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Process Development using Co-immobilized Lipases for Biodiesel Production

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Abstract. Biodiesel has become more attractive recently because of its environmental benefits and the fact that is made from renewable resources. Among various processes for transesterification, enzymatic process has many advantages: low energy consumption, simple separation process and simple processes composition. A new process for biodiesel production using a mixture of *Rhizopus oryzae* and *Candida rugosa* lipases was developed successfully. In this study, biodiesel production from Jatropha and waste oil was investigated in a packed-bed reactor using co-immobilized lipases. Immoblilzed lipases were packed in a jacketed glass column (ID 25mm x 130mm). Temperature was controlled at 45℃ by circulating water. In circulation process, the maximum conversion yield was about 80 % at the feed flow rate 0.8 mL/min for 24 h. When the continuous process was operated for 72 h under the optimized conditions for biodiesel production, the normal conversion yield was about 90 % at the feed flow rate 0.1 mL/min.

Keywords: biodiesel, lipase, co-immobilization, transesterification, continuous process.

1. Introduction

Demand for oil has increased dramatically cosmopolitanly with the rise in use of vehicles. However, after a treaty proposed in the Kyoto Protocol in Kyoto, in 1997, restrictions on the use of pollutant started, and the policy of bioenergy obligations was strongly forced to meet standards in World Summit on Sustainable Development held in Johannesburg, in 2002. Therefore, the development of alternative energy is urgently needed. Even though lots of renewable energy is being produced, there are difficulties in putting to practical use of it [1-3]. However, biodiesel has similar disposition to diesel, and there is no need to transform it to be used as a fuel for vehicles [6]. Most of all, the reason why bioenergy is getting the spotlight is that it rarely produces pollution. Biodiesel, fatty acid methyl ester, is produced by transesterification. Biodiesel is being produced by synthesis with the use of acid-base catalysis. However, there is an engine corrosion triggered by catalysis in the process of using acid-base catalysis, so it needs multistage process and neutralization cleaning process [12]. Moreover, difficulties of collection of by-products, contamination caused by waste water, and the costs of disposal become serious concerns. Therefore, the need for eco-friendly new process which is capable for meeting a demanding for Biodiesel is being on the rise. For these reasons, enzymatic process which is using lipase as catalysis in the process of making biodiesel is in the spotlight. This process has advantages to reduce the costs and save energy in temperate condition compared to chemical process [3-6]. Particularly, it does not cause saponification due to not affected by free fatty acid (FFA) which is applicable for nonedible crop. So, it turns out to solve the food problem. In addition to these, the enzymatic process which does not contain catalysis that corrodes engine can simplify the process and the

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costs of Biodiesel could be reduced by using of glycerol acquired as a by-product. However, in the enzymatic process, low conversion rate, low reaction rate and the high-priced lipase are blocking a commercialization [12]. Thus, the purposes of studies being carried out nowadays is development of new continuous process that augments conversion rate, reaction rate and reacting dose by using lipase with high activity to commercialize enzymatic process [3, 5].

2. Materials and methods

2.1. Materials

C. rugosa lipase, *R. oryzae* lipase, 3-Aminopropyltriethoxysilane, and Glutaraldehyde were purchased from Sigma Chemical Co. (USA). MOPs-free acid was supplied by Bio Basic Inc (Canada). Silica gel was obtained from the Grace Davison Co. (USA). All other chemicals used were of reagent grade.

2.2. Biodiesel process design

The continuous process was performed in a packed-bed reactor. Fig. 1 shows a packed-bed reactor system of improved biodiesel production process. The reactor 1 and 2 were composed of a water jacketed glass column (ID 25mm x 130mm). Temperature was controlled at 45 ℃ by circulating water in the water jacket. Oil, water and methanol were mixed with an agitator and preheated in substrate tank 3 and 4. Mixture enters the reactor using a peristaltic pump. Biodiesel production was investigated in a packed-bed reactor using co-immobilized lipases (20g).

2.3. Analytical method

Determination of ester content was analyzed based on EN 14103. The fatty acid methyl ester (FAME) contents were analyzed using gas chromatography (GC) M6000D (Younglin. Co. Ltd., Korea) equipped with a column (30 m × 25 μm, HP-INNOWAX 1909IN-133 , Agilent, USA) [5]. The sample injected volumes were 1μL, the split injector was used with a split ratio of 50:1 and a temperature of 250℃. The oven temperature was raised from 140 ℃ to 245 ℃ at a rate of 5 ℃/min, and finally was maintained for 10min at 245℃. The FID detector was set at 250℃. Methyl heptadecanoate included as the internal standard for GC.

