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Production of biodiesel fuel from triglycerides and alcohol using immobilized lipase

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Abstract

Transesterification reaction was performed using triglycerides and short-chain alcohol by immobilized lipase in non-aqueous conditions. The long-chain fatty acid ester, which is the product of this reaction, can be used as a diesel fuel that does not produce sulfur oxide and minimize the soot particulate. Immobilized *Pseudomonas fluorescens* lipase showed the highest activity in this reaction. Immobilization of lipase was carried out using porous kaolinite particle as a carrier. When methanol and ethanol were used as alcohol, organic solvent like 1,4-dioxane was required. The reaction could be performed in absence of solvent when 1-propanol and 1-butanol were used as short-chain alcohol. The activity of immobilized lipase was highly increased in comparison with free lipase because its activity sites became more effective. Immobilized enzyme could be repeatedly used without troublesome method of separation and the decrease in its activity was not largely observed.

Keywords: Enzyme biocatalysis; Lipase; Transesterification; Immobilized enzyme; Biodiesel fuel

1. Introduction

The biodiesel fuel from vegetable oil does not produce sulfur oxide and minimize the soot particulate one-third times in comparison with the existing one from petroleum. Because of these environmental advantages, biodiesel fuel can be expected as a substitute for conventional diesel fuel. At present, biodiesel has been produced chemically using vegetable oil in Europe and USA. However, requirement of removal of catalyst and excessive energy requirements are the major drawbacks of such chemical process. Because enzymatic methods may conquer the problems for the reaction, several researches have been carried out using lipase [1–8]. Enzymes are generally effective biocatalyst for having substrate specificity, functional group specificity and stereo specificity in aqueous media. Besides, chemical reactions can be also raised directly using lipase in an organic media. The esterification reaction of alcohol and carboxylic acid is difficult in aqueous media, whereas this reaction takes place easily in organic solvent. However, the production of biodiesel fuel by enzymatic method has not been adopted industrially, because of the high cost of enzyme catalyst. In order to use the enzyme catalyst repeatedly, the process of immobilization must be carried out using appropriate method. In this research, the transesterification reaction for the production of

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biodiesel fuel was performed from triglycerides and short-chain alcohol using immobilized lipase obtained by effective method employing an inorganic porous particle as a carrier in non-aqueous condition.

2. Experimental

2.1. Materials

Lipase powder from *Pseudomonas fluorescens* (Lipase AK), *P. cepacia* (Lipase PS), *Mucor javanicus* (Lipase M), *Candida rugosa* (Lipase AY) and *Rhizopus niveus* (Newlase F) were used as enzymes. These enzymes were received from Amano Pharmaceutical Company. Triolein (Tokyo Kasei Co.) and safflower oil (Nisshin Seiyu Co.) were used as substrate triglyceride. Methanol, ethanol, 1-propanol and 1-butanol were used as short-chain alcohols without being furthermore purified. The carrier particle used for immobilization of enzyme was porous kaolinite that was baked after granulation. This porous kaolinite particle, Toyonite 200-M, was offered by Toyo Denka Kogyo Co.

2.2. Methods

2.2.1. Immobilization of enzyme

In a l00 ml beaker, 0.6 g of lipase was dissolved with 60 ml of phosphate buffer solution (pH 7). The aqueous solution obtained was then filtered under reduced pressure. Then, 3 g of Toyonite 200-M was mixed with the filtrate in a 200 ml flask and shaken continuously for 6 h by a shaker at room temperature. The solution was filtered under reduced pressure. Consequently, the residue was obtained which is immobilized lipase on Toyonite 200-M particle. The immobilized enzyme produced was perfectly dehydrated under high vacuum of 8×10^{-3} Pa for 3 days. The quantity of enzyme immobilized onto the carrier particle was determined by using Lowry method.

2.2.2. Enzymatic transesterification reaction

Triolein (30 g, 33.9 mmol) and alcohol were put into a l00 ml three-necked flask fitted with cooler and thermometer. The molar ratio of triolein:alcohol chosen was 1:3. Immobilized lipase (in the case of immobilized *P. fluorescens* lipase, 2.84 g including 0.l g of lipase) or free lipase (0.l g) that was same amounts as including enzyme was added into it. Then, it was heated in an oil bath at constant temperature with continuous stirring. The conversion of product was analyzed by gas chromatogram (Shimazu GC-14A) equipped with silicone SE-30 as a column, taking sample at fixed interval. The column temperature was set at 220°C. The injector and detector temperatures were set at 250 and 260°C, respectively. Water content in the reaction mixtures was analyzed by Karl Fisher moisture titrator (Kyoto Denshi Kogyo MKS-500).

