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Study on the kinetics of enzymatic interesterification of triglycerides for biodiesel production with methyl acetate as the acyl acceptor

Yuanyuan Xu, Wei Du*, Dehua Liu*

Department of Chemical Engineering, Tsinghua University, Beijing 100084, PR China

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Abstract

A new route for biodiesel production using methyl acetate instead of methanol as the acyl acceptor was proposed in our previous research, and it has been found that this novel route could enhance the stability of the immobilized lipase greatly. In this paper, the kinetics of lipase-catalyzed interesterification of triglycerides for biodiesel production with methyl acetate as the acyl acceptor was further studied. First, a simplified model based on Ping Pong Bi Bi with substrate competitive inhibition mechanism was proposed to describe the reaction kinetics of the interesterification. During our further study, it was observed that three consecutive and reversible reactions occurred in the interesterification of triglycerides and methyl acetate. So, a kinetic model based on mass balance of three second-order reversible reactions was developed and the reaction rate constant, k, was determined by solving the differential rate equations of the reaction system. The results showed that $k_{DG–MG}$ (0.1124) and $k_{MG–TA}$ (0.1129) were much higher than $k_{TG–DG}$ (0.0311), which indicated that the first step reaction was the limit step for the overall interesterification.

Keywords: Biodiesel; Kinetics; Lipase; Interesterification; Methyl acetate

1. Introduction

Biodiesel is defined as fatty acid methyl esters and used as an alternative fuel for diesel engine [1]. Conventionally, biodiesel was produced by transesterification of triglycerides and alcohols in the presence of an acid or an alkaline catalyst. In recent years, the use of lipases as biocatalysts for biodiesel production has become of great interest due to its environment friendly. However, some alcohols such as methanol inactivated the lipases to some extent and the enzymatic stability was poor. In order to enhance the stability of the lipase, three-step methanolysis was adopted, however, glycerol, as one of the products was easy to adsorb on the surface of lipase resulting in serious negative effect on the enzymatic activity [2–4]. In order to solve the above-mentioned problems, we previously reported that using methyl acetate as acyl acceptor instead of methanol for biodiesel production could enhance the stability of the lipase significantly and in the process, triacetylglycerol instead of glycerol would be produced and it has been demonstrated that triacetylglycerol had no negative effect on the activity of the lipase. Moreover, triacetylglycerol was an important by-product with a higher value than glycerol and this novel route was thought to be very promising for large scale production of biodiesel [5,6].

In this paper, the interesterification kinetics of triglycerides and methyl acetate for biodiesel production was studied further. First, a simple model based on Ping Pong Bi Bi with competitive substrate inhibition was proposed to describe the reaction kinetics of the interesterification. However, during our further studies, some intermediates such as diacyl monoacetyl glycerol and monoacyl diacetyl glycerol were detected in the process of interesterification of triglycerides and methyl acetate. Considering that the mechanism was actually more complex due to the presence of

* Corresponding authors. Fax: +86 10 62794742.
E-mail addresses: duwei@tsinghua.edu.cn (W. Du), dhliu@tsinghua.edu.cn (D. Liu).

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three consecutive reactions from triglycerides to the products, a kinetic model based on a material balance involving three consecutive reversible reactions was developed further.

2. Materials and methods

2.1. Materials

Immobilized lipase from *Candida antarctica* (Novozym 435) was a generous gift from Novo Nordisk (Denmark). Palmitic acid methyl ester, stearic acid methyl ester, oleic acid methyl ester, linoleic acid methyl ester, linolenic acid methyl ester and heptadecanoic acid methyl ester were purchased from Sigma and chromatographically pure. Diacyl monoacetyl glycerol and monoacyl diacetyl glycerol were separated from the reaction products by countercurrent chromatography in our laboratory. Refined soybean oil (palmitic acid 12.1%, stearic acid 3.0%, oleic acid 23.2%, linoleic acid 55.6%, linolenic acid 6.1%)was purchased from local supermarket and all other chemicals were obtained commercially and were of analytical grade.

2.2. Interesterification of triglycerides and methyl acetate for biodiesel production

Batch reactions were carried out in a 50 ml flask containing 10 g reaction mixtures of soybean oil and methyl acetate using 0.5 g immobilized lipase. Different molar ratios of triglyceride/methyl acetate were tried and the reaction mixture was incubated in a 40 °C shaker. Samples (5 μl) were withdrawn at different time intervals and mixed with 300 μl of 1.4 mM heptadecanoic methyl ester (severed as internal standard) for gas chromatographic analysis.

