# Kinetics of glycerol effect on biodiesel production for optimal feeding of methanol

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**Abstract**−The enzymatic production of biodiesel has been considered as an eco-friendly process. *Candida antarctica* lipase B (CalB) has been studied for its application for biodiesel production because of its high activity and stability. Enzyme deactivation caused by alcohol and effect of glycerol has to be resolved for the industrial application of this process. In traditional kinetic studies of biodiesel production, the effects of alcohol and oil were only considered in the kinetic equation, while the effect by glycerol was neglected. A new kinetic model incorporating glycerol effect is proposed in this paper. The proposed kinetic equation is applied by predicting the supplying rate of methanol in a fed-batch addition of methanol. The conversion rate was improved from 59.7% to 94.6% in a fed-batch by considering glycerol effect.

Key words: Biodiesel, Enzyme, Kinetic Model, Oil, Glycerol, Lipase

## INTRODUCTION

Development of renewable and clean energy sources has become an urgent task due to the serious consequences of the current energy crisis and environmental pollution. Considered one of the promising renewable energy sources for fuel, biodiesel has been thus widely studied [1]. It is being produced by methanolysis of triglycerides such as animal fats and plant oils. For the production of biodiesel, an alkali-catalysis process has been established because of its higher conversion of oils to methyl esters. This process, however, has several drawbacks, including the difficulty of glycerol recycling, removal of the catalyst, and wastewater treatment [2]. To overcome these drawbacks, an enzymatic process using lipase has recently been developed [3-6]. The lipase-catalyzed process overcomes the problems of the conventional process, such as energy-intensiveness, wastewater treatment, and difficulty in glycerol recovery [7,8]. There are three major components in the lipase-catalyzed process: oil, alcohol as the substrate, and lipase as the catalyst. Fatty acid methyl ester (FAME) content, the major component of biodiesel, can be increased by increasing the amount of methanol in the 0.1-1.5 molar equivalents of methanol to soybean oil. When more than 1 : 1.5 molar equivalents of methanol are added, the conversion to FAME starts to decrease significantly, mainly because of insoluble alcohol, even though the stoichiometric ratio is three to one to convert oil to FAME [9, 10]. When the amount of alcohol exceeds its solubility limits, methanol to oil molar ratios >1 [11], lipase becomes deactivated by insoluble alcohol that exists as droplets in the hydrophobic phase [12,13].

Researchers have reported that stepwise addition of methanol avoids much of the deactivation of lipase resulting from a high concentration of methanol [9,10]. In a fed-batch process, the methanol feeding rate can be maintained below the solubility limit by using

a kinetic model. Consequently, the enzyme can maintain its activity and thus promote the conversion in a repeated reaction cycle.

In addition to alcohol, glycerol decreases the enzyme activity [14,15] as well as increases their stability [9]. The glycerol inhibition is due to mass transfer limitation in immobilized enzymes. Glycerol forms a hydrophilic phase that is not completely miscible with oil. This hydrophilic phase serves to partition the alcohol from the oil phase, thereby decreasing the concentration of alcohol in the reaction medium and causing a concomitant decrease in the conversion rate [14]. Moreover, this glycerol-alcohol immiscible phase inhibits the enzyme reaction. During the course of transesterification, glycerol accumulates in the mixture to such an extent that the reactivity of the enzyme is decreased [14].

There are many kinetic studies in the literature of enzymatic processes [4,8,12,16,17]. For biodiesel production, however, most of the earlier kinetic studies described only the reaction rate containing alcohol and substrate oil [12,17]. Because glycerol is also produced during the reaction and the presence of glycerol decreases the rate and extent of conversion [14], the effect of glycerol has to be considered when modeling the reaction for biodiesel production. A new strategy of optimal feeding for biodiesel production is reported in this paper on the basis of reaction kinetics considering glycerol effect.

## MATERIALS AND METHODS

### 1. Materials

Soybean oil (Ottogi Corporation, Korea) consists of 99.5% triacylglycerol, 0.1% diacylglycerol, 0.2% monoacylglycerol, and under 0.1% free fatty acid. Immobilized lipase (triacylglycerol hydrolase, EC 3.1.1.3; Novozym 435, Novo Nordisk, Denmark) was used for methanolysis after incubation in soybean oil for 24 h at 40 °C. Analytical grade n-hexane and methanol were purchased from Fisher Scientific, UK. All other chemicals were of the highest purity available.

