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ARTICLE *in* INDUSTRIAL & ENGINEERING CHEMISTRY RESEARCH · OCTOBER 2000

Impact Factor: 2.59 · DOI: 10.1021/ie990865p

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Kinetics of the Enzymatic Alcoholysis of Palm Kernel Oil in Supercritical CO_2

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Recently, the use of supercritical CO_2 ($SCCO_2$) as a solvent in enzyme-catalyzed reactions has been a matter of considerable research because of its favorable transport properties that can accelerate mass-transfer-limited enzymatic reactions. Further, biocatalysts, especially lipases, have substrate specificity and catalyze the syntheses of esters from a variety of acids and alcohols in $SCCO_2$. Fatty acid esters obtained from alcoholysis of vegetable oils have many applications, such as antifriction agents, food preservatives, and emulsifiers. However, despite the industrial importance of the products obtained from alcoholysis of vegetable oils, conversions of such reactions in $SCCO_2$ have hardly been reported in the literature. Hence, the main objective of this work is to study the ethanolysis of palm kernel oil in $SCCO_2$ as catalyzed by two commercial lipases. The experiments were performed in a reactor vessel in the temperature range of 40−70 °C and from 60 to 200 bar using a water concentration of 0−10 wt % and oil/ethanol molar ratios from 1:3 to 1:10. The initial reaction rate was found to be a function of both pressure and the oil/ethanol molar ratio. A simple model, capable of representing the conversion−time relationship curve, is proposed.

Introduction

Over the past few years, biomodification of oils and fats has been of considerable interest because of the fact that oleochemicals are derived from renewable resources and hence can be produced worldwide.[1] The large-scale production of such materials together with progressive advances in bioreactor technology has promoted the appearance of new techniques to produce oleochemicals. Because lipases can selectively lower the activation energies for a reaction, provide higher substrate specificities, and provide a considerable enhancement in reaction rates relative to nonenzymatic reactions, they have been widely applied in triglyceride technology.[1]

Among several important processes for lipid modification are the hydrolysis reactions, synthesis of esters, and transesterification of fats and oils, as catalyzed by lipases. In these reactions the triglyceride reacts with a fatty acid (acidolysis), an alcohol (alcoholysis), or another ester (interesterification), resulting in a rearrangement of the triglyceride fatty acid groups to produce a new triglyceride as a consequence of the competitive hydrolysis and esterification reactions.

Among several Brazilian raw materials of interest in the production of high-value-added products, palm fruit is one of the most prominent. It is commonly used for the production of an edible oil and the kernel oil is used in the manufacture of soaps. Though direct application of alkyl esters is scarce, they can be used as intermediates in the oleochemical production or as biodiesel.[2]

Several researches have reported an alternative method to produce esters through enzymatic reactions using lipases as catalysts.[3−9] Because biocatalysts have high specific activity and a low impact on the environment, they have become increasingly important for industry. For example, immobilized lipases are used as catalysts for reactions involving biomodification of triglycerides.[10] In this sense, supercritical fluids (SCF), and in particular carbon dioxide, have currently received widespread attention as a possible medium for enzymatic reactions.[11] The main advantage of SCF over liquid solvents is that their high diffusivity, low viscosity, and low surface tension can accelerate the mass transfer in enzymatic reactions.

Nevertheless, enzymatic alcoholysis of vegetable oils in supercritical CO_2 ($SCCO_2$) has hardly been discussed in the literature.[12,13] Furthermore, no comprehensive study concerning process conversion or reaction kinetics for this type of reaction has been reported. Hence, this study is aimed at providing experimental data on the lipase-catalyzed ethanolysis of palm kernel oil in $SCCO_2$ by investigating the influence of operating variables on both the process conversion and reaction kinetics as well as the effect of pressure and the oil/ethanol molar ratio on the initial reaction rate.

Experimental Section

Materials. Palm kernel oil was used as purchased without any pretreatment. The fatty acid composition of palm kernel oil was determined using a gas chromatograph (HP 5890) with a flame ionization detector. The following instrumentation and conditions were used: H_2 as the carrier gas, a modified poly(ethylene glycol) column (FFAP; 2−25 m × 0.20 mm i.d. × 0.30 μm film), column temperature 180−210 °C (2 °C/min), injector temperature 250 °C, and detector temperature 280 °C. The fatty acid composition in palm kernel oil was determined to be 9% caprylic acid, 47% lauric acid, 15% myristic acid, 9% palmitic acid, and 20% oleic acid, resulting in an average molecular weight of the oil as 701.9 g·gmol^{-1}. Ethyl alcohol of analytical grade was

