Appl Biochem Biotechnol (2010) 161:365–371
DOI 10.1007/s12010-009-8829-z

Development of Batch and Continuous Processes on Biodiesel Production in a Packed-Bed Reactor by a Mixture of Immobilized *Candida rugosa* and *Rhizopus oryzae* Lipases

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Received: 27 May 2009 / Accepted: 19 October 2009 /
Published online: 7 November 2009

Abstract In this study, transesterification and esterification were investigated in batch and continuous process using immobilized *Candida rugosa* and *Rhizopus oryzae* lipases. In the case of batch process, stepwise reaction method was investigated to prevent the lipase deactivation. Reaction conditions were as follows: temperature, 45 °C; agitation speed, 250 rpm; enzyme concentration, 20%; and water contents 10%. And then, conversion yield was 98.33% at 4 h. In the case of continuous process, circulation and long-term continuous system were investigated for development of efficient mass transfer system. Optimal reaction conditions were as follows: temperature, 45 °C; flow rate, 0.8 mL/min; and water contents, 10%. And then, conversion yield of biodiesel was 97.98% at 3 h. Especially, the maximum conversion yield using a mixture of immobilized lipases exceeded over 90% for 108 h in long-term continuous system under optimal reaction conditions (45 °C; flow rate, 0.8 mL/min; and water contents, 10%). These results should help in determining the best method for the biodiesel production and improving the design and operation of large scale by enzymatic systems.

Keywords Transesterification · Esterification · Circulation process · Long-term continuous process · Packed-bed reactor

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Introduction

The production of fuels such as biodiesel, bioethanol, and biobutanol from various biomass is seen as a possible strategy to reduce greenhouse gas emissions. Among various alternative fuels, biodiesel is a renewable and eco-friendly substitute for petroleum based diesel fuel. It is a mixture of alkyl esters of fatty acids derived from the transesterification of edible and non-edible feedstock with alcohol [1, 2]. Alkyl ester is generally produced by chemical esterification and transesterification. However, the chemical process has some disadvantages, such as the environmental problems caused by using organic solvents and the high operating costs related to the severe reaction conditions. Moreover, the raw materials of high and uniform quality were needed for biodiesel production in the alkaline-catalyzed process [3–8]. Therefore, many investigators have been intensively studying enzymatic processes for biodiesel production to solve those problems [6, 7, 9–11]. Enzymatic transesterification process for biodiesel production using lipase looks attractive and encouraging for reasons of various advantages such as easy product separation, minimal wastewater treatment needs, easy glycerol recovery, and the absence of side reactions [2].

Lipases are enzymes that can produce biodiesel from various oils and alcohols [12]. The use of lipases as biocatalysts in enzymatic processes for biodiesel production offers some advantages. Because lipases catalyze the transesterification under mild conditions, they may reduce the process cost in terms of energy consumption and capital equipment requirements. Furthermore, biodiesel can be produced by lipases without any organic solvents: thus, the enzymatic transesterification process is environmentally benign [3, 11–15].

In our previous work, a new process for biodiesel production using a mixture of immobilized *Rhizopus oryzae* and *Candida rugosa* lipases was developed successfully and optimal conditions were obtained [12, 16]. However, because of mass transfer inhibition, biodiesel production process using a mixture of immobilized lipases was carried out in small scale. To enhance the efficiency of biodiesel production, the effects of a mixture of immobilized lipases in batch and continuous reaction processes were investigated.

Materials and Methods

Materials

C. rugosa lipase and 3-aminopropyltriethoxysilane was purchased from Sigma Chemical Co. (USA), and *R. oryzae* lipase and glutaraldehyde were purchased from Fluka Co. (Switzerland). Silica gel was obtained from the Grace Davison Co. (USA). All other chemicals were of reagent grade.

Batch and Continuous Production of Biodiesel

Biodiesel was produced using a mixture of immobilized *C. rugosa* and *R. oryzae* lipases. Three ~9 mmol of methanol was initially added to the reaction medium containing 3 mmol of soybean oil and 0.53 g (20% *w*/*w* oil) of a mixture of immobilized *C. rugosa* and *R. oryzae* lipases. An equivalent amount of methanol was then added once or twice during biodiesel production.