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## 2. Transesterification

Reactions were carried out in a 500 mL spinner flask filled with soybean oil at 40 °C. Methanol was separately incubated at 40 °C. Predetermined molar ratios of soybean oil and methanol were prepared and the reactions were initiated by the addition of 5% immobilized lipase (w/w soybean oil) [9,17]. Stirring of 150 rpm was maintained throughout the process. Pharmercia liquid chromatography PUMP P-500 and a programmable controller were used for feeding methanol accurately.

## 3. Sample Analysis

Sample analysis followed the Korean standard method KS M 2413. At regular intervals, a 1 ml sample was withdrawn from the reactor for subsequent analysis. A Hewlett Packard 6890 gas chromatograph equipped with a flame ionization detector (FID) and a DB-WAX fused silica capillary column (30 m×0.32 mm×0.25 μm) was used to determine the fatty acid methyl ester composition of the samples. A 50 mg aliquot was withdrawn from the sample bottle and transferred to a GC vial containing 10 mg of 99.7% methyl heptadecanoate (Fluka) as an internal standard and then mixed with 1 ml heptane. Sample volumes of 1 μl were injected into the GC. Injector and detector temperatures were set to 250 °C. The oven initiating temperature of 150 °C was held for 2 min, elevated to 250 °C at 5 °C/min, and then held for 18 min. The carrier gas was helium at 11 psig.

## 4. Feeding Strategy

Initially, 1 : 0.4 molar equivalents of substrate oil and methanol were supplied to the reactor, and lipase was added to start the reaction. Only the amount of consumed methanol, which was determined in following kinetic equations, was added to maintain the ratio of methanol and oil When the total methanol concentration remaining in the reactor (initial conc.+additional conc.) equaled three times the molar equivalent of the substrate oil, the methanol feeding was stopped.

In the case of adding methanol (A), the concentration can be expressed according to the following equations for a given time t:

$$SV = S_0 V_0 + ut \cdot \rho/Mw \tag{1}$$

$$dS/dt = (\rho/Mw - S) u/V \tag{2}$$

Where, S and $S_0$ are concentration of substrate and initial concentration of substrate, $V_0$ is initial volume, respectively. And 'ut d/Mw' and '$\rho$/Mw' are mol/L change after time t and density/molecular weight, respectively.

In the case of non-adding components such as oil (S) and product, the molar concentration after time 't' is given by the following equations:

$$SV = S_0 V_0 \tag{3}$$

$$u = dV/dt \tag{4}$$

$$dS/dt = -Su/V \tag{5}$$

From these equations, the concentration of each component in a fed-batch reaction mode can be expressed as follows:

Component S as oil (TAG):
$$dS/dt = F_S = -1/3F_k - Su/V \tag{6}$$

Component A as alcohol (Methanol):

$$dA/dt = F_A = -F_k + (\rho/Mw - A)u/V \tag{7}$$

Component P as biodiesel:
$$dP/dt = F_P = F_k - Pu/V \tag{8}$$

Component G as glycerol:
$$dG/dt = F_G = 1/3F_k - Gu/V \tag{9}$$

And volume after time (v) equal to $V_0 + \int u \, dt$ \tag{10}

The feeding rate of alcohol is related to the substrate concentration by Eq. (11):

$$u = (-)3/(\rho/Mw) \cdot d(SV)/dt. \tag{11}$$

In these equations, '$F_k$' denotes the initial reaction rate adapted from Al-Zuhair et al. [12]:

$$\upsilon = F_k = \frac{v_{max}}{1 + (K_{IA}/[A])[1 + ([S]/K_S)] + (K_{IS}/[S])[1 + ([A]/K_A)]}$$
$$= \frac{dP}{dt} \tag{12}$$

## RESULTS AND DISCUSSION

### 1. Kinetics of the Glycerol Effect

The changes of both the methanol and oil concentrations were measured to determine the constants in Eq. (12). Each reaction mixture had different molar ratios of soybean oil to methanol. The initial reaction rate for each substrate concentration was determined by the slope of a line that best fits to the product concentration versus time curve. The kinetic parameters for the initial reaction rates (v) were determined by a multiple regression method for each reaction mixture using MATLAB and EXCEL solvers. The resulting kinetic parameters ($V_{max}$, $K_S$, $K_A$, $K_{IS}$ and $K_{IA}$) are shown in Table 1.