3. Results and discussion

When methanol was used as a substrate, it did not dissolve well with triolein or safflower oil. Therefore, appropriate organic solvent was necessary to carry out the transesterification reaction. 1,4-Dioxane, benzene, chloroform and tetrahydrofuran were used as solvent and immobilized *P. fluorescens* lipase as catalyst at 50°C. The enzymatic activity was highest with 1,4-dioxane. The enzymatic activity increases with the high amount of 1,4-dioxane, which is shown in Fig. 1. The activity of immobilized *P. fluorescens* lipase was extremely low with benzene, chloroform and

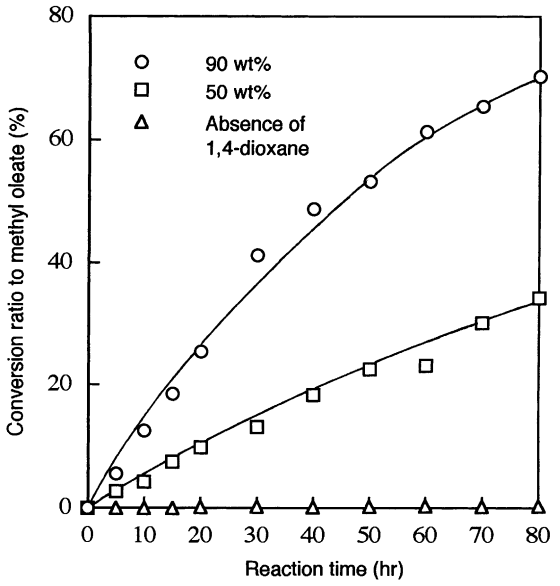

Fig. 1. Effect of additional amount of 1,4-dioxane on production of methyl oleate by immobilized *P. fluorescens* at 50°C.

tetrahydrofuran. When ethanol was used, the reaction did not take place homogeneously as in methanol. For the practical application of making diesel fuel from the discarded vegetable oil, it is not desirable to use solvent. It is because, after completion of the reaction the solvent must be removed by distillation, extraction and so on, which need additional energy and effort. The reaction, on the contrary, occurred homogeneously, when propanol or butanol was used as substrate. Therefore, organic solvent is not necessary in this reaction.

3.1. Types of lipase

Lipases can be used as biocatalyst in this transesterification reaction. The reactions were performed using immobilized *P. fluorescens*, *P. cepacia*, *M. javanicus*, *C. rugosa* and *R. niveus* lipases with 1-propanol and 1-butanol as substrate at 50°C. The each immobilized enzyme was added in such an amount that the total amount of lipase in immobilized enzyme analyzed by Lowry method became same. The conversion to propyl oleate and butyl oleate is shown in Figs. 2 and 3, respectively. Immobilized *P. fluorescens* lipase showed the highest enzymatic activity among

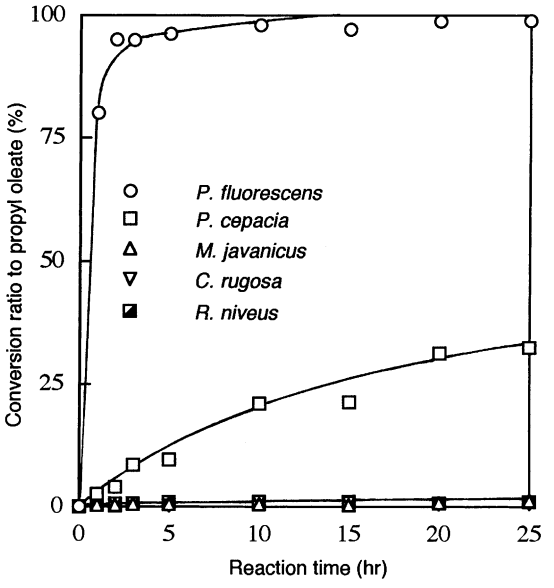

Fig. 2. Difference of reaction behavior by the kind of immobilized lipase on production of propyl oleate at 50°C.