The continuous production of biodiesel was carried out in a packed-bed reactor system (Fig. 1). A jacketed glass column (ID 40 mm×100 mm) was used as a packed-bed reactor,

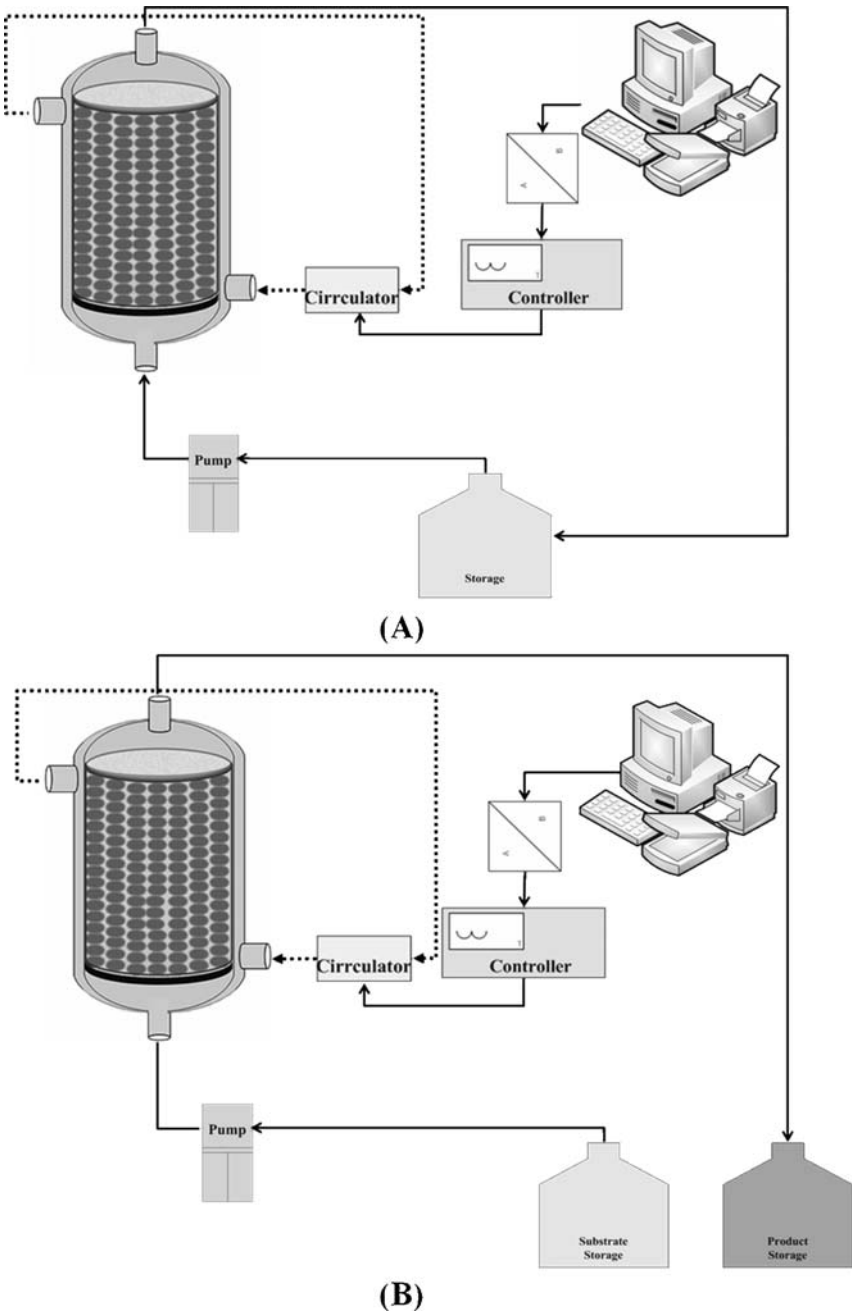

Fig. 1 Schematic diagram of circulation and continuous production process for biodiesel production using immobilized and co-immobilized lipases: **a** circulation process and **b** continuous process

where the immobilized lipases (100 g) were charged. Temperature was controlled at 45 °C by circulating water in the water jacket.

