바이오디젤 생산을 위한 가교 방법을 통한 Lipase 고정화

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Lipase immobilization using cross-linking method for esterification of oil to biodiesel

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INTRODUCTION

Lipase can be used as an efficient catalyst for biodiesel(FAME; Fatty Acid Methyl Ester) production. Biodiesel has drawn attention as a non-toxic, biodegradable and renewable source of energy [1]. Conventionally the synthesis of alkyl ester is accomplished by chemical transesterification from which short reaction times and high yields are obtained [1]. However, pretreatment of the substrate is required when water is present, drawbacks such as difficulties in the recovery of catalyst and glycerol, as well as high energy requirements are disadvantages in alkali or acid catalyzed process [1]. As biodiesel shows scope to replace petrolium-based fuels partially there is an increasing number of research reports on biodiesel production by biological means [2]. Generally, lipases are commonly used in an immobilized state for easy separation from the product and reuse. In this study, ws used immobilized lipase for the production of biodiesel from oil. Cross-linking method on the silica-gel was used for immobilization because it serves stronger binding and stability than other methods.

The objects of this study are to develop a suitable immobilization method of lipase and its application of biodiesel production from oil with suitable activity and stability.

EXPERIMENTAL

1. Materials

Lipase OF (Meito, Japan) was gifted by Mugunghwa Co. Ltd. and NOVO 435 was gifted by Enzymtech. Silica-gel was gifted by Chong Gun Dang Pharm., 3-aminoproxyethoxysilane and glutaraldehyde are purchased by Sigma and Fluka, respectively. Other used reagents were experimental grade or higher.

2. Methods

(1) Immobilization method of Lipase

One gram of dry silica-gel was pretreated with 35% hydrogen peroxide solution and mixed with 3-aminopropylethoxysilane (10% in 20 mL dissolved acetone). The suspension was incubated at 50℃ for 2 h with constant mixing, washed throughly with water before drying 120℃ for 2 h, and then 2%(v/v) glutaraldehyde was added to the silica gels suspended in 100 mM of phosphate buffer (pH 7). Finally, enzyme solution was added. The suspension was stirred at 25℃ for 2 h and the immobilized lipase recovered by filteration was washed. After resuspending in 100 mM phosphate buffer (pH 7), the immobilized enzyme was analyzed.

(2) Assay of lipase activity

Lipase activity was measured by Winkler & Stuckmann method [3]. Thirty miligram of *p*-nitrophenyl palmitate, 207 mg of sodium deoxycholate and 100 mg gum arabic was dissolved in solution of 90 mL of 100 mM phosphate buffer (pH 7.0) and 10 mL of iso

propyl alcohol. Substrate solution (2.4 mL) and 0.1 mL of lipase solution were mixed and incubated in 37℃ water bath for 15minutes. The absorbance of the solution was measured by UV-VIS spectrophotometer at 410 nm. One unit of lipase activity was indicated by 1 μM of *p*-nitrophenol / mL · min.

(3) Determination of the degree of silanization

The degree of silanization was determined by ninhydrin method [4]. To 1 mL of 3-APTES solutions in distilled water, 1 mL ninhydrin reagent was added, and reacted in a covered boiling water bath at 100℃ for 20min. The reactants were then cooled below 30℃ in a cold-water bath and the contents were diluted with 5 mL of 50%(v/v) ethanol / water. The absorbance level at 570nm was measured.

(4) Determination of the degree of glutaraldehyde modification

The degree of glutaraldehyde modification was determined by Bersthorn's method [5]. To 2mL of glutaraldehyde solution, 2mL of 0.4%(w/v) MBTH was added, and reacted in a covered boiling water bath at 100℃ for 3min. The reactants were then cooled rapidly in an ice bath. After adding 5mL of 0.4%(w/v) $FeCl_3$, gently shaked at 25℃ for 15 min. Absorbance level at 634nm was measured.

(5) Protein quantitative analysis and determination of the amount of binding protein

The amount of protein was determined by the Bradford method [6]. The amount of protein bound to the carriers was determined by the difference between initial and residual protein concentrations.

(6) Determination of degree of conversion from oil to biodiesel

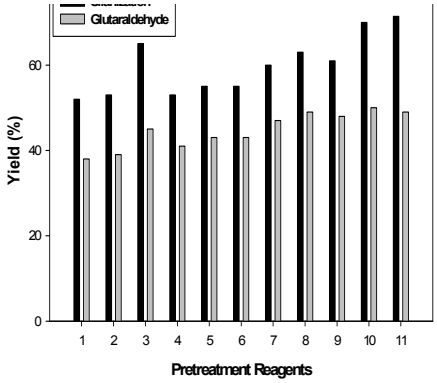
Fatty acid methyl ester (biodiesel) is analysed by gas chromatography M600D (Younglin, Seoul, Korea) with Innowax column.

RESULTS AND DISCUSSION

1. Immobilization of lipase

(1) Pretreatment

Some reagents were used for pretreatment of silica-gel. Silanization and Glutaraldehyde modification yield is Criteria of immobilization of enzyme. Fig. 1 shows that 35% hydrogen peroxide is suitable for pretreatment.