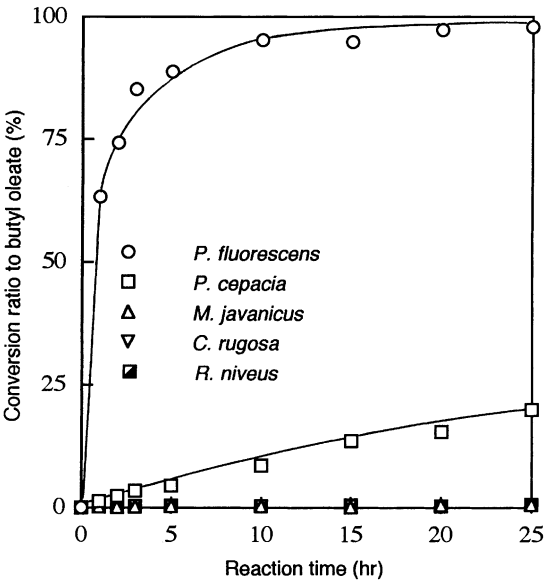

Fig. 3. Difference of reaction behavior by the kind of immobilized lipase on production of butyl oleate at 50°C.

others. The reaction was faster with 1-propanol than 1-butanol. The reaction did not proceed significantly in case of immobilized *P. cepacia* lipase, as the conversion ratio to propyl oleate was only 32% and that of butyl oleate was 20% even after 25 h. Rest of the immobilized lipases was inactive. Thus, immobilized *P. fluorescens* lipase was selected for the further study.

Immobilization of lipase was carried on the surface of Toyonite 200-M, which is a porous particle having the average particle diameter of 155 μm, the specific surface 52 m^2/g and the average pore diameter 0.06 μm. Methyl methacrylate group was chemically introduced on the surface of carrier using silane-coupling agent. In our previous research, immobilized *P. cepacia* lipase using Toyonite 200-M was employed in transesterification reaction of low molecular weight compounds [9] or ring opening polymerization reaction of lactones [10] in organic solvent. It has been seen that the activity of immobilized lipase became remarkably higher than free lipase. In an organic medium, free lipase is aggregated at considerable degree. Therefore, most of the active site of an enzymes are confined inward.

It is explained that when an enzyme is immobilized, its active sites become more effective, as each

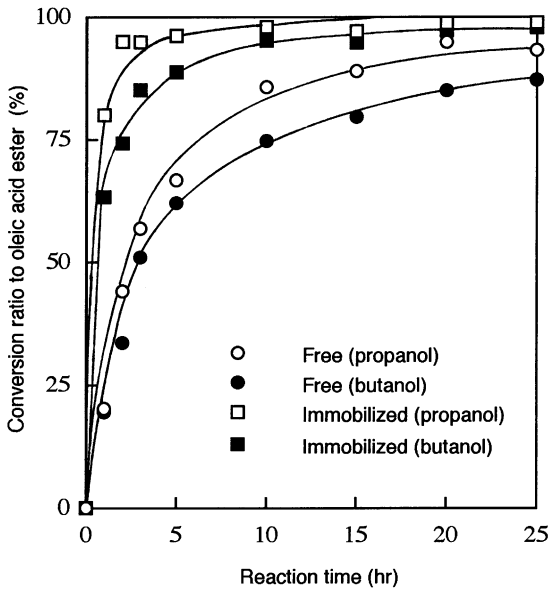

Fig. 4. Effect of immobilization of *P. fluorescens* on production of propyl oleate and butyl oleate at 50°C.

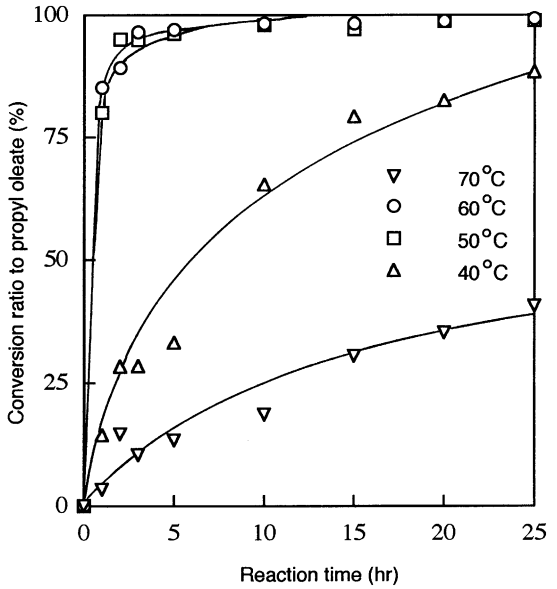

Fig. 5. Effect of reaction temperature on production of propyl oleate by immobilized *P. fluorescens* lipase.

and every enzymes are dispersed on the surface of the carrier particle. In this research, the transesterification reaction was carried out using free and immobilized *P. fluorescens* lipase with 1-butanol and 1-propanol as alcohol. The results of reaction are shown in Fig. 4. In this experiment, the immobilized lipase was used in such an amount that the total amount of lipase in immobilized enzyme became same to that of free lipase. In case of 1-proponal as substrate, the reaction has completed within 10 h when immobilized enzyme was used. However, the conversion to product was 90% at 25 h with free *P. fluorescens* lipase. In case of 1-butanol also, the rate of reaction was seen higher with immobilized lipase than free one.