FAME was calculated using the following equations.

$$C = \frac{(\sum A) - A_{mh}}{A_{mh}} \times \frac{C_{mh} - V_{mh}}{m} \times 100$$

$\sum A$ is the total peak area from the methyl ester in C_{14} to that in $C_{24:1}$;

A_{mh} is the peak area corresponding to methyl heptadecanoate;

C_{mh} is the concentration, in milligrams per millilitre, of the methyl heptadecanoate solution being used;

V_{mh} is the volume, in millilitres, of the methyl heptadecanoate solution being used;

m is the mass, in milligrams, of the sample.

3. Results and discussion

3.1. Circulation process of biodiesel production using co-immobilized lipase

In our previous work, biodiesel was produced using a mixture of *Rhizopus oryzae* lipase and *Candida rugosa* lipase was successfully developed and the optimal conditions investigated [7]. In addition, biodiesel production from jatropha and waste oil was investigated in a packed-bed reactor using co-immobilized lipases [5]. Flow rate of the important parameters during the continuous transesterification carried out in the packed-bed reactor. The circulation process feed flow rate was increased from about 78% to 97% with an increase of conversion from 0.4mL/min to 0.8mL/min. It implies that the feed flow rate is important factor on the conversion of biodiesel [8, 9]. Previous studies based on the optimized process jatropha oil and waste oil had a circulation process [5]. Shown in Fig. 1 the FAME contents were about 80.0% during 24 h of continuous process. The result was shown that higher FAME conversion yield than previous experimental of Tamalampudi S. et al. They used jatropha oil for biodiesel production and the result was about 80% FAME contents for 60h [11]. Conversion of waste oil than jatropha oil was lower because waste oil was its high

content of the FFA(Free Fatty Acid). Usually the oil for transesterification reaction should be less than 0.5% FFA content, but waste oil is greater than the range [9, 10].

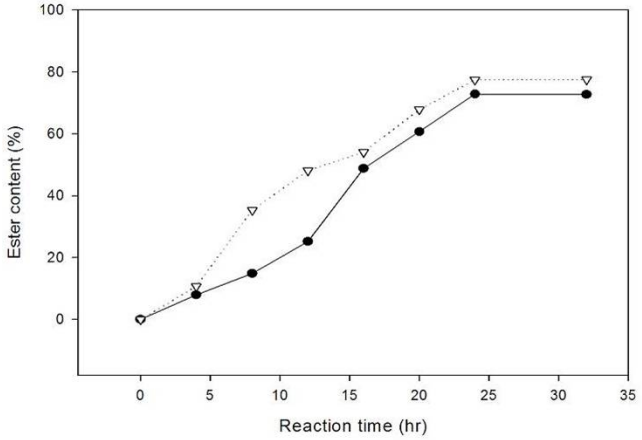

Fig. 1 Circulation production of biodiesel by a mixture of *C. rugosa* and *R. oryzae* lipases in packed-bed reactor: jatropha oil (▽), waste oil (●). Experiments were carried out triplicate and the variations were less than 5%.

3.2. Continuous process scale-up of biodiesel production used packed bed reactor

Continuous process to produce in large quantities to biodiesel, scale-up process was required. In our previous work, various processes (batch process, circulation process and continuous process) were produced as a biodiesel. In spite of using lots of co-immobilized lipase, however, the amount that was converted to biodiesel was not enough [5]. In an effort to solve these problems, we did scale-up continuous process by using two reactors. Before we could see reaction, circulation reactor operated circulation process until biodiesel conversion yield reached about 60% (Fig. 1) in a mine substrate tank containing oil. Since then, we opened T-valve and some of oil in mine substrate tank circulated continuously and others were sent to continuous reactor to be turned into biodiesel by undergoing one more reaction process. The reason why two reactors were used was to increase conversion yield and the amount of oil converted to biodiesel in the process of circulation processing. Moreover, high conversion yield with few amount could be gained because each reactor was stuffed with 20g of lipase. In the process of reaction, reactants (oil, methanol, and water) were added from serve tank to main tank. At this time, a conversion yield of main tank was not reduced, but maintained at 60% by circulation process. As denoted in Figure 2, jatropha oil and waste oil, were adjusted in continuous process. The reactants were exhausted in both serve substrate tank and main substrste tank after 72hours reaction. At this point, the conversion yield was maintained by 90%. Likewise, FFA(ferr fatty acid) was higher in waste oil than in jatropha oil, so it was considered that a conversion yield was reduced because substrate was inhibited [9, 10].