Pretreatment Reagents	
1	None
2	Sonication
3	H_2SO_4+H_2O_2
4	H_2O_2 5%
5	H_2O_2 10%
6	H_2O_2 15%
7	H_2O_2 20%
8	H_2O_2 25%
9	H_2O_2 27%
10	H_2O_2 30%
11	H_2O_2 35%

Fig. 1 Silanization and glutaraldehyde modification yield with various pretreatment reagents.

(2) Silanization

Three kinds of silanization reagents were tested for silanization. 3-APTES showed good activity through all concentrations (Fig. 2). When 10% of 3-APTES was used, it showed 4.5 U / 100 mg carrier.

(3) Glutaraldehyde modification

Glutaraldehyde is widely used as a coupling reagent for immobilization of enzyme. However, certain impurities in the glutaraldehyde solution or Schiff base formation may cause reduction of activity of immobilized enzyme.

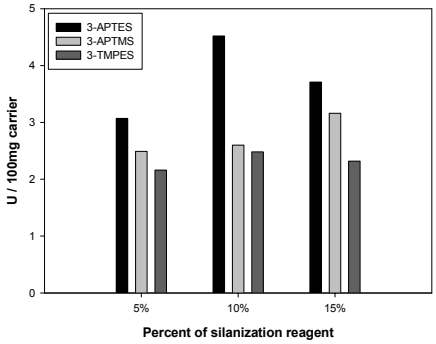


Fig. 2 Immobilized lipase activity with various kinds of silanization reagents and concentration.

Adequate concentration of glutaraldehyde is needed for immobilization. Different glutaraldehyde concentrations (1-5%) were tested. Fig. 3 shows that 2% concentration of glutaraldehyde is proper.

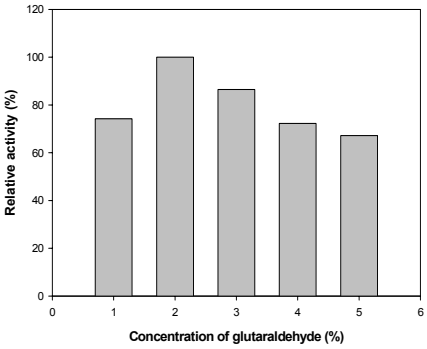


Fig 3. Relative activity with various concentrations of glutaraldehyde.

(4) Stability of immobilized lipase

The activities of immobilized lipase and commercially immobilized lipase(NOVO 435) are compared during 20 times of reuse (Fig. 4).

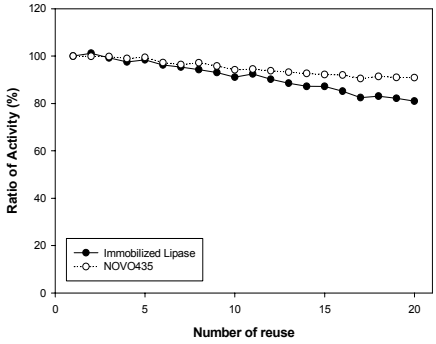


Fig. 4 Comparison of reuse between immobilized Lipase OF and NOVO 435.

After 20 times of hydrolysis reaction, immobilized Lipase OF was maintained about 80% of initial activity. In case of NOVO 435, 90% of activity was maintained. In the report of Tien-Chieh Hung et. al. [7], immobilized lipase with similar method was shown 80% of

initial activity after 10 times of reuse. In view of so far achived, immobilized lipase is relatively stable.

(5) Immobilization and esterification reaction of Lipase OF

Lipase OF was immobilized on silica-gel with predetermined conditions. One gram of carrier was pretreated. 3-APTES (10%) was used for silanization and 2% glutaraldehyde was treated as a coupling reagent. Lipase OF with 22.4 U of activity and 0.5mg/ml protein was loaded on the treated carrier. After reaction, 0.46 mg of protein was immobilized on the carrier and it showed 7.8 U/g-matrix of activity. The protein binding yield was 92%, and activity recovery yield was 34.8%.

For esterification reaction, 1:1 molar ratio of olive oil (9.65 g) and methanol (0.35 g) mixture and 1 gram of immobilized lipase were used. After 12 h and 24 h of reaction, 1molar ratio of methanol (0.35 g) was fed, respectively. Finally, 41.2% of olive oil was converted to biodiesel after 30 h (Fig. 5).

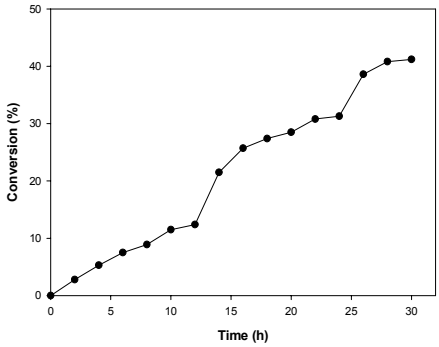


Fig. 5 Biodiesel conversion using immobilized Lipase OF.

CONCLUSIONS

Lipase OF was immobilized on silica-gel surface. Hydrogen peroxide with 35% concentration was adequate for pretreatment reagent and 10% of 3-APTES was chosen for silanization. For coupling reagent, 2% glutaraldehyde was proper. Immobilized lipase showed good stability. It maintained 80% of initial activity and showed better results compare to other study. About 92% of protein was immobilized and 34.7% of activity was recovered. Esterification reaction using immobilized lipase with 7.8 U/g-matrix showed about 41.2% of conversion ratio.

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