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Kinetic study on lipase-catalyzed biodiesel production from waste cooking oil

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ABSTRACT

A kinetic model of the biodiesel production from waste cooking oil using *Candida* sp. 99-125 lipase as catalyst was established in this paper. The limited reaction steps in the model were considered to be the hydrolysis of glycerides and the esterification of free fatty acids, while the methanolysis of glycerides were considered to be negligible. Matlab programming platform was used to estimate the parameters of the model. A series of experiments were conducted to verify the validity of the model. The results showed that the model could adequately simulate the reaction results of biodiesel production at different lipase loadings, substrate ratios and initial water concentrations. The agreement between experimental data and calculated values was good, which could prove the validity of the kinetic model. Moreover, the results of biodiesel production using a twelve-step methanol feeding method and a three-step methanol feeding method could be simulated well by the kinetic model. The results indicated that the model could also simulate the biodiesel production using methanol step-wise addition.

1. Introduction

Biodiesel is a renewable resource derived from oil crops, engineered microalgae, animal fats, and waste cooking oil (WCO). Recently, biodiesel has attracted increasing research interest because of its low emissions and its desirable chemical characteristics, such as being non-toxic and biodegradable [1]. Traditionally, biodiesel can be produced by using chemical catalysts, lipase, and engineered microalgae. The preferred industrial process for producing biodiesel typically uses alkali or acid as the catalyst for the transesterification reaction owing to its low price and availability [2]. Many researchers have investigated the process of biodiesel production using an alkaline or acid catalyst, and they obtained good results [3,4]. However, the chemical-catalysis method requires high temperature and complicated downstream processes including the removal of inorganic salts from the product, the recovery of salt-containing glycerol, and the treatment of the wastewater [5]. Also, in order to prevent the generation of soap, the process also requires rigorous feedstock specifications such as low contents of water and free fatty acids [6].

In order to solve these problems, scientists started to use lipase to catalyze the reaction, because it can provide a solution to the aforementioned problems [7], while the reaction can be carried out at lower temperature and pressure, which could reduce energy consumption [8]. Wang et al. [9] investigated biodiesel production from soybean oil deodorizer distillate using Novozym 435 and Lipozyme TLIM in a *tert*-butanol system. The result demonstrated that the yield of biodiesel was 97.0% at the optimum condition with reaction temperature 40 °C in 12 h. There are many researchers focusing on biodiesel production from vegetable oil using lipase as catalyst. However, it is impractical to produce biodiesel using edible oil as feedstock in China, because of the restriction from national regulation. Therefore, the reasonable and viable way to produce biodiesel is to use WCO as the raw material.

Only a few researchers are interested in biodiesel production from WCO, and their methods seem to be effective [10–12]. Chen et al. [13] investigated biodiesel production from WCO in the presence of immobilized *Candida* lipase in a fixed bed reactor. The highest yield of 91.08% was obtained at optimum condition of 25:15:10:100 of lipase/hexane/water/WCO weight ratio, 2.1 ml/min reactor flow, and 45 °C reaction temperature. Most researchers are focusing on a series of experiments to determine the optimal conditions for biodiesel production. On the other hand, the kinetic mechanism of the reaction has not been investigated completely. Until now, most of the kinetic mechanisms of biodiesel production using lipase are based on the transesterification of triacylglycerols [14–16]. The reaction was believed to take place directly by alcoholysis of triacylglycerols. However, according to

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the former research, the esterification of free fatty acids was also found in the biodiesel production from WCO [17]. Some researchers assumed that the reaction in WCO contains two consecutive steps, the hydrolysis of triacylglycerols and the esterification of the fatty acids [18]. Unfortunately, the exact mechanism and the rate expressions in biodiesel production from WCO have not been clarified. It is therefore necessary to clarify the kinetic mechanism for biodiesel production from WCO.

In order to investigate the kinetic mechanism for biodiesel production from WCO, computer simulation could be a useful tool. In this paper, MATLAB programming platform was used to calculate the rate expressions of the kinetic model. A series of biodiesel production experiments were carried out in a low aqueous media. Methanol was chosen as the substrate because of its low cost [19]. Lipase from *Candida* sp. 99-125 made in our laboratory was used as catalyst [20]. A kinetic model was proposed to describe the kinetics of the esterification and hydrolysis reactions. After fitting the equations to the experimental data to estimate the rate constants, a series of experiments at different lipase loadings, substrate ratios and initial water concentrations were carried out to verify the kinetic model. Finally, the kinetic model was used to simulate biodiesel production using methanol step-wise addition.