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10.1021/ie990865p CCC: $19.00
Published on Web 10/21/2000

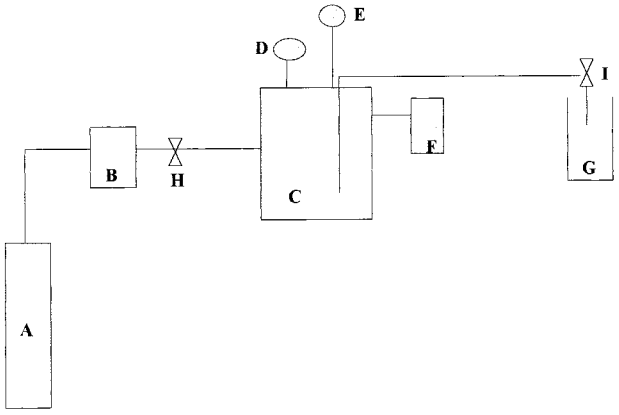

Figure 1. Schematic diagram of the experimental apparatus: A, CO_2 cylinder; B, syringe pump; C, reactor; D, temperature controller; E, pressure transducer; F, liquid pump; G, cold trap; H, check valve; I, micrometering valve.

used as the substrate, and carbon dioxide with a purity higher than 99.99% was used as the solvent. Two commercial immobilized lipases were kindly supplied by NOVO Nordisk Bioindustrial-Brazil (Araucária, PR): *Rhizomucor miehei* (Lipozyme IM) immobilized on a macroporous anion-exchange resin (0.15 U/g, 4% water and diameter in the range of 0.2−0.6 mm) and *Candida antarctica* (Novozym 435) immobilized on a macroporous anionic resin (0.12 U/g, 1.4% water, and a diameter in the range of 0.3−0.9 mm). The optimum activities for both enzymes are achieved at 40−60 °C for Novozym 435 and at 30−70 °C for Lipozyme IM.[14]

Analytical Method. The glycerol content evolved during enzymatic alcoholysis was determined using the method described by Cocks and van Rede.[15] The reaction conversion was then calculated by determining the glycerol concentration, assuming a maximum glycerol yield at the end of the reaction of 12% of the mass of oil.[16]

Experimental Apparatus and Procedure. The experimental setup, as shown in Figure 1, consists basically of a CO_2 cylinder, a high-pressure pump (ISCO 260D), a liquid pump (TSP 4100), and a 300 mL reactor (Autoclave Engineers, model 300BG) equipped with a mechanical stirrer (MagneDrive II), a heating mantle, an internal cooling loop capable of keeping the temperature constant within 0.1 °C, a pressure transducer, and a sampling tube. Palm kernel oil at preestablished concentrations, enzyme (5 wt % of substrates), and water (0−10 wt % of substrates) were placed in the reactor. Afterward, the reactor was closed, flushed, and pressurized with CO_2. Before reaching the desired pressure, a preestablished amount of ethanol was fed into the reactor through the use of the metering liquid pump. The substrates and enzyme were then continuously mixed with an agitation level of 1000 rpm. The amount of allowable reactants was determined based on the phase equilibrium data from Bharath et al.[17] and is reported elsewhere.[18]

To assess the influence of process variables on the reaction conversion, the reaction time was set at 4 h. Typically, samples of approximately 2 mL were withdrawn after the reaction completion, or during the course of the reaction, and then analyzed using the previously described method. A Taguchi experimental design with two levels and four variables (temperature, water and enzyme concentrations, and an oil/ethanol molar ratio) was adopted. The experimental design along with the variable ranges is presented in Table 1, columns 1−5. Duplicate runs were made for all experimental conditions, and the variations around the average values were typically about 3%. The process conversion was then modeled by empirical expressions.

Table 1. Process Conversions Obtained for the Enzymatic Alcoholysis of Palm Kernel Oil in $SCCO_2$

	experimental conditions[a]				reaction conversion (%)	
exp.	T (°C)	P (bar)	W	R	Lipozyme IM	Novozym 435
1	40	73	0	1:3	7.7	54.5
2	40	73	10	1:10	16.7	63.2
3	70	200	0	1:3	11.9	24.7
4	70	200	10	1:10	14.2	35.4
5	40	200	10	1:3	11.7	14.1
6	40	200	0	1:10	25.4	21.3
7	70	73	10	1:3	15.5	12.1
8	70	73	0	1:10	18.4	42.9
9	55	136	5	1:6.5	26.4	20.4

[a] Reaction time: 4 h. W = water concentration, wt % of substrates. R = oil/ethanol molar ratio.