During the course of the transesterification process, glycerol accumulated in the mixture and formed a layer on the surface of the immobilization matrix of the enzyme [15]. This could reduce the activity by limiting the diffusion of the substrate and product because of its insolubility in oil [15]. Glycerol decreased both the rate and extent of the conversion for biodiesel production [14]. To examine the glycerol inhibition, experiments were conducted under various initial concentrations of glycerol. The result was similar to the previous report [14]. Fig. 1(a) shows the conversion was reduced when the amount of glycerol increased. A conversion of only 37.5% was obtained at 0.8 mol of glycerol/*l*.

Most of the earlier kinetic studies were performed using the initial reaction rate [12,17]. Because product effect cannot be considered when using the initial reaction rate equation, it cannot sufficiently express the effect by the products. In other words, product inhibi-

Table 1. Numerical values of the kinetic parameters from the experiments

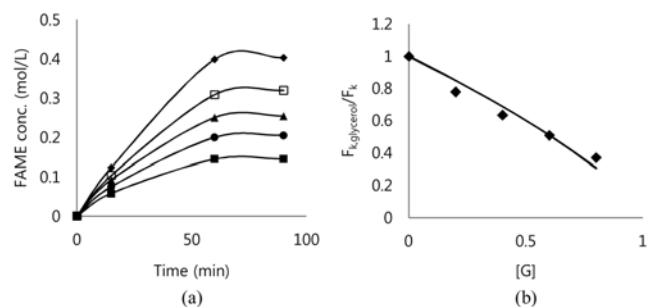| Parameter | Value |
|---|---|
| $V_{max}$ | $6.01 \times 10^{-3}$ mol/L min |
| $K_{IA}$ | $1.17 \times 10^{-9}$ mol/L |
| $K_S$ | $4.57 \times 10^{-2}$ mol/L |
| $K_{IS}$ | $1.07 \times 10^{-4}$ mol/L |
| $K_A$ | $2.03 \times 10^{-4}$ mol/L |

Fig. 1. The effect of glycerol in the production of biodiesel. Reaction mixtures of soybean oil/methanol (molar ratio 1 : 0.4) and 5% Novozyme 435 (w/w soybean oil) were incubated at 40 °C for 24 h and agitated at 150 rpm. (a) amount of added glycerol was 0 (◆), 0.2 (□), 0.4 (▲), 0.6 (●), or 0.8 (■) mol/L. (b) Fk,glycerol/Fk plotted against experimental data (◆) and a function of glycerol concentration; [G] (—).

tion is neglected in the initial reaction rate model. However, since glycerol affects both the extent and rate of conversion, the equation must be modified to include the glycerol term for the simulation of biodiesel production.

The rate of production '$F_{k, glycerol}$' can, therefore, be represented by the equation

$$F_{k, glycerol} = F_k \cdot f([G]) \tag{15}$$

The following equation, derived in the case of ethanol inhibition in alcohol fermentation, was adapted for this study [18,19]:

$$F_{k, glycerol} = F_k \cdot (1 - [G])^n \tag{16}$$

For very low concentrations of glycerol, $F_{k, glycerol}$ approaches $F_k$. The empirical exponent (n) was determined to be 0.74 from the ex-

periment shown in Fig. 1(b).

## 2. Model Validation in Batch

The equation for $F_{k, glycerol}$ (Eq. (16)) was applied to batch reactions and compared with the experimental results for validation. Fig. 2 shows the time courses of methanolysis of soybean oil for various methanol concentrations (molar ratio of oil : methanol=1 : 0.1-1 : 2) in a batch mode. The FAME content was increased with increasing amounts of methanol with 0.1-1.5 molar equivalents of methanol vs. soybean oil. The model equation successfully simulated the reaction profile. When methanol, higher than 1 : 1.5 molar equivalents, was added, the FAME content decreased significantly below the predicted value. In particular, the final FAME content was much lower than the predicted value in the reaction mixture consisting of a molar ratio of oil to methanol of 1 : 2 and 1 : 3. This result showed that the lipase was inactivated when the methanol content was above 1 : 1.5. The proposed model was suitable for low concentrations, but not for high concentrations of methanol. However, because the equation was used for concentrations below the 1 : 1.5 molar equivalents of methanol, which is below the inhibition concentration and usually adopted, this model gave a good result in our study and can be used for the simulation of biodiesel production.