2.3. Analysis of the samples

Samples prepared as described above were analyzed by a GC-14B gas chromatograph (Shimadzu Corp., Kyoto) connected to a DB-1ht capillary column (0.25 mm × 15 m; J&W Scientific, Folsom, CA). The column temperature was kept at 100 °C for 0.5 min, raised to 380 °C at 15 °C/min and maintained at this temperature for 6 min. The temperatures of the injector and detector were set at 250 and 390 °C, respectively.

The initial reaction rate was determined within the limits of less than 5% of interesterification and it was represented as the molar concentration of biodiesel produced per minute.

3. Results and discussion

3.1. Kinetic study of interesterification of triglycerides and methyl acetate for biodiesel production

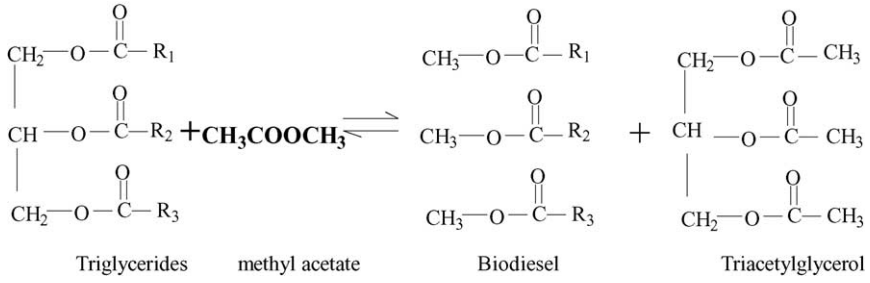
The interesterification of triglycerides and methyl acetate for biodiesel production was shown in Scheme 1.

Since there were only substrates without any extra organic solvent existing in this reaction system, the concentrations of the two substrates were strictly interdependent. Therefore, it was impossible to perform a classic kinetic study by fixing the concentration of one substrate and changing the concentration of the other substrate. In our experiments, the effect of substrate concentrations on the reaction rate was investigated as follows: 0.5 g Novozym 435 was used to catalyze the interesterification of triglycerides and methyl acetate and the two substrates were mixed at different ratios, while the total substrate weight was kept fixed (10 g). The concentration ranges were 0.5–1 and 0.1–6 mol/l for triglycerides and methyl acetate, respectively. In these ranges, competitive inhibition by methyl acetate was observed, so a kinetic model based on Ping Pong Bi Bi with competitive substrate inhibition was proposed here [7].

The equation of this mechanism was given as follows:

$$V_i = \frac{V_{max}[TG][A]}{K_{mTG}[A](1 + ([A]/K_i)) + K_{mA}[TG] + [TG][A]} \quad (1)$$

where V_i was the initial reaction rate; [TG] and [A] the initial molar concentrations of triglycerides and methyl acetate, respectively; K_{mTG} and K_{mA} the apparent Michaelis constants for triglycerides and methyl acetate, respectively; K_i the apparent inhibition constant of methyl ac-



Scheme 1. Interesterification of triglycerides and methyl acetate for biodiesel production.

etate and V_{max} the initial maximum velocity of the reaction.

Since the concentration of triglycerides and methyl acetate were interdependent, [TG] could be expressed as a function of [A], and Eq. (1) was converted to Eq. (2):

$$V_i = \frac{V_{max}[A](\rho_{mix} - M_A[A])/M_{TG}}{K_{mTG}[A](1 + ([A]/K_i)) + (K_{mA} + [A])(\rho_{mix} - M_A[A])/M_{TG}} \quad (2)$$

where ρ_{mix} (=900 g/l) was the density of reaction mixture, M_A (=74) and M_{TG} (=882) the molecular weights of methyl acetate and triglycerides, respectively. The kinetic constants in Eq. (2) could be determined by nonlinear regression method, and the software Datafit was used to perform the nonlinear regression by Levenberg–Marquardt method with double precision. The fitting results were as follows: V_{max} = 1.9 mol/l min; K_{mTG} = 1 mol/l; K_{mA} = 16 mol/l; K_i = 0.0455 mol/l.

Comparison between modeled and experimental data was presented in Fig. 1 and a good agreement was obtained. As can be seen from Fig. 1, the initial reaction rate increased with increasing of the concentration of methyl acetate when the molar concentration was below 0.94 mol/l, while when more methyl acetate was added to this reaction, the initial reaction rate decreased gradually. The reduction in initial reaction rate partly attributed to the inhibition of methyl acetate and the kinetic parameter K_i indicated the inhibition extent of methyl acetate.