Results and Discussion

It is apparent from Table 1 that the highest reaction conversion (63.2%, 4 h) in $SCCO_2$ was obtained using Novozym 435 as the catalyst. This result might be very important from an economic point of view because a product with a low value can be used to produce high-value-added substances at a relatively low pressure and near room temperature. Moreover, it is relevant to call attention to the much lower solvent costs using a supercritical medium, approximately 2 mL of CO_2/g of oil, compared with recent results in a conventional solvent, 40 mL of *n*-hexane/g of oil.[19] Of course, not only the solvent price but also other important parameters such as environmental aspects, equipment specification, solvent recycling, and energetic and material balances should also be taken into consideration for the conception of a real process.

From the results obtained in the experimental design, a standard statistical modeling technique was employed in constructing empirical models in order to evaluate the effect of process variables on the reaction conversion. The empirical models were built assuming that all variable interactions were significant, estimating the parameters related to each variable interaction and main variable effects, and discarding the meaningless parameters by using the Student's t test considering a confidence level of 95%. The objective of using the Student's t test is to evaluate whether the parameters were significantly different from zero. This test takes into account the standard deviation of each parameter according to a well-known procedure available in many textbooks.[20] The parameters were estimated through the maximum likelihood method.[21] Related to the parameter analysis, it is important to mention that a parameter with a negative value implies a negative effect of the variable on the process.

For the system containing Lipozyme IM, inspection of Table 2 reveals that the oil/ethanol molar ratio was the variable that more positively affected the process conversion. Compared to a recent investigation performed in a conventional medium,[19] this was an expected result, because, in a supercritical medium, ethanol, one of the substrates, is more susceptible to migrate to the lightest phase.[13] The cross interactions between temperature−pressure and temperature−oil/

Table 2. Regression Results for the System Containing Lipozyme IM and Novozym 435 in $SCCO_2$*

Lipozyme IM ($\eta = a_0 + a_1P + a_2R + a_3W + a_4TP + a_5TR + a_6PR + a_7T^2$)			Novozym 435 ($\eta = a_0 + a_1T + a_2P + a_3R + a_4W + a_5TP + a_6TR + a_7T^2$)		
effect	parameter	σ	effect	parameter	σ
	(a_0) 24.27	0.54		(a_0) 20.12	1.58
P	(a_1) 0.61	0.19	T	(a_1) −2.39	0.55
R	(a_2) 3.48	0.19	P	(a_2) −11.29	0.55
W	(a_3) −0.66	0.19	R	(a_3) 4.83	0.55
TP	(a_4) −2.57	0.19	W	(a_4) −0.72	0.55
TR	(a_5) −2.19	0.19	TP	(a_5) 8.76	0.55
PR	(a_5) 0.51	0.19	TR	(a_6) 1.80	0.55
T^2	(a_7) −9.1	0.57	T^2	(a_7) 14.83	1.67

[a] η = calculated reaction conversion. σ = parameter standard deviation.

ethanol molar ratio also had a significant effect on the reaction conversion, with both of them showing a negative effect.

In a second step, the same procedure was adopted using Novozym 435 as the catalyst. After carrying out all of the experiments, an empirical model was built for this system. It can be noted from Table 2 that the pressure was the variable that more strongly affected the process conversion, showing that this variable has a negative effect on the reaction conversion using this particular enzyme, probably causing enzyme activity loss. The oil/ethanol molar ratio also had a significant effect, and hence the same discussion applied to the system containing Lipozyme IM can be done in this case. Temperature also presented a significant negative effect, and therefore an increase in this variable may lead to inactivation of the enzyme. The interaction between temperature and pressure also had an important effect on the reaction conversion. From this comparative study, it becomes clear that enzymes may exhibit different behavior, thus affecting the reaction conversion under the same processing conditions.

The next step toward improving the process conversion was to conduct experiments at the optimized values for the two enzymes. For Lipozyme IM, the optimum condition was found to be 51 °C, 146 bar, R = 1:10, and W = 0 with a predicted reaction conversion of 31.0%, whereas the experimental result was verified to be 29.3%. For Novozym 435, the following values were calculated: 40 °C, 73 bar, R = 1:9.8, and W = 0, resulting in a reaction conversion of 61.1% while the experiment led to 60.9%.[18]

Taking into account these figures, a kinetic study was performed using Novozym 435 as the catalyst, keeping the temperature and W at their optimum values (40 °C and 0 wt %, respectively) and perturbing the oil/ethanol molar ratio and pressure around the optimized values, R = 1:9.8 and 73 bar, respectively. Figure 2 presents the reaction conversion for three values of oil/ethanol molar ratio, 1:9.8, 1:6, and 1:12, obtained by monitoring the reaction up to 8 h and taking samples every 30 min. Note that initially there is a relatively sharp increase of glycerol production, followed by an asymptotic behavior at larger times. Thus, from a practical standpoint, the reaction might be interrupted to meet economic aspects—small gains after a certain time.