## 3. Feeding in Fed-batch Mode

Using the proposed model equation ($F_{k, glycerol}$) and calculated kinetic parameters, the feeding rate of methanol was determined (Fig. 3). When the feeding rate of methanol was used without considering glycerol effect, the experimental result was different from the simulation result (Fig. 3(a)). The final FAME content was much lower than the simulation value. Without considering glycerol effect, the methanol feeding rate was determined only by the methanol and oil concentrations (Eq. (12)). Because the predicted reaction rate was higher than the experimental rate, the lipase was deactivated by an excess of methanol.

After the glycerol effect was incoporated, the simulation result accurately predicted the experimental result (Fig. 3(b)). These results
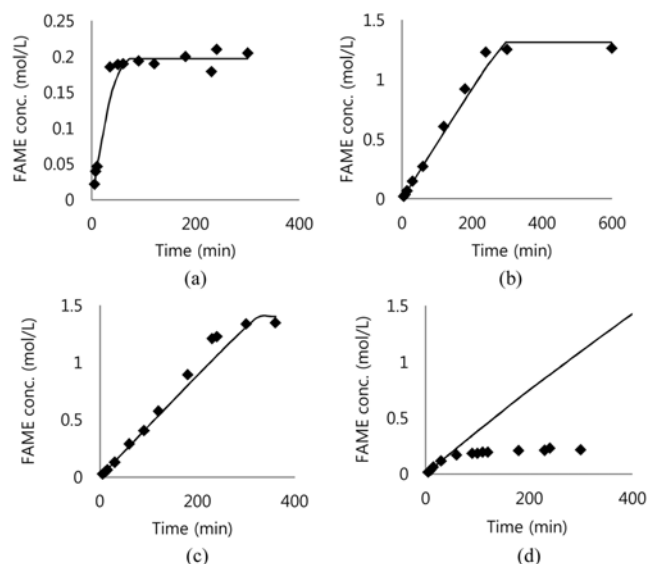


Fig. 2. Comparison of the model prediction (—) and experimental data for the reaction rate (◆). Reaction mixtures of soybean oil/methanol ratio was (a) 1 mol : 0.2 mol, (b) 1 mol : 1.4 mol, (c) 1 mol : 1.5 mol, (d) 1 mol : 2 mol and 5% Novozyme 435 (w/w soybean oil) were incubated at 40 °C for 24 h and agitated at 150 rpm.
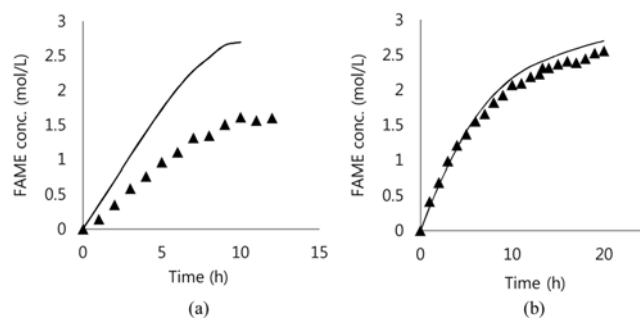


