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Production of Biodiesel via Enzymatic Ethanolysis of the Sunflower and Soybean Oils: Modeling

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Abstract Biodiesel has become attractive due to its environmental benefits compared with conventional diesel. Although the enzymatic synthesis of biodiesel requires low thermal energy, low conversions of enzymatic transesterification with ethanol (ethanolysis) of oils to produce biodiesel are reported as a result of deactivation of the enzyme depending on the reaction conditions. The synthesis of biodiesel via enzymatic ethanolysis of sunflower and soybean oils was investigated. Kinetic parameters for the overall reactions were fitted to experimental data available in the literature with the Ping Pong Bi-Bi mechanism including the inhibition effect of the ethanol on the activity of lipase Novozyme® 435. The model was applied to a batch reactor and the experimental conversions were successfully reproduced. The modeling of a semibatch reactor with continuous addition of ethanol was also performed and the results showed a reduction of roughly 3 h in the reaction time in comparison with the batch-wise operation.

Keywords Biodiesel · Enzymatic ethanolysis · Ping Pong Bi-Bi · Sunflower oil · Soybean oil

Introduction

The shortage of the fossil fuels and the environmental degradation due to gas emissions and/or spills has contributed for the increase in the search for alternative fuels. Biodiesel is an alternative for the diesel derived from oil, which can be synthesized through the catalytic transesterification reaction of edible/non-edible oils or animal fats with an alcohol yielding

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fatty acid alkyl esters and glycerol as by product. It is environmentally friendly since it is derived from renewable and biodegradable resources and produces less gas emissions compared with the conventional fuels.

Overall transesterification reaction for the production of biodiesel is shown in Fig. 1. An excess of alcohol is usually required because the reaction is reversible.

Several vegetable oils can be used as raw materials such as soybean, palm, sunflower, cotton, etc. Metanol and ethanol are the most used alcohols. In Brazil, ethanol is advantageous due to its enormous availability.

Acid, alkaline, and biocatalysts can be employed commercially for the synthesis of biodiesel. The reaction is known to be faster in the presence of alkaline catalysts, but when the concentration of water and fatty acids in the oil is high, the acid catalysts are preferable. As far as biocatalysts are concerned, lipases catalyze transesterification reactions of the triglycerides present in vegetable oils by acting on their ester bond. The difficulties during the glycerol recovery, catalyst removal, and purification steps, which are characteristics of the reactions with alkaline and acid catalysts, are not found in transesterification routes that use immobilized lipases. Despite the high costs associated with the enzymes and their inhibition due to the presence of the alcohol, the positive aspects offered by this technology encourage the researches in this field in order to overcome the drawbacks so that the process can be used in industrial scale.

The rate of an enzymatic reaction depends on factors such as substrates concentration, enzyme load, pH, and temperature. Several studies in the literature report that the kinetics of the enzymatic transesterification reaction wherein raw materials derived from natural products are involved should be modeled using a model that accounts for the effects of the inhibition of the enzyme since some species present in the raw materials affect the activity of the enzyme. According to studies performed by Shimada et al. [1], the inhibition of the enzyme is a consequence of the contact of the enzyme with the immiscible polar phase, containing the alcohol and glycerol, in the presence of the nonpolar oil phase. The stepwise reaction has been mentioned in the literature (e.g., Watanabe et al. [2] and Shimada et al. [1]) as an alternative to overcome such inhibition. As mentioned by Dossat et al. [3] and Al-Zuhair et al. [4], a model widely used to describe the kinetics of this kind of reaction is based on the Ping Pong Bi-Bi mechanism which takes into consideration the influence of the alcohol and the substrates on the activation of the enzyme.

Kinetic data obtained experimentally can be modeled for scale-up purposes and also for the evaluation of the influence the different reaction parameters on the enzymatic transesterification reaction. The experimental enzymatic ethanolysis of edible oils aiming at the production of biodiesel has been reported in the literature (e.g., Selmi and Thomas [5] and Hernández-Martín and Otero [6]). However, works that deal with modeling of the transesterification reaction for the production of biodiesel including the inhibition effect of the ethanol are seldom reported. Therefore, the objective of this work is to employ the Ping Pong Bi-Bi kinetics in a model of a semibatch reactor to investigate the influence of the concentration of the alcohol on the conversion of the ethanolysis of sunflower and soybean oils with lipase Novozym® 435 as the catalyst.

