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Biodiesel production from crude canola oil by two-step enzymatic processes

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ABSTRACT

Crude canola oil (CCO) contains about 100–300 ppm of phospholipids, which have shown negative effects on biodiesel/buffer solution phase separation, resulting in low biodiesel production yield. Therefore, phospholipids should be removed before transesterification by a degumming process for efficient production of biodiesel. In this study, two-step enzymatic processes (degumming and transesterification) were carried out for the production of biodiesel from CCO. Degumming of CCO was performed using phospholipase A2 as a degumming reagent. The initial phospholipid content was reduced to less than 5 ppm by enzymatic degumming. The effects of three formulations of enzyme catalyst on the efficiency of transesterification were investigated. As a result, conversion rates of degummed CCO to fatty acid methyl esters (FAME) were 68.56%, 70.15%, and 84.25%, respectively. Lipase formulation composed of a 1:1 (vol:vol) enzyme mixture of *Rhizopus oryzae* and *Candida rugosa* showed the best performance among those tested. In order to recover and reuse the lipase catalyst efficiently, a 1:1 enzyme mixture of *R. oryzae* and *C. rugosa* was immobilized on silica gel. The immobilized lipase was used in subsequent transesterification optimization experiments. Optimization of transesterification was performed by response surface methodology (RSM). A total of 20 experiments based on RSM were carried out, and the optimal reaction conditions appeared to be 24.4% (w/w) immobilized catalyst, 13.5% (w/w) buffer solution, and 15.8% (w/w) methanol based on oil mass. Conversion rate of degummed CCO to FAME was determined to be 88.9% under optimal conditions.

1. Introduction

As global warming and the oil crisis have become more serious, alternative energies such as bioethanol, biodiesel, solar cells, and fuel cells have attracted much attention. Especially, biodiesel has many advantages for the improvement of environmental pollution and production of renewable resources. Generally, biodiesel is produced via a transesterification process utilizing vegetable oil or animal fat as a raw material [1–4].

Canola oil, a vegetable oil used in biodiesel production, contains approximately 300 ppm of phospholipids. Phospholipids contained in canola oil decrease the efficiency of biodiesel separation, resulting in low productivity of transesterification. These problems are easily resolved by a degumming process, which involves extraction of gum from vegetable oil and Fig. 1 shows these problems and improvement [5].

Transesterification processes are generally based on chemical, physical, and enzymatic methods. The physical process is economically infeasible due to heavy energy consumption, whereas the chemical process has high reaction efficiency but is characterized by a complicated reaction step and generation of waste water. In comparison, the enzymatic process using immobilized enzyme has many advantages, including low energy consumption, simplified post-processing, and production of high purity glycerol. However, the enzymatic process has not been commercialized due to its slow reaction rate and expensive enzyme cost [6–9].

In our previous work, a new process for biodiesel production using a mixture of *Rhizopus oryzae* and *Candida rugosa* lipases was successfully developed and optimal conditions were investigated. The mixing of 1, 3-specific lipase and non-specific lipase removed the acyl-migration step which is rate determining step of three steps of biodiesel production mechanism, and enzyme activity was notably enhanced [10–13].

In this study, Degumming process was carried out by using crude canola oil containing 120 ppm of phospholipids and phspholipase A2 as a degumming reagent. The transesterification process utilized free lipase and co-immobilized lipase (from *R. oryzae*, *C. rugosa*). Consequently, optimization of factors such as

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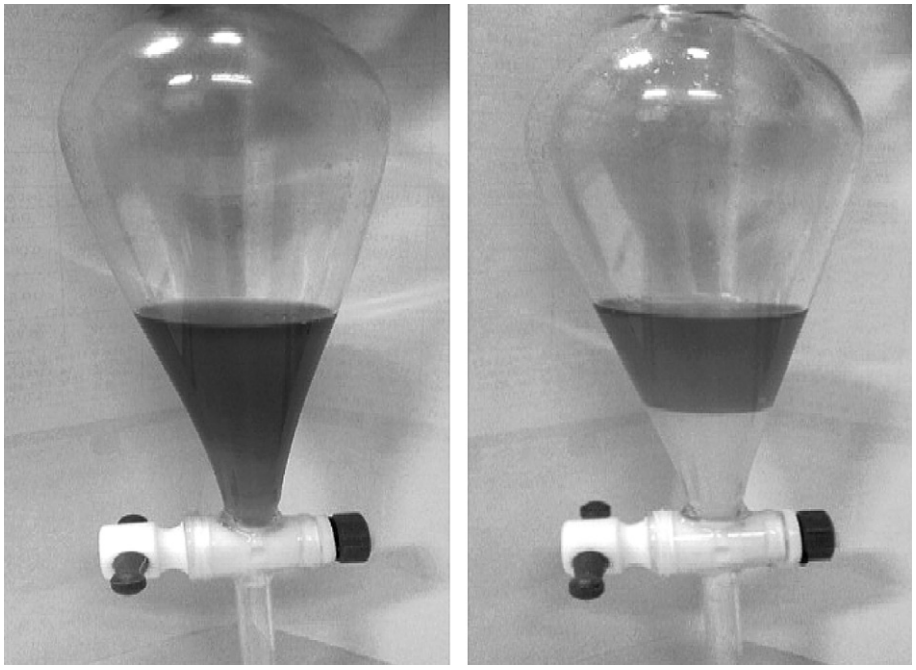