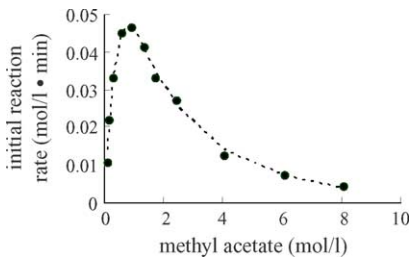


Fig. 1. Comparison of model prediction (- - -) and experimental data (●) of initial reaction rate. Reaction conditions: 10 g mixture of soybean oil and methyl acetate, 0.5 g Novozym 435, 40 °C, 150 oscillations/min.

3.2. Reaction mechanism of interesterification of triglycerides and methyl acetate for biodiesel production

During the course of the interesterification of triglycerides and methyl acetate for biodiesel production, some intermediates (monoacyl diacetyl glycerol and diacyl monoacetyl glycerol) were detected. So we hypothesized that three consecutive reversible reactions occurred during the interesterification of triglycerides and methyl acetate. The scheme of the interesterification of triglycerides and methyl acetate could be shown as follows [8]:Overall reaction:

$$TG + CH_3COOCH_3 \rightleftharpoons 3RCOOCH_3 + TA$$

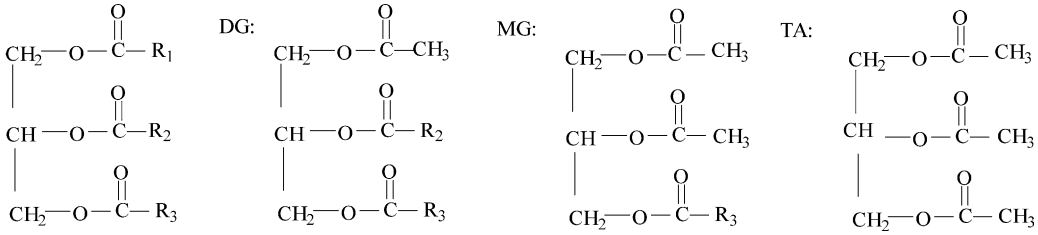
Stepwise reactions:

$$TG + CH_3COOCH_3 \underset{k_2(DG \rightharpoonup TG)}{\overset{k_1(TG \rightharpoonup DG)}{\rightleftharpoons}} RCOOCH_3 + DG$$

$$DG + CH_3COOCH_3 \underset{k_4(MG \rightharpoonup DG)}{\overset{k_3(DG \rightharpoonup MG)}{\rightleftharpoons}} RCOOCH_3 + MG$$

$$MG + CH_3COOCH_3 \underset{k_2(TA \rightharpoonup MG)}{\overset{k_5(MG \rightharpoonup TA)}{\rightleftharpoons}} RCOOCH_3 + TA$$

TG: DG: MG: TA:



To describe a simple mathematical model for the interesterification reaction system, the following assumptions were adopted: (i) since there is only little water (0.2%, w/w) in the reaction mixtures and negligible free fatty acid was detected in the system, the hydrolysis reaction could be negligible and it was assumed that only these three consecutive reactions occurred; (ii) the enzyme used in this study has no positional specificity, so it acts concurrently on any acyl group; (iii) mass transfer limitation in the reaction system was neglected. Based on the above assumptions, the differential equations characterizing the stepwise reactions were as follows:

$$\frac{d[TG]}{dt} = -k_1[TG][A] + k_2[DG][ME],$$

$$\frac{d[DG]}{dt} = k_1[TG][A] - k_2[DG][ME] - k_3[DG][A] + k_4[MG][ME],$$

$$\frac{d[MG]}{dt} = k_3[DG][A] - k_4[MG][ME] - k_5[MG][A] + k_6[TA][ME],$$

$$\frac{d[ME]}{dt} = k_1[TG][A] - k_2[DG][ME + k_3[DG][A] - k_4[MG][ME] + k_5[MG][A] - k_6[TA][ME],$$

$$\frac{d[A]}{dt} = -\frac{d[ME]}{dt},$$

$$\frac{d[TA]}{dt} = k_5[MG][A] - k_6[TA][ME] \quad (3)$$

where [TG], [DG], [MG], [ME] and [TA] were the molar concentrations of triglycerides, diacyl monoacetyl glycerol, monoacyl diacetyl glycerol, biodiesel and triacetylglycerol, respectively. The above equations could also be rearranged to give the following equations:

$$\frac{d[TG]}{dt} = -k_1[TG][A] + k_2[DG][ME],$$

$$\frac{d[TG + DG]}{dt} = -k_3[DG][A] + k_4[MG][ME],$$

$$\frac{d[TG + DG + MG]}{dt} = -k_5[MG][A] + k_6[TA][ME] \quad (4)$$

Besides, some mass balances equations were as follows:

$$[TG] + [DG] + [MG] + [GL] = [TG]_0,$$

$$3[TG] + 2[DG] + [MG] + [ME] = 3[TG]_0,$$

$$[ME] + [A] = [A]_0 \quad (5)$$

where $[TG]_0$ and $[A]_0$ were the initial concentration of triglycerides and methyl acetate, respectively. The concentration change of each component with time could be obtained experimentally and the nonlinear curve fitting software Matlab was used for fitting the system of differential equations (4) and (5) into the experimental data.

Based upon the obtained rate constants and the kinetic scheme, the concentration of each composition at different reaction could be calculated. If the calculated value well agreed with the experimental data, it indicated that the kinetic scheme employed was adequate.

3.3. Determination of rate constants of the interesterification for biodiesel production

The concentration of each composition (triglycerides, diacyl monoacetyl glycerol, monoacyl diacetyl glycerol, biodiesel or triacetylglycerol) at different times was obtained experimentally when the substrate molar ratio of triglycerides and methyl acetate was 1:12. By fitting the above differential equations (4) and (5) to the experimental data, the rate constants were calculated and listed in Table 1. However, the rate constants (k_1–k_6) were not really "constant" and were actually related to the concentration of substrates which varied with time [9]. So the rate constants listed in Table 1 were just average values. Comparison between modeled and experimental data was presented in Fig. 2 and a good agreement was obtained. Therefore, the mechanism of three consecutive reversible reactions proposed in this study was adequate for the interesterification of soybean oil and methyl acetate.

Table 1
Reaction rate constants in the three consecutive reversible reactions

Reaction direction	Rate constants (l/mol min)	Value
TG → DG	k_1	0.0311
DG → TG	k_2	0.0176
DG → MG	k_3	0.1124
MG → DG	k_4	0.1271
MG → TA	k_5	0.1129
TA → MG	k_6	0.0915

Reaction conditions: 5 g soybean oil, 5 g methyl acetate, 0.5 g Novozym 435, 40 °C, 150 oscillations/min.

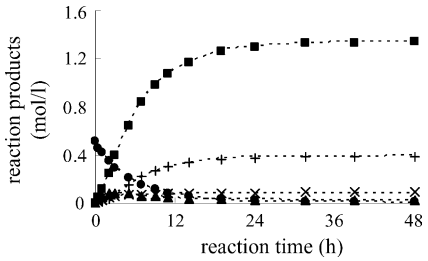


Fig. 2. Comparison of modeling results (dashed lines) and experimental data (symbols) for the interesterification of soybean oil and methyl acetate. Reaction conditions: 5 g soybean oil, 5 g methyl acetate, 0.5 g Novozym 435, 40 °C, 150 oscillations/min, biodiesel (■), triglycerides (●), diacyl monoacetyl glycerol (▲), monoacyl diacetyl glycerol (×), triacetylglycerol (+).

In terms of the reaction rate constants in Table 1, the forward reaction rate constant in the first reaction ($k_{1(TG–DG)} = 0.0311$) was much lower than that in the second and third forward reactions ($k_{2(DG–MG)} = 0.1124$, $k_{3(MG–TA)} = 0.1129$). The results indicated that the first forward reaction (TG–DG) was the limit step during the overall reactions. Since both the second and third forward reaction rates were high, the intermediates (diacyl monoacetyl glycerol and monoacyl diacetyl glycerol) were easily converted to biodiesel and the experimental results also showed that the concentrations of the intermediates were low during the course of the interesterification.

4. Conclusions

The kinetics of lipase-catalyzed interesterification of soybean oil with methyl acetate for biodiesel production was studied in this paper. Firstly a simplified model based on the Ping Pong Bi Bi with substrate competitive inhibition mechanism was proposed to describe the interesterification kinetics. Considering that the mechanism was actually more complex due to the presence of three consecutive reactions from triglycerides to the products, a three-consecutive-reversible-reaction model was developed successfully to reflect the reaction mechanism. Based on the kinetic model, the reaction rate constants for three consecutive reactions were calculated and the results indicated that the first

step reaction (TG–DG) was the limit step for the overall interesterification.

Acknowledgements

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