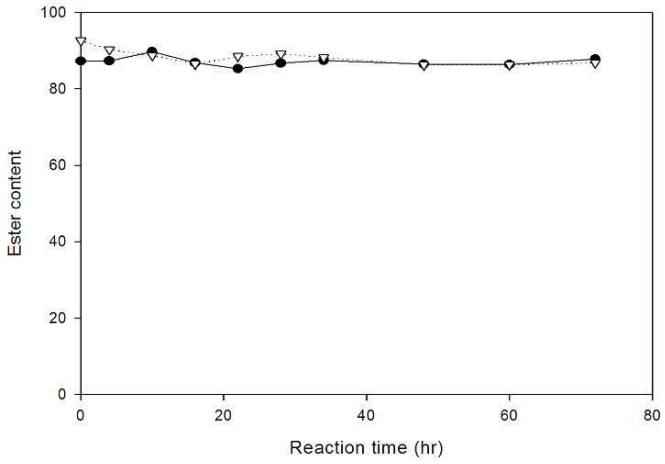

Fig. 2 Continuous production scale-up of biodiesel by a mixture of *C. rugosa* and *R. oryzae* lipases in packed-bed reactor: jatropha oil (▽), waste oil (●). Experiments were carried out triplicate and the variations were less than 5%.

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5. References

[1] Shimada Y, Watanabe Y, Sugihara A, Tominaga Y. Enzymatic alcoholysis for biodiesel fuel production and application of the reaction to oil processing. *Journal of Molecular Catalysis B: Enzymatic*. 2002, **17**:133-142.

[2] Muniyappa PR, Brammer SC, Noureddini H. Improved conversion of plant oils and animal fats into biodiesel and co-product. *Chemical and Biomolecular Engineering Research and Publications*. 1996, **56**:19-24.

[3] Tan T, Lu J, Nie K, Deng L, Wang F. Biodiesel production with immobilized lipase: A review. *Biotechnology Advances*. 2010, **28**:628-634.

[4] Lee DH, Park CH, Yeo JM, Kim SW. Lipase Immobilization on Silica Gel Using a Cross-linking Method. *Journal of* Industrial *and Engineering Chemistry*. 2006, **12**:777-782.

[5] Lee JH, Kim SB, Park CH, Tae BS, Han SO, Kim SW. 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. *Applied Biochemistry and Biotechnology*. 2010, **161**:365-371.

[6] Dizge N, Keskinler B. Enzymatic production of biodiesel from canola oil using immobilized lipase. *Biomass and Bioenergy*. 2008, **32**:1274-1278.

[7] Lee DH, Kim JM, Shin HY, Kang SW, Kim SW. Biodiesel production using a mixture of immobilized *Rhizopus oryzae* and *Candida rugosa* lipases. *Biotechnology and Bioprocess Engineering*. 2006, **11**:522-525.

[8] Feng Y, Zhang A, Li J, He B. A continuous process for biodiesel production in a fixed bed reactor packed with cation-exchange resin as heterogeneous catalyst. *Bioresource Technology*. 2011, **102**:3607-3609.

[9] Santacesaria E, Tesser R, Serio MD, Guida M, Gaetano D, Agreda AG. Kinetics and Mass Transfer of Free Fatty Acids Esterification with Methanol in a Tubular Packed Bed Reactor: A Key Pretreatment in Biodiesel Production. *Industrial & Engineering Chemistry Research*. 2007, **46**:5113-5121.

[10] Ma F, Clements LD, Hanna MA. The effects of catalyst, free fatty acids, and water on transesterification of beef tallow. *Transactions of the ASAE*. 1998, **41**:1261-1264.

[11] Tamalampudi S, Talukder MR, Hama S, Numata T, Kondo A, Fukuda H. Enzymatic production of biodiesel from Jatropha oil: A comparative study of immobilized-whole cell and commercial lipases as a biocatalyst. *Biochemical Engineering Journal*. 2008, **39**:185-189.

[12] Bajaj A, Lohan P, Jha PN, Mehrotra R. Biodiesel production through lipase catalyzed transesterification: An overview. *Journal of Molecular Catalysis B: Enzymatic*. 2010, **62**:9-14.