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Novel strategy for lipase-catalyzed synthesis of biodiesel using blended alcohol as an acyl acceptor

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ABSTRACT

As a novel strategy, blended alcohols consisting of methanol and ethanol were used as acyl acceptors for biodiesel synthesis from soybean oil by lipase-catalyzed transesterification. Based on enzyme screening, Novozym 435 from *Candida antarctica* was selected for the reaction. The effects of the molar proportion of methanol in the blended alcohol, temperature, and enzyme loading were investigated for optimization of the reaction. In addition, the relative consumption rates of methanol and ethanol during the transesterification were studied. Among six proportions tested, 0 (100 mol% ethanol), 20, 40, and 60 mol% methanol in the blended alcohols exhibited high yields of biodiesel. For the optimum temperature, 30 °C was selected. The highest yield of biodiesel, over 95 wt%, was obtained at an enzyme loading of 5–10 wt% loading. In the lipase-catalyzed transesterification, the reactivity of methanol was significantly higher than that of ethanol.

1. Introduction

Biodiesels are defined as monoalkyl esters of long-chain fatty acids derived from vegetable oils or animal fats, for use in diesel engines [1,2]. Biodiesel can be used in any mixture with petroleum diesel as it has very similar characteristics, but it has lower exhaust emissions. Biodiesel has better properties than those of petroleum diesel in that it is renewable, biodegradable, non-toxic, and essentially free of sulfur and aromatics [3]. The use of biodiesel fuel has the potential to reduce levels of pollutants and of potential or probable carcinogens [4].

The fundamental reaction in biodiesel production is the transesterification reaction, which can be catalyzed either chemically or enzymatically. Enzymatic transesterification has certain advantages over the chemical catalysis of transesterification, because it allows less energy intensity and easy recovery of glycerol [5–8].

In the biodiesel production, methanol is most widely used because of its economic feasibility and accessibility in most countries [9,10]. In addition, with a chemical catalyst, methanol makes high equilibrium conversion due to highly reactive intermediate methoxide [11,12]. However, for enzymatic transesterification, methanol has a critical problem that methanol has a stronger denaturing activity compared with longer aliphatic alcohols [13,14]. Chen and Wu [15] reported that the degree of deactivation is inversely proportional to the number of carbon atoms in the alcohol. In general, lipases are known to have a propensity to act on long-chain fatty alcohols more readily than on short-chain ones [16,17]. To overcome drawbacks of methanol, trials using stepwise addition of methanol to reaction mixtures have been performed in previous studies [18–21].

For the biodiesel production, ethanol is also used as an acyl acceptor because ethanol less likely triggers enzyme deactivation. From the literatures, the efficiency of the enzymatic transesterifiaction of triglycerides with methanol (methanolysis) was low compared to that of ethanol in systems without a solvent. Mittelbach and Tritthart [22] reported transesterifications of the primary alcohols, i.e., methanol, ethanol, and 1-butanol, with and without solvents; the ester yields from ethanol and 1-butanol were significantly higher than that from methanol. Abigor et al. [23] also found that in the conversion of palm kernel oil to alkyl esters using *Pseudomonas cepacia* lipase, ethanol gave the highest conversion, i.e., 72%, whereas the methyl ester yield from methanol was only 15%. However, ethanol is still used not as often as methanol because of its higher cost.

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Methanol and ethanol, which are acyl acceptors used for synthesis of biodiesel, have their own advantages and disadvantages. Thus, using a blended alcohol of methanol and ethanol as an acyl acceptor for lipase-catalyzed transesterification could be an innovative strategy for overcoming the drawbacks of each alcohol.

In this study, lipase-catalyzed transesterification was carried out to explore the effect of a blended alcohol of methanol and ethanol on biodiesel synthesis in a solvent-free system. Enzyme screening of six lipases from different sources was performed. The optimum reaction parameters with the selected lipase were also determined. The effect of the proportion of methanol in the blended alcohol was thoroughly studied as the major parameter, and other parameters such as temperature and enzyme loading were also investigated. In addition, changes in the molar proportion of methanol in the residual blended alcohol during the reaction were also explored.

2. Materials and methods

2.1. Materials

The refined soybean oil (Sajo Haepyo®, Seoul, Korea) used in this study was purchased from a local market (Seoul, Korea). Novozym 435 from *Candida antarctica*, Lipozyme TL IM from *Thermomyces lanuginosa*, and Lipozyme RM IM from *Rhizomucor miehei* were purchased from Novo Nordisk Bioindustry Ltd. (Seoul, Korea). Free-type lipase OF from *Candida rugosa* was purchased from Meito Sangyo Co., Ltd. (Nagoya, Japan). Free-type lipase PS from *Pseudomonas fluorescence* and lipase AYS from *C. rugosa* were purchased from Amano Enzymes (Troy, VA, USA). Tricaprin was purchased from Sigma Aldrich Co. (Seoul, Korea). The other chemicals used in this study were of analytical grade unless otherwise noted.