Fig. 1. Effect of phospholipids on biodiesel separation: biodiesel from CCO (left), degummed CCO (right).

the amounts of methanol, buffer, enzyme concerned with transesterification was successfully performed by response surface methodology (RSM).

2. Materials and methods

2.1. Materials

Crude canola oil (CCO) was used as a raw material for degumming and transesterification process. The properties of CCO included 0.64 mgKOH/g of acid value and 138 ppm of phosphorus contents. Phospholipase A2 and *C. rugosa* lipase, *R. oryzae* lipase were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Silica gel was provided by Chong Kun Dang Pharmaceutical Co. (Grace Davison, USA). All other chemicals were of reagent grade.

2.2. Degumming of CCO using phospholipase A2

Exactly 2 ml of 1.0 M citrate buffer (pH 5.5) was added to CCO (100 g), followed by mixing at 70 °C for 30 min with constant stirring (300 rpm). Three milliliters of distilled water and 50 μl of phospholipase A2 (50 ppm w/w oil) were added to the mixtures, followed by homogenization at 10,000 rpm for 1 min. The mixture was then incubated at 50 °C with stirring at 500 rpm for 4 h.

2.3. Preparation of free enzyme solution

Three formulations of free lipase (*R. oryzae*, *C. rugosa*, 1:1 (vol:vol) mixed *R. oryzae* and *C. rugosa*) were investigated for their effects on transesterification in order to select the immobilized lipase. One gram of *R. oryzae* and *C. rugosa* lipase were diluted in distilled water at 25 °C with stirring at 300 rpm for 1 h, followed by centrifugation at 4000 rpm for 15 min at 4 °C. The supernatant was used for the transesterification experiment.

2.4. Transesterification of canola oil using free enzyme solution

Degummed canola oil (10 g) was initially added to the reaction medium containing 4.5 ml of methanol (1.5 mol/mol of oil), 1 ml of free lipase solution (10% w/w oil), and 5 ml of 0.5 M sodium phosphate buffer (pH 7). The mixture was then incubated at 37 °C with stirring at 150 rpm for 60 h. An equivalent amount (1.5 mol/mol of oil) of methanol was then added three times (0, 20, 40 h) during the transesterification process since excessive methanol exerted a negative effect on the lipase catalytic reaction.

2.5. Preparation of activated silica gel for lipase immobilization

One gram of dry silica gel was mixed with 10% 3-aminopropyltriethoxysilane in 20 ml of acetone, followed by incubation at 50 °C for 2 h at 150 rpm. The silica gel was then washed with distilled water and dried at 60 °C for 2 h. The dried silica gel was suspended in 20 ml of 1 mM phosphate buffer solution (pH 7). Two milliliters of 25% (w/v) glutaraldehyde was added to the solution, followed by incubation at 20 °C for 2 h to activate the silica gel. The activated silica gel was then washed with distilled water and dried at 60 °C for 2 h.

2.6. Immobilization of lipase

The activated silica gel (1 g) was mixed with 0.5 ml of lipase solution, followed by incubation at 20 °C for 24 h. Immobilized lipase was recovered by filtration, washed with distilled water, and then dried overnight at room temperature.

2.7. Immobilized lipase activity measurement

Ten milliliters of isooctane containing 10% (w/v) soybean oil were added to 10 ml of 50 mM sodium phosphate buffer (pH 7), followed by the addition of 200 mg of immobilized lipase into the prepared solution. The reaction mixture was incubated at 50 °C for 30 min at 150 rpm. Two milliliters of supernatant were transferred