Analytical Method

Biodiesel was analyzed using a GC M600D (Younglin. Co. Ltd., Korea) with an HP-innowax 1909IN-133 column (30 m×25 μm, Agilent, USA). Samples were collected from the reaction mixture and then centrifuged to obtain the upper layer. One microliter of the treated sample was injected into the GC, and the column temperature was raised from 150 to 180 °C at a rate of 15 °C min^{-1}, and then from 180 to 240 °C by increasing the temperature at a rate of 5 °C min^{-1}, after which the temperature was maintained at 240 °C for 1 min. The injector and the detector temperature were both set at 260 °C, respectively.

Results and Discussion

Batch Production of Biodiesel Using Stepwise Reaction Method by a Mixture of Immobilized Lipases

Biodiesel was produced using a mixture of immobilized *C. rugosa* and *R. oryzae* lipases (1:1). In our previous studies, the deactivation of lipase can be induced by accumulated methanol and many immobilized lipases were used to increase the conversion yield [5, 7]. Therefore, in order to prevent lipase deactivation caused by high methanol concentration, the concentration of methanol ranging from 3 to 6 mmol was initially added to the reaction medium containing 3 mmol of soybean oil and 0.53 g of the mixture of immobilized lipases and then added twice more during biodiesel production [5, 7, 11]. Biodiesel was produced at 45 °C and 250 rpm for 4 h. Three ~6 mmol of methanol was added to the reaction medium every 1~2 h during biodiesel production. As shown in Fig. 2, when 4.5 mmol of methanol was added every 1.5 h, biodiesel conversion was 98.33% at 4 h. However, when 6 mmol of methanol was added every 2 h, biodiesel conversion was only 73.56%. In the

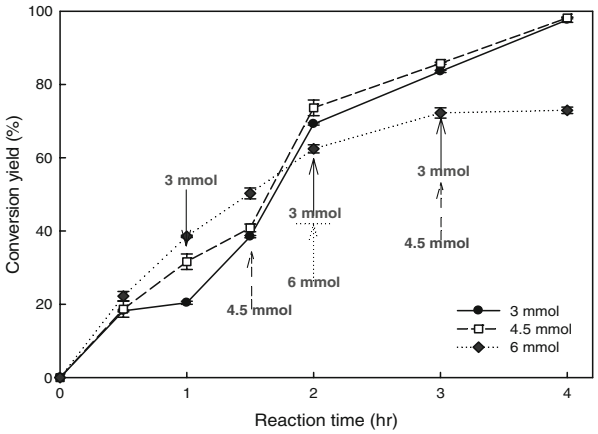

Fig. 2 Biodiesel production using various stepwise reaction methods by a mixture of *R. oryzae* and *C. rugosa* lipases (1:1)

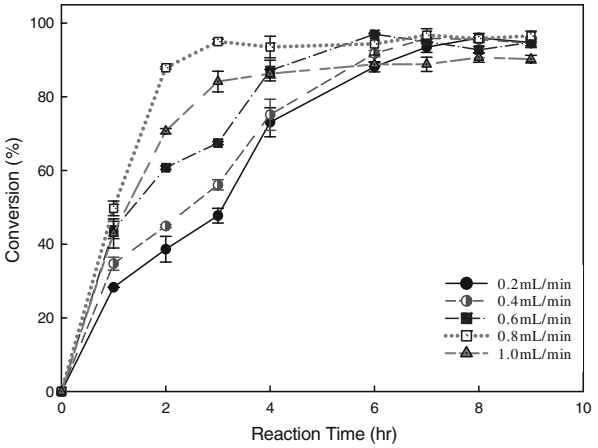

Fig. 3 Biodiesel production by a mixture of *C. rugosa* and *R. oryzae* lipases in packed-bed reactor using circulation reaction method

former case, immobilized lipases were deactivated by accumulated methanol in the reaction medium. This result implies that initial concentration of methanol is important factor for the maintenance of lipase activity [5, 11]. Moreover, accumulated methanol in the reaction medium should be decreased for high activity of lipase. According to these results, it is the practical method of methanol addition is to initially add 4.5 mmol of methanol to the reaction medium and then to add 4.5 mmol of methanol is added every 1.5 h during biodiesel production.