2.2. Lipase-catalyzed transesterification

For the lipase-catalyzed transesterifications, blended alcohols of methanol and ethanol were prepared for use as acyl acceptors. The molar proportions of methanol in the blended alcohols were 0, 20, 40, 60, 80, and 100 mol%; 0 mol% methanol indicates 100 mol% ethanol. The reaction was performed in a 25 mL screw-capped Erlenmeyer flask. A substrate (3 g) consisting of a mixture of soybean oil and the blended alcohol in a molar ratio of 1:3 were combined in the flask and a lipase (2.5 to 15% of total substrate weight) was added. The reaction mixture was agitated in an orbital shaker water-bath (model G76; New Brunswick Scientific Co., Inc., New Brunswick, NJ, USA) operating at 300 rpm and temperatures from 20 to 50 °C. Individual samples were removed at selected times and analyzed. All trials were conducted in duplicate.

2.3. Analysis of products

When the reaction was complete, the product mixtures were filtered through a 0.45 μm nylon microfilter to remove lipase. The yield of fatty acid esters in the reaction mixture was determined using samples (30 mg) of the products obtained using the different reaction conditions dissolved in 1 mL of chloroform. Tricaprin (1 mg) was used as an internal standard. A gas chromatograph (model 3800; Varian, Palo Alto, CA, USA) equipped with a DB-1ht column (15 m × 0.25 mm i.d.; J&W Scientific, Folsom, CA, USA) and a flame ionization detector (FID) was used for analysis. The column temperature was initially held at 120 °C for 3 min, increased to 370 °C at a rate of 20 °C/min, and held for 3 min at 370 °C. The carrier gas was helium, and the total gas flow rate was 50 mL/min. The injector and detector temperatures were both 370 °C. The yield of biodiesel was calculated as follows:

$$\text{Yield of biodisel (wt\%)} = \frac{\text{fatty acid methyl and ethyl ester (mg)}}{\text{reaction mixture (mg)}} \times 100$$

2.4. Methanol and ethanol residues after transesterification

To determine the molar proportion of methanol in the residual blended alcohol in the reaction mixture by gas chromatography, a distillation was firstly carried out using a distillation apparatus (Catalog No. 751351-0005, Kimble Chase, Vineland, NJ, USA). In brief, the reaction mixture (4 g) and toluene (8 mL) were placed in a 50 mL round-bottomed flask, which was connected to a Dean–Stark distillation receiver. The temperature of the condenser in the distillation system was kept at −20 °C using a cooling circulator (model CW-05G; Jeio Tech, Seoul, Korea). The alcohols and toluene in the reaction mixture were distilled by heating with a heating mantle. The distilled fraction was analyzed by gas chromatography, using a gas chromatograph (model 3800; Varian, Palo Alto, CA, USA) equipped with a Supelcowax 10 (30 m × 0.25 mm i.d.; Sigma Aldrich Co., Seoul, Korea) column and a FID. The column was initially held at 40 °C for 2 min, and programmed to rise to 115 °C at a rate of 5 °C/min. The carrier gas was helium, and the total gas flow rate was 50 mL/min. The injector and detector temperatures were both 220 °C.

3. Results and discussion

3.1. Enzyme screening

There have been numerous reports on biodiesel synthesis using various lipases. In particular, three immobilized lipases, namely Novozym 435 [24–26], Lipozyme TL IM [27–29], and Lipozyme RM IM [18] have been studied widely. However, in these previous studies, transesterifications were carried out with only a single acceptor such as methanol or ethanol.

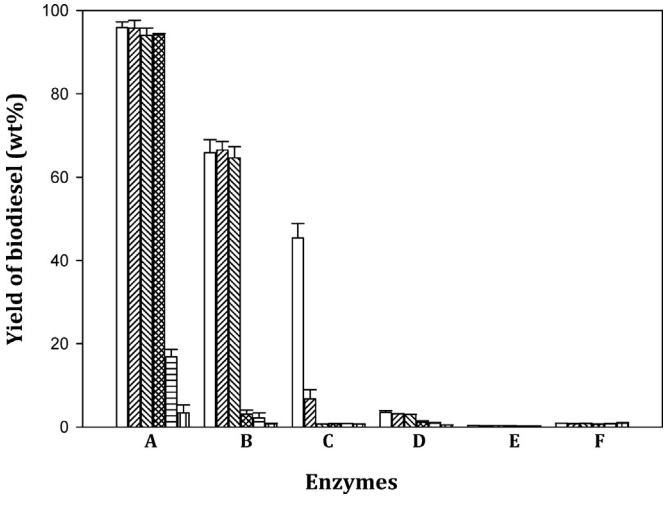

Fig. 1. Enzyme screening for synthesis of biodiesel by transesterification of soybean oil and blended alcohols. Enzymes: A, Novozym 435 (from *Candida antarctica*); B, Lipozyme RM IM (from *Rhizomucor miehei*); C, Lipozyme TL IM (from *Thermomyces lanuginose*); D, lipase PS (from *Burkholderia cepacia*); E, lipase AYS (from *Candida rugosa*); F, lipase OF (from *Candida rugosa*). The reaction was performed at a molar ratio of 1:3 (soybean oil to alcohol), temperature of 40 °C, and enzyme loading of 5% of total substrate weight for 12 h. The molar proportions of methanol in the blended alcohols were 0 (100 mol% ethanol) (□), 20(▨), 40(▥), 60(▧), 80(▩), and 100 mol% (▤).