about 30 min by a shaker incubator. 1.2-Fold stoichiometric excess of methanol was mixed with preheated FA for about 15 min. The reaction was initiated by adding 0.2 g Novozym 435. Methanolysis of CPO were performed under the same conditions described above except that FA was replaced by CPO.

2.6. HPLC analysis of BD

After the specified time of methyl esterification or methanolysis in isooctane, whole sample was centrifuged at 4000 rpm and 25 °C for 10 min, and the BD in upper layer was analyzed. For reactions in the absence of isooctane, whole sample was first mixed with 40 ml isooctane at 60 °C for 15 min, and centrifuged. The method used for HPLC analysis of BD was previously reported [21,22]. HPLC (Waters 2695, USA) was equipped with a UV detector (Waters 2487, USA) and a prevail-C18 5u column (4.6 mm × 250 mm, Altech Inc., USA). The UV wavelength and the column temperature were set at 210 nm and 40 °C, respectively. The mobile phase consisted of three different components: hexane, isopropanol and methanol. Reservoir A contained methanol and reservoir B contained a mixture of isopropanol and hexane (5:4, v/v). The gradient went from 100% A to 50% A + 50% B linearly over 30 min. The flow rate of the mobile phase was 1 ml/min and the sample injection volume was 10 μl. BD was quantified according to the external calibration curves. Methyl oleate, methyl palmitate, methyl stearate, methyl linoleate and methyl myristate were used as standards for BD because oleic, palmitic, stearic, linoleic and myristic acids are major fatty acids in palm oil. BD yield was based on CPO and calculated as the amount of BD produced (actual mass in gram) divided by theoretical mass in gram.

3. Results and discussion

3.1. C. rugosa lipase-catalyzed hydrolysis of CPO

Among the lipases from various sources, *C. rugosa* lipase has been reported as one of the most active and versatile enzyme [23–25]. Hence, this lipase was chosen for converting CPO to FA as an intermediate for BD production. The time course of the CPO hydrolysis at different lipase concentrations (wt.% of CPO) is shown in Fig. 1. The complete conversion of CPO to FA was achieved after 4, 10 and 20 h for lipase loading of 0.1, 0.05 and 0.02 wt.% of CPO, respectively. The reaction at a low lipase loading (e.g. 0.01 wt.% of CPO) progressed slowly and could not reach the equilibrium because of a time limit of 20 h. Lipase is a surface active enzyme and binds with substrate at oil–water interface [26,27]. Since interfacial area depends on water to oil ratio (v/v) [28,29], the optimal water (buffer) content was investigated at a lipase loading of 0.1 wt.% of