Continuous Production of Biodiesel in a Packed-Bed Reactor by a Mixture of Immobilized Lipase

In our previous studies, biodiesel was produced in various processes, but the inhibition by mass transfer became an issue for process scale-up [11, 15, 16]. In packed-bed reactor system, substrate was passed on catalysts layer directly and contact surface of substrate and

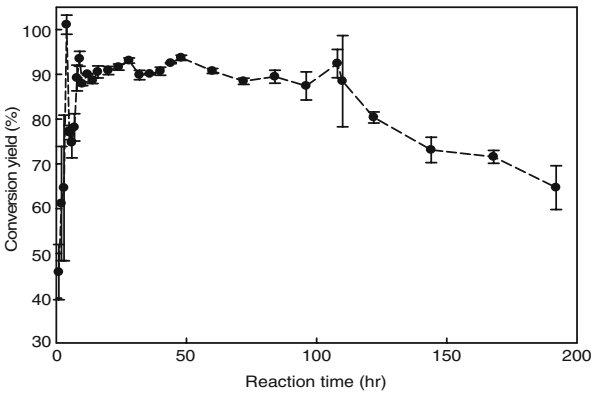

Fig. 4 Continuous production of biodiesel by immobilized and co-immobilized *C. rugosa* and *R. oryzae* lipases in packed-bed reactor

catalyst can be increased [17, 18]. Therefore, in this study, packed-bed reactor system was applied to increase the mass transfer. Biodiesel was produced continuously in a packed-bed reactor at optimized reaction conditions. Figure 3 shows the effect of the feed flow rate on the conversion yield of biodiesel by a mixture of immobilized *C. rugosa* and *R. oryzae* lipases using circulation reaction method. The biodiesel production increased as the feed flow rate increased to 0.8 mL/min, and then maximum conversion yield was 97.98% at the feed flow rate 0.8 mL/min at 3 h. However, at 0.6 and 1.0 mL/min of the feed flow rate, reaction rate for accomplishment of maximum conversion yield increased to 7 and 8 h, respectively. This result implies that the feed flow rate is important factor on the conversion yield of biodiesel.

The packed-bed reactor was also employed for the long-term operation. Figure 4 shows the continuous production of biodiesel in a packed-bed reactor at optimal reaction and feed flow rate of 0.8 mL/min. The conversion yield of biodiesel by immobilized lipases was maintained at about 90% for longer than 108 h. However, as accumulated glycerol increased in packed-bed reactor, conversion yield rapidly decreased to 61.22%. These operation time and conversion yield are significantly long and high, compared with other enzymatic processes in packed-bed reactor [18–20]. Therefore, these immobilization processes were effective for the continuous production of biodiesel. Base on the results of long-term operation and high level of biodiesel conversion in a packed-bed reactor, it is suggested that a continuous process by immobilized *C. rugosa* and *R. oryzae* lipases has potential for application to industrial biodiesel production

Conclusion

In this study, biodiesel was produced continuously by a mixture of immobilized *C. rugosa* and *R. oryzae* lipases (1:1). In circulation system, as the feed flow rate increased to 0.8 mL/min, conversion yield of biodiesel increased and then maximum conversion yield of biodiesel reached to 97.98% at the feed flow rate 0.8 mL/min. Moreover, in long-term operation system, the conversion yield of biodiesel by immobilized lipases was maintained at about 90% for longer than 108 h. Base on the results of long-term operation and system, it is suggested that this continuous process by immobilized lipases has a potential for application of scale-up to industrial biodiesel production.

Acknowledgements This study was supported by the Brain Korea 21 program issued by the Ministry of Education, Korea.

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