Triglycerides + 3 Alcohol ↔ 3 Esters + Glycerol

Fig. 1 Overall transesterification reaction between triglycerides and alcohol

Kinetic Modeling

Al-Zuhair et al. [4] considered in their model the global reaction for the synthesis of biodiesel, as shown in Fig. 1, instead of three consecutive reactions starting from tri-, di-, and monoglyceride, respectively, because only small amounts of mono- and diglycerides were detected over the reaction. The rate expression given by Eq. 1 was then used in this work.

$$V_\mathrm{i} = \frac{V_\mathrm{m} \cdot [\mathrm{oil}] \cdot [\mathrm{alc}]}{\mathrm{Km}_\mathrm{oil} \cdot [\mathrm{alc}]\left(1 + \frac{[\mathrm{alc}]}{K_\mathrm{i}}\right) + \mathrm{Km}_\mathrm{alc}[\mathrm{oil}] + [\mathrm{oil}][\mathrm{alc}]} \tag{1}$$

Where [oil] and [alc] are the molar concentrations of oil and alcohol, respectively. Km_oil and Km_alc are the binding constants for the oil and alcohol. K_i represents the inhibition constant of the alcohol, whereas V_m denotes the maximum initial rate for the reaction.

In this work, a pseudo-homogeneous kinetic model with the rate per unit of enzyme load, as used by Steinigeweg and Gmehling [7], combined with the Ping Pong Bi-Bi model was applied to describe the reaction rate as given by Eq. 2.

$$(-r_\mathrm{i}) = -\frac{1}{m_\mathrm{cat}}\frac{1}{V}\frac{\mathrm{dN_i}}{\mathrm{dt}} = \frac{V_\mathrm{m} \cdot [\mathrm{oil}] \cdot [\mathrm{alc}]}{\mathrm{Km}_\mathrm{oil} \cdot [\mathrm{alc}]\left(1 + \frac{[\mathrm{alc}]}{K_\mathrm{i}}\right) + \mathrm{Km}_\mathrm{alc}[\mathrm{oil}] + [\mathrm{oil}][\mathrm{alc}]} \tag{2}$$

Where m_cat is the mass of enzyme, V is the reaction mixture volume, and $\mathrm{dN_i/dt}$ represents the number of moles of species i reacted per unit time.

The experimental data taken from Hernández-Martín and Otero [6], who studied the ethanolysis of sunflower and soybean oils catalyzed by lipase Novozym® 435, were used to fit the kinetic parameters (V_m, Km_oil, Km_alc, and K_i). The reactions were carried out at ambient pressure, room temperature, initial molar ratio ethanol (pure) to sunflower oil of 20.6:1, 14.4:1 for soybean oil, enzyme load of 1 g (50% *w*/*w* based on weight of oil), and using hexadecane as a solvent. Since the kinetic experimental data were referred as conversion of triglycerides, it was convenient to consider both oils as pseudo-components in the reaction model. Thus, the properties of the mixture of triglycerides and ethyl esters were calculated by averaging the individual properties with the corresponding mole fraction of their constituents.

The fitting of the parameters was performed with the applicative Mathcad v.14 by means of the routine *Minimize* employing the Quasi-Newton numerical method. The objective function is presented in Eq. 3, where n denotes the number of experimental data.

$$\mathrm{FO}(V_\mathrm{m}, \mathrm{Km}_\mathrm{oil}, \mathrm{Km}_\mathrm{alc}, K_\mathrm{i}) = \sum_{j=1}^{n}\left[\left.\frac{\mathrm{dN_i}}{\mathrm{dt}}\right|_{\mathrm{calc\,j}} - \left.\frac{\mathrm{dN_i}}{\mathrm{dt}}\right|_{\mathrm{expj}}\right]^2 \tag{3}$$

The kinetic parameters obtained maybe considered as apparent values due to the possibility of internal and/or external diffusion limitations. The results for the parameters in the model were:

- Sunflower oil: V_m=0.00024 $\mathrm{mol.h^{-1}.mgcat^{-1}.L^{-1}}$, Km_oil=0.000006331 mol/L, Km_alc= 0.04 mol/L, and K_i= 0.00028797 mol/L.

- Soybean oil: V_m=0.0002 mol.h^{-1}.mgcat^{-1}.L^{-1}, Km$_{\text{oil}}$=0.00000294 mol/L, Km$_{\text{alc}}$= 0.4 mol/L, and K_i= 0.000167 mol/L.

Results and Discussion

In order to validate the kinetic parameters, a batch reactor was modeled using a set of four ordinary differential equations representing the mole balances applied to the reactants and products, which was numerically solved with the *Odesolve* routine of Mathcad v.14 with the adaptive step-size algorithm that combines the fourth-order with fifth-order Runge-Kutta methods. In the reaction with sunflower oil, the initial concentrations for the reactants, products, and solvent were: oil=0.4603 mol/L, ethanol=9.47 mol/L, ester=0, glycerol=0, and hexadecane=0.1780 mol/L, and the reaction mixture volume=4.9616 cm^3. For soybean oil, the initial conditions were: oil= 0.5233 mol/L, ethanol=7.52 mol/L, ester=0, glycerol=0, hexadecane=0.2031 mol/L, reaction mixture volume=4.332 cm^3.