2. Materials and methods

2.1. Materials

The WCO used in the experiments was purchased from Shanghai Luming Evvironm en Science Co., Ltd., China. The WCO contained 80.17% of free fatty acids (FFAs), 1.9% of monoacylglycerols (MAGs), 6.78% of diacylglycerols (DAGs), and 11.15% of triacylglycerols (TAGs). The FFA composition was as following (wt%): C14:0, 2.12%; C16:0, 20.70%; C18:0, 6.07%; C18:1, 67.57%; C18:2, 3.55%. Based on the FFA composition, the average molecular weight of FFAs in the WCO was measured at 274. Lipase from *Candida* sp. 99-125, which was made in our laboratory, was used in the experiments as catalyst. Other chemicals used in this paper were analytic grade.

2.2. Biodiesel production from WCO

The reaction was carried out in a batch reactor. The reaction condition was based on the study by Nie et al. [17]. The temperature was controlled at 40 °C, and the mixture was stirred with an electric stirrer at 220 rpm. During the experiments, methanol and water were added into 150 g WCO in the first step in order to avoid the deactivation of lipase. Then the mixture was kept stirring under the reaction condition for 20 min. Finally, lipase was added into the mixture, and the reaction was started. The results were substituted into the rate expressions to estimate the parameters, and were also used to compare with the simulated results. All of the experimental data presented in this paper were the means of three parallel experiments.

2.3. Using methanol step-wise addition for biodiesel production

According to the former studies, methanol was found to have inhibitive effect on enzyme [21,22]. Since too much methanol would inactivate the enzyme, typically, methanol step-wise addition was used in the production of biodiesel. In order to verify the validity of the kinetic model, it is necessary to carry out biodiesel production using methanol step-wise addition, and to compare the experimental data with the simulated results. Therefore, a series of experiments using methanol step-wise addition were carried out under different reaction conditions to verify the model.

Normally, a twelve-step methanol feeding method was used in our laboratory for the biodiesel production [17]. The total molar amount of methanol added into the reaction was equal to 1.3 times of the molar amount of FFAs in the WCO. The detail of the twelve-step methanol feeding method was demonstrated in Table 1. The initial water concentration was 2 wt%, based on the WCO weight. The enzyme loadings used in the experiments were 0.2 wt% and 0.4 wt%, respectively based on the WCO weight.

Table 1
Methanol feeding method.

Time (h)	Methanol added into the reaction (mmol/g)
0	0.481
3	0.481
6	0.481
9	0.375
11.5	0.375
14	0.375
16.5	0.375
19.5	0.229
21.5	0.229
23.5	0.229
25.5	0.229
27.5	0.104

2.4. Analytical method

The concentrations of fatty acid methyl esters (FAMEs) and other species in the reaction system were measured by gas chromatography (GC) analysis (The Shimadzu GC-2010 Gas Chromatography, Japan). DB1-ht capillary column (0.25 mm × 15 m; J&W Scientific) was used for this analysis, and highly pure nitrogen was used as carrier gas. The temperature programming was designed to heat up the column through two steps: first, the column was heated from 100 °C to 180 °C at a rate of 10 °C/min; then the column was heated from 180 °C to 350 °C at a rate of 20 °C/min. Hydrogen flame ionization detector was used and the temperature was set at 350 °C. The temperature of vaporizing chamber was set at 350 °C. Octodecane was used as internal standard.

2.5. Kinetic model development

The mechanism used in this paper was similar to the mechanism in the studies by Cheirsilp et al. [23,24]. The mechanism was based on the Michaelis–Menten's equilibrium model, and could be divided into two groups of reactions: esterification and hydrolysis. The esterification of FFAs was considered to be the main step of the reaction for biodiesel production, since the FFAs were the main content in the WCO. The TAGs, DAGs, and MAGs in the WCO were considered to be hydrolyzed to produce FFAs. The newly produced FFAs also reacted with methanol to produce FAMEs. These two routes were considered to occur simultaneously. On the other hand, the methanolysis steps were ignored in the reaction.

In this paper, some assumptions were made in order to simplify the kinetic model: (1) The mass transfer limitations in the reaction were negligible, therefore it could be ignored. (2) Although there were many different substitutions of TAGs, DAGs, MAGs and FFAs in the WCO, they could be treated as a single constitute, respectively. (3) Methanol was considered to be the main inhibitor in the WCO that would affect the lipase. Other inhibitors in the WCO were considered to be negligible. The inhibition of enzyme activity caused by methanol in the kinetic model followed a competitive inhibition mechanism. (4) The reaction in the kinetic model followed an ordered Bi mechanism. (5) The limiting steps of the reaction were considered to be the esterification of FFAs and the hydrolysis of glycerides, other steps in the mechanism were considered to be in rapid equilibrium.

The reaction network of the mechanism was illustrated in Fig. 1, where E represents free lipase. From Fig. 1, a series of equations