Generally, the activity of immobilized enzyme is decreased in an aqueous system, regardless the method of immobilization. In contrast, it is noticed that the activity of an immobilized enzyme is higher than free enzyme in this study.

3.2. Effect of temperature on the reaction

The effect of temperature on this transesterification reaction was examined at the temperature range from 40°C to 70°C with both free and immobilized lipase. The behavior of reaction with 1-propanol using free and immobilized *P. fluorescens* lipase is shown in Figs. 5 and 6, respectively. The conversion ratio to

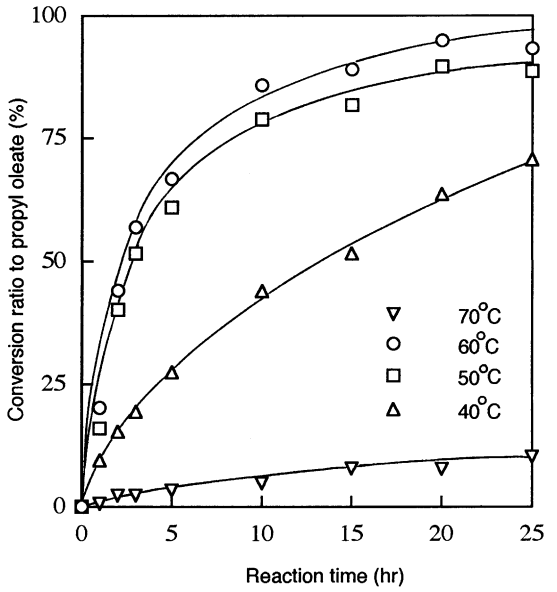

Fig. 6. Effect of reaction temperature on production of propyl oleate by free *P. fluorescens* lipase.

propyl oleate was observed highest at 60°C, whereas the activity highly decreased at 70°C in case of free *P. fluorescens* lipase. When immobilized enzyme was used, the rate of reaction seems to be increased at the same temperature that can be seen comparing Figs. 5 and 6. In particular, the comparison of conversion ratio at 70°C reveals the fact that thermal stability of enzyme increases because of immobilization. The conversion ratio to propyl oleate was almost same at 50 and 60°C when immobilized lipase was used.

3.3. Effect of water content on the reaction

It is well-known that the activity of an enzyme in non-aqueous medium is affected by water content in it. In this research, the effect of water content on enzymatic activity was examined adding a small amount of water in the reaction mixture of 1-praponal and triolein as substrates and immobilized *P. fluorescens* lipase as an enzyme. The reaction was performed taking water content ranging from 0 to 1 wt.% of the total amount of reaction mixture with the constant temperature of 50°C and the duration of 0.5 h. Since the conversion ratio of this reaction increases linearly up to 0.5 h, the conversion ratio is taken at 0.5 h as the comparison of enzymatic activity. The result is shown in Fig. 7. It is observed that the conversion ratio was highest at 0.3 wt.% of water content. The activity of an enzyme at 0.3 wt.% is about 17% higher than in absence of water. It is further realized that the enzymatic activity gradually decreased at more than 0.3 wt.% of water content.

3.4. Repeated use of immobilized enzyme

The main advantage of immobilization of an enzyme is that an expensive enzyme can be repeatedly used. It was observed, how the reaction behavior changes when an immobilized enzyme is used repeatedly. The change of enzymatic activity examined by immobilized *P. fluorescens* lipase at 1 h is shown in Fig. 8. The activity of an enzyme was decreased to about two-third, when it was used for second time but the decrease in its activities could be hardly observed in its further use. When an immobilized enzyme was used for the first time, some amount of enzymes was desorbed. The desorbtion of an enzyme could not be observed after further repeated use. Therefore, immobilized enzymes can be used time and again. Immobilized enzyme can be separated by the process of decantation, after completion of the reaction and does not require special method of separation.