The comparison between the predicted and experimental conversions for the sunflower and soybean oils is illustrated in Figs. 2 and 3, respectively. In the case of sunflower oil, the modeling reproduced the experimental conversion data with an absolute percent mean deviation of 4.6%. The model predicts rather successfully the experimental data and 90% conversion was achieved in approximately 4 h.

For the ethanolysis of soybean oil the modeling predicted the experimental data with an absolute percent mean deviation of 7%.

Watanabe et al. [2] and Shimada et al. [1] investigated the reaction experimentally with sequential feedings of alcohol and considered this method as an option to reduce the inhibition due to high concentration of ethanol. In their work, the transesterification reaction was carried out with sequential addition of less than stoichiometric quantities of ethanol to a fixed amount of oil. Likewise, the use of a semibatch approach might be an alternative to conduct the reaction avoiding the loss of activity of the enzyme caused by the inhibition. Based on the kinetics discussed above, the modeling of a semibatch reactor with continuous feed of ethanol was conducted in order to investigate the influence of the concentration of ethanol on the conversion. Such a model applied to a semibatch reactor comprises a system of four ordinary differential equations representing mole balances on

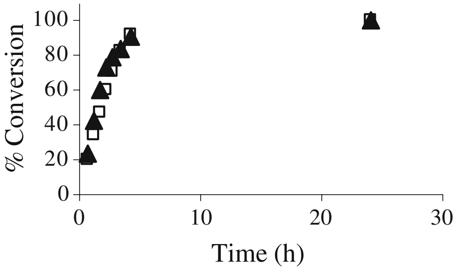

Fig. 2 Sunflower oil conversion for a batch reactor. (*filled triangle*) Exp., (*empty square*) Calc. Conditions: 25 °C, 1 g enzyme (50% *w/w*), ethanol/oil molar ratio=20.6:1

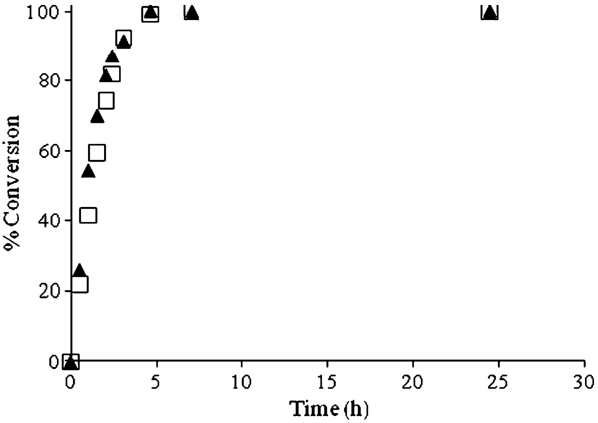

Fig. 3 Soybean oil conversion for a batch reactor. (*Filled triangle*) Exp., (*empty square*) Calc. Conditions: 25 °C, 1 g enzyme (50% *w/w*), ethanol/oil molar ratio=14.4:1

each species and an overall mass balance. The model is described by the following set of equations:

$$\frac{\mathrm{d}N_{\mathrm{oil}}}{\mathrm{d}t}=\frac{-m_{cat}\left[V_{\max}N_{oil}(t)\right]\left[\theta_{alc}-3\left(1-\frac{N_{oil}(t)}{N_{oil0}}\right)\right]}{Km_{\mathrm{oil}}\left(\theta_{\mathrm{alc}}-3\left(1-\frac{N_{\mathrm{oil}}(t)}{N_{\mathrm{oil0}}}\right)\right)\left(1+\frac{N_{oil0}\left(\theta_{\mathrm{alc}}-3\left(1-\frac{N_{\mathrm{oil}}(t)}{N_{\mathrm{oil0}}}\right)\right)}{V\cdot K_{\mathrm{i}}}\right)+Km_{\mathrm{alc}}\left(\frac{N_{\mathrm{oil}}(t)}{N_{\mathrm{oil0}}}\right)+N_{\mathrm{oil}}(t)\left[\theta_{\mathrm{alc}}-3\left(1-\frac{N_{\mathrm{oil}}(t)}{N_{\mathrm{oil0}}}\right)\right]} \tag{4}$$

$$\frac{\mathrm{d}N_{\mathrm{oil}}}{\mathrm{d}t}=\frac{1}{3}\frac{\mathrm{d}N_{\mathrm{alc}}}{\mathrm{d}t}=-\frac{1}{3}\frac{\mathrm{d}N_{\mathrm{ester}}}{\mathrm{d}t}=-\frac{\mathrm{d}N_{\mathrm{glyc}}}{\mathrm{d}t} \tag{5}$$