Fig. 3. The comparison of the model prediction and experimental data. (a) Fed-batch methanol feeding without considering glycerol inhibition. (b) Fed-batch methanol feeding considering glycerol inhibition. (b) Fed-batch methanol feeding considering glycerol inhibition. Initially the reaction mixture of soybean oil/methanol was 1 mol : 0.4 mol. Only the amount of consumed MeOH was added to maintain the ratio, and when the total methanol concentration was three times the substrate oil molar equivalents, the methanol feeding was stopped. (▲) Experimental data and (—) model prediction data in the fed-batch reaction.
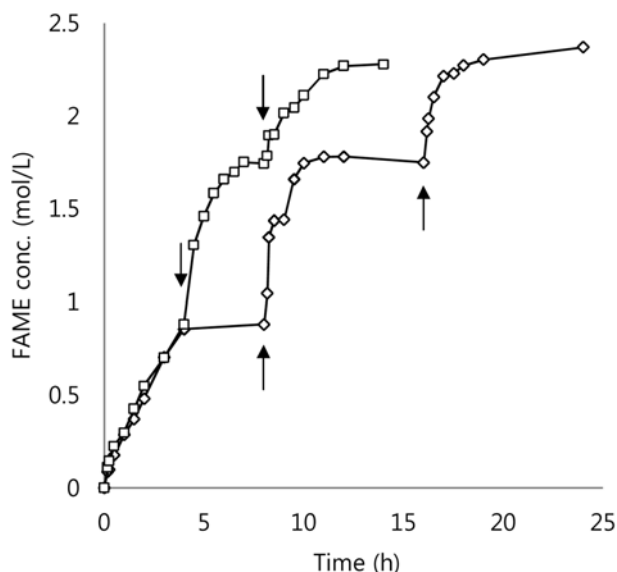
**Fig. 4. Times courses of transestrification by stepwise addition. Stepwise addition every 8 hours (◇) and stepwise addition every 4 hours (□). Upward and down ward arrows indicate the addition of methanol (1.3 mol/L) every 8 hours and 4 hours, respectively.**

indicate that kinetic equations containing only the initial reaction rate are not applicable for the real transesterifications of vegetable oil. Therefore, it is clear that a model equation ($F_{k, glycerol}$) that incorporates glycerol inhibition must be used to simulate the transesterification of oils. In addition, the equation can be used to determine the optimal alcohol feeding rate and to design various types of bioreactors for biodiesel production.

As shown in Fig. 3, the conversion of soybean oil was improved from 59.7% to 94.6% in 20 hours using the simulation results of our model equations. Although the methanol inhibition was negligible for our fed-batch reactor, the glycerol could retard the reaction, causing the decrease in the conversion from 100%. If the glycerol can be removed during the reaction, the conversion could be improved even further [14]. Using this strategy, it is also possible to improve the reusability of the enzyme by keeping the methanol concentration under its solubility limit.

For the comparison of the feeding strategy with the three-step addition strategy, three-step transesterifications of soybean oil were conducted by stepwise addition of methanol at 8-hour or 4-hour intervals (Fig. 4). After the three-step addition, the conversion of soybean oil reached 87.8% and 84.4% for the 8 and 4 hour intervals, respectively. Although the final conversion after the 8-hour addition feeding was higher than 4-hour addition, the conversion rate by 4-hour addition was faster than the 8-hour addition. When using the optimal feeding strategy, the final conversion was 94.6%, and this result was higher than either three-step addition strategy. The lower conversion was probably due to the enzyme deactivation resulted from the partially high concentration of methanol in each feeding step.

Thus, proposed model equstion which considered with not only methnol inhibition but also glycerol effect can be used to design an enzymatic biodiesel process by minimizing the methanol inhibition

and glycerol effect.

## CONCLUSIONS

In a lipase-catalyzed process, lipase is known to be deactivated by insoluble alcohol when the alcohol amount exceeds its solubility limits, and glycerol is also known to retard the reaction rate during biodiesel production. In this study, a new kinetic model incorporating the effect of glycerol was proposed for feeding of methanol in biodiesel production. The proposed model equations were verified with experimental results using soybean oil as a model substrate in batch and fed-batch modes. The suggested model equation and strategy can be used for efficient process design in biodiesel production.

## NOMENCLATURE

[A]   : alcohol concentration [mol/L]
[S]   : substrate concentration (i.e., the ester bond on the triglyceride) [mol/L]
[G]   : glycerol concentration [mol/L]
[P]   : product concentration (i.e., biodiesel) [mol/L]
$V_{max}$ : maximum reaction rate [mol L/min]
$S_0$   : initial concentration of substrate [mol/L]
t     : time [min]
u     : dV/dt, change in volume per unit time [L/min]
$\upsilon$     : initial reaction rate [L mol/min]
$V_0$   : initial volume [L]
$V_t$   : volume at time t
$ut \cdot \rho/Mw$ : concentration change after time t [mol/L]
$\rho/Mw$: density/molecular weight
$K_S$   : dissociation constant for the substrate [mol/L]
$K_A$   : dissociation constant for the alcohol [mol/L]
$K_{IS}$  : inhibition constant for the substrate [mol/L]
$K_{IA}$  : inhibition constant for the alcohol [mol/L]

## ACKNOWLEDGEMENTS

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