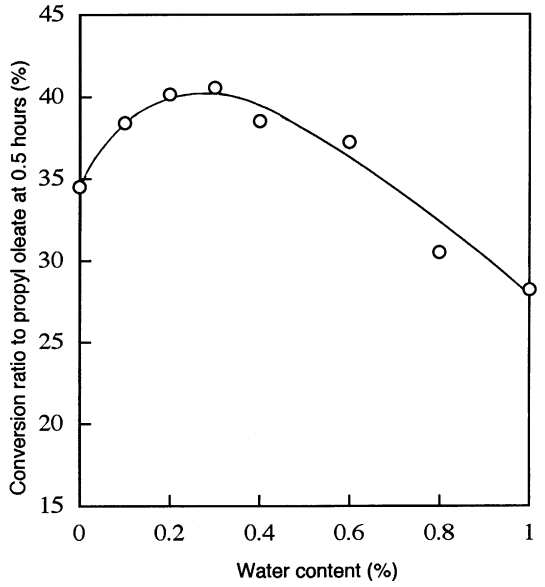

Fig. 7. Effect of water content on enzymatic activity of immobilized *P. fluorescens* lipase at 50°C.

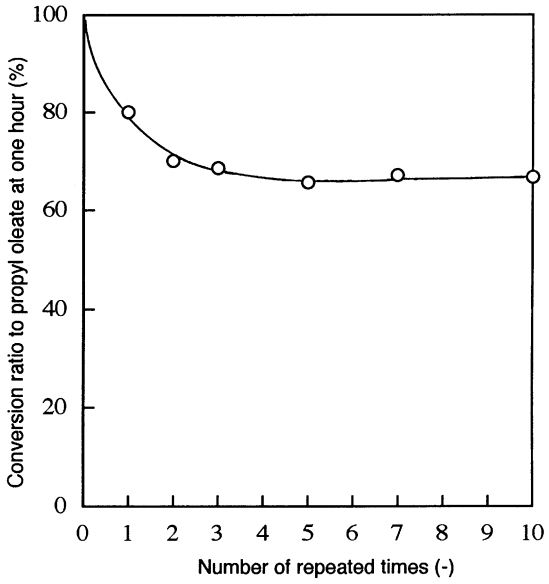

Fig. 8. Change of enzymatic activity by repeated use of immobilized *P. fluorescens* lipase at 50°C.

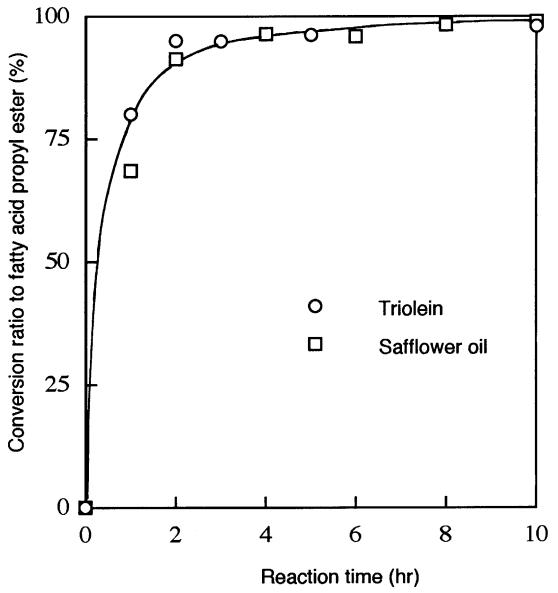

Fig. 9. Comparison of triolein and safflower oil in transesterification using immobilized *P. fluorescens* lipase.

3.5. When safflower oil was used as substrate

In order to find the differences in the behavior of the reaction between safflower oil and triolein, transesterification reaction was carried out using 1-propanol as alcohol. The enzymatic activity of immobilized *P. fluorescens* lipase was examined. The results of reaction of triolein and safflower oil were comparatively studied, which is shown in Fig. 9. In both cases, the behavior of reaction was observed to be similar.

4. Conclusions

Transesterification reaction was carried out using triglyceride triolein (which is one of the main components in some vegetable oil) and short-chain alcohol by some kind of immobilized lipase. In order to be able to use repeatedly, enzyme catalyst must be immobilized by appropriate method, since the enzymes are generally very expensive. Immobilization of lipase was carried out using porous kaolinite particle (Toyonite 200-M) as a carrier. Methanol, ethanol, 1-propanol and 1-butanol was used as alcohol. Immobilized *P. fluorescens* lipase has the highest activity than other immobilized lipases in these reactions. The activity of immobilized lipase was highly increased in comparison with free lipase. When methanol and ethanol were used as alcohol, the reactions need an appropriate organic solvent like 1,4-dioxane. On the other hand, the reaction could be performed without solvent when 1-propanol and 1-butanol was used. The decrease in activity of the immobilized enzyme was hardly observed even it is repeatedly used. The transesterification reaction was also carried out using safflower oil. The results closely resemble with the reaction of triolein. It is expected that the long-chain fatty acid ester produced by this reaction can be used as a diesel fuel that does not produce sulfur oxide and minimize the soot particulate.

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