$$\frac{\mathrm{d}N_{\mathrm{alc}}}{\mathrm{d}t}=3\frac{\mathrm{d}N_{\mathrm{oil}}}{\mathrm{d}t}+\frac{v_0N_{\mathrm{oil0}}\theta_{\mathrm{alc}}}{V_0} \tag{6}$$

$$V=V_0+v_0t \tag{7}$$

For the ethanolysis of sunflower oil V_0=4.9616 cm^3, N_{oil0}=0.002284 mol, and θ_{alc}=20.6 represent the initial conditions for the reaction mixture volume, moles of oil, and ethanol to oil molar ratio, respectively. In the case of soybean oil, the corresponding values are: V_0= 4.3315 cm^3, N_{oil0}=0.002266 mol, and θ_{alc}=14.4. The mol balance for the oil is given in Eq. 4, which combines the Ping Pong Bi-Bi rate expression, Eq. 2, with the stoichiometry of the reaction and the conversion defined in terms of oil. Equation 5 gives the stoichiometry relating the reaction rates of each species (e.g. oil, alcohol, glycerol, and esters). Equation 6 shows the molar balance for ethanol including a continuous flow of this component, ν_0, whereas Eq. 7 gives the mixture volume as a function of time. Similarly to the case of the batch reactor, the model was solved with the *Odesolve* routine of Mathcad v.14.

In order to compare the conversions obtained with batch and semibatch strategies, the initial conditions of the reaction mixture volume and concentrations of the reactants have the same values in both modes. The goal was to investigate the behavior of the conversion by varying the flow rate of ethanol added to the system continuously, ν_0. In contrast to the works of Watanabe et al. [2] and Shimada et al. [1], the initial alcohol to oil molar ratio was kept the same as the in source of the experimental data and, therefore, above the stoichiometric ratio. The effect of ethanol flow rate on the conversion of sunflower oil in a semibatch reactor is illustrated in Fig. 4, which shows the conversions over a period of 6 h for ethanol flow rates of 0.1, 1, and 10 ml/h. A similar behavior was obtained for soybean oil. The improvement in the conversion was basically due to the increasing in the ethanol flow rate that yields high ethanol to oil molar ratios. It appears that, under the given operation conditions, the inhibition of the alcohol is not relevant. These results are in compliance with the findings of Hernández-Martín and Otero [6] who observed rather insignificant inhibition of Novozym® 435 by ethanol with alcohol to oil molar ratios ranging from 14.3:1 to 28.8:1. Additionally, Irimescu et al. [8] reported a satisfactory performance of this enzyme in the ethanolysis of triacylglycerol using even high molar ratio (e.g. 77:1). The increasing in the conversion caused by the reduction in the inhibition effects might be better evaluated by starting the reaction with a concentration of ethanol lower than that used in the batch mode and, consequently, ethanol to oil molar ratios lower that 20.6:1 as in the case of the reaction with sunflower. However there was no experimental data available so that the kinetic parameters could be determined.

Conclusion

A model based on the Ping Pong Bi-Bi mechanism with competitive ethanol inhibition was employed to describe the kinetics of the transesterification reaction of sunflower and soybean oils with ethanol catalyzed by lipase Novozym® 435. The kinetic constants were fit to experimental data, and the model was found to be qualitatively consistent with the proposed mechanism since it reproduced the experimental conversions rather adequately. The results of the model applied to a semibatch reactor with continuous addition of ethanol using the same initial conditions as in the batch mode showed improvements in the conversion basically due to the increased in the ethanol to oil molar ratio rather than the decreased in the inhibition effects.

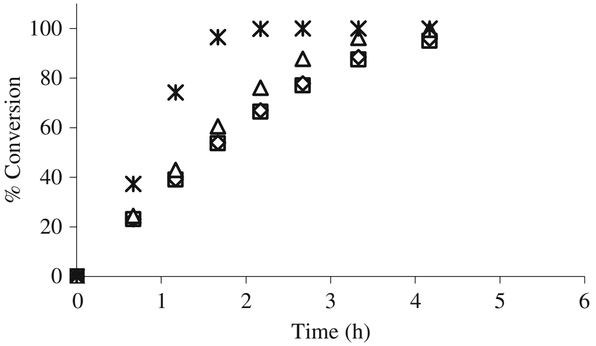

Fig. 4 Conversion results for batch and semibatch reactors. *(empty square*) batch mode, (*empty diamond*) semibatch with 0.1 ml/h, (*empty triangle*) semibatch with 1.0 ml/h, (*asterisk*) semibatch with 10 ml/h. Conditions: 25 °C, 1 g enzyme (50% *w*/*w*), initial molar ethanol/oil ratio=20.6:1

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