According to the experimental design results (Tables 1 and 2), we have noticed that the variable under study (R) affects positively the reaction conversion, thus showing no inhibition caused by an excess of ethanol. As presented in Figure 2, this trend, however, is not

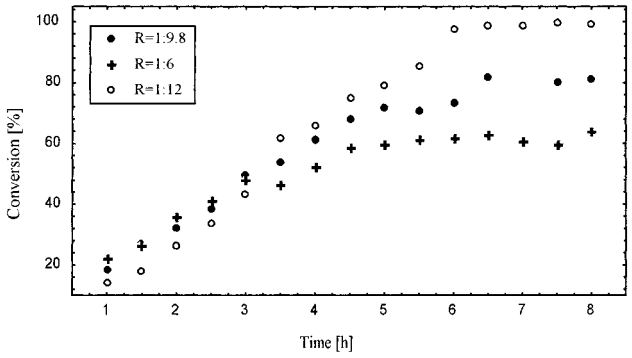

Figure 2. Effect of the ethanol/oil molar ratio (R) on the ethanolysis of palm kernel oil catalyzed by Novozym 435 at T = 40 °C, P = 73 bar, and W = 0.

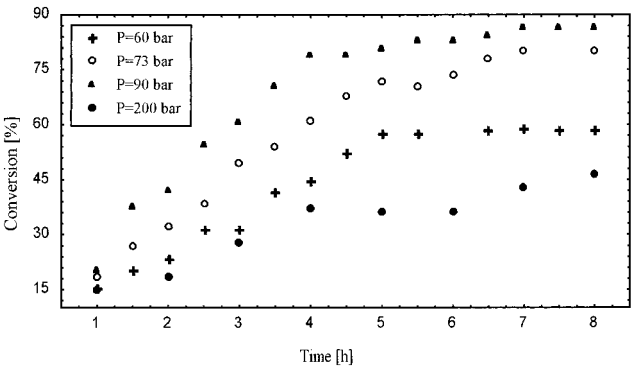

Figure 3. Effect of pressure on the ethanolysis of palm kernel oil catalyzed by Novozym 435 at T = 40 °C, R = 1:9.8, and W = 0.

verified for the entire curve especially for short times where the reaction conversion decreases with increasing ethanol concentration. This trend inverts approximately after 4 h probably because of the natural ethanol consumption during the reaction development and its migration to the lightest phase. Perhaps an interesting alternative to be investigated toward improving process efficiency, avoiding the ethanol inhibition at the beginning of the reaction could be the use of a fed-batch process, in which ethanol would be added continuously to the reaction vessel.

The effect of pressure on the reaction kinetics was evaluated by carrying out experiments at 60, 73, 90, and 200 bar while keeping T = 40 °C, R = 1:9.8, and W = 0. It can be noticed from Figure 3 that a rise in pressure results in an enhancement in the initial reaction rate and reaction conversion except for 200 bar. The initial rates were determined by taking the slopes of the glycerol production vs time curves at the beginning of the reaction (linear part). One can observe from this figure a monotonic increase of glycerol production, leading to a reaction conversion after 7 h as high as 90% at 90 bar. This intriguing phenomenon has been the subject of intense research recently. In fact, it has been observed by Cernia et al.[22] that the initial reaction rates generally exhibit an unusual behavior in the range of 6−25 MPa. As shown in Figure 4, in the subcritical region a rise in pressure leads to a sharp increase in the reaction rate and then a gradual decrease at higher pressures. The rate of glycerol production rapidly increases from 13.09 (60 bar) and 16.08 (73 bar) up to 28.15 at 90 bar and then dramatically decreases to 6.66 at 200 bar. The fact that the initial rate of enzyme-catalyzed reactions in SCF is maximized near the solvent critical region has just been corroborated in the

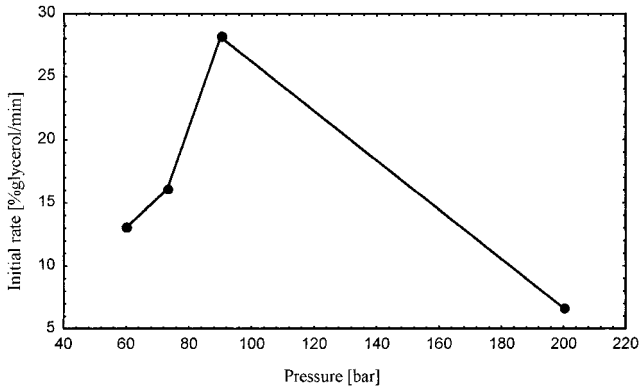

Figure 4. Effect of pressure on the initial reaction rate of palm kernel oil catalyzed by Novozym 435 at $T = 40$ °C, $W = 0$, and $R = 1{:}9.8$.

literature.[22] Though the role of $SCCO_2$ molecules during enzymatic reactions is, so far, not well understood, it has been argued that the pressure could induce modifications of both the structure and function of the enzyme, leading to a change in the enzyme activity.[22]

Kinetic Modeling. In an attempt to represent the experimental kinetic data, a simplified mechanism was proposed by considering the equation

$$[\mathrm{T}] + 3[\mathrm{A}] \underset{k_2}{\overset{k_1}{\rightleftharpoons}} [\mathrm{G}] + 3[\mathrm{M}] \qquad (1)$$

where [T], [A], [G], and [M] stand for the molar concentrations (mmol/mL) of triglycerides, ethanol, glycerol, and esters, respectively.

The following assumptions have been made: (i) reversible reaction; (ii) no loss of enzyme activity during reaction; (iii) no limitations by mass-transfer restrictions, and (iv) no consideration of intermediate reaction products, mono- and diglycerides. In fact, these substances have not been experimentally detected from the thin layer chromatographic (TLC) analyses. Then, two differential equations are obtained:

$$\frac{\mathrm{d}[\mathrm{G}]}{\mathrm{d}t} = k_1[\mathrm{T}][\mathrm{A}]^3 - k_2[\mathrm{G}][\mathrm{M}]^3 \qquad (2)$$

$$\frac{\mathrm{d}[\mathrm{T}]}{\mathrm{d}t} = \frac{1}{3}\frac{\mathrm{d}[\mathrm{A}]}{\mathrm{d}t} = -\frac{\mathrm{d}[\mathrm{G}]}{\mathrm{d}t} = -\frac{1}{3}\frac{\mathrm{d}[\mathrm{M}]}{\mathrm{d}t} \qquad (3)$$

We have noticed from the experimental results of the conversion−time relationship curves that the initial rates are quadratically dependent on the ethanol concentration and decay exponentially with increasing pressure. Thus, to account for the effect of pressure and ethanol concentration on the kinetic modeling, the following empirical expression for the parameter k_1 is suggested:

$$k_1 = (a_0 + a_1[\mathrm{A}] + a_2[\mathrm{A}]^2)\{a_3 \exp[a_4(\mathrm{P})]\} \qquad (4)$$

where a_0, a_1, a_2, a_3, and a_4 are adjustable parameters.

These parameters along with k_2 were estimated through the maximum likelihood method solving eqs 2 and 3 using the commercial software Maple V, resulting in the following values: $a_0 = 5.658$ mL^3/mmol^3·min; $a_1 = -7.824 \times 10^{-1}$ mL^4/mmol^4·min; $a_2 = 3.153 \times 10^{-2}$ mL^5/mmol^5·min; $a_3 = 2.378 \times 10^{-4}$ mL^3/mmol^3·min; $a_4 = -1.542 \times 10^{-2}$ bar^{-1}, and $k_2 = 2.084 \times 10^{-4}$ mL^3/mmol^3·min.

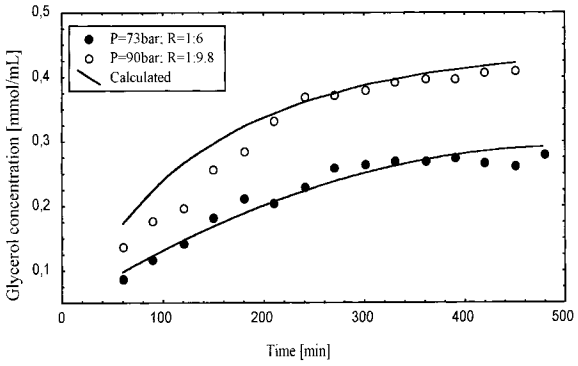

Figure 5. Experimental and calculated glycerol concentrations for the ethanolysis of palm kernel oil catalyzed by Novozym 435 at $T = 40$ °C and $W = 0$.

It should be stressed that a global fitting with all of the experimental kinetic data present in Figures 2 and 3 has been performed. As an example, Figures 5 depicts experimental and calculated reaction conversion values for two process conditions. Though the proposed model may be considered somewhat oversimplified in relation to a more complex approach (for example, Ping-Pong Bi−Bi mechanism[23,24]), it has proven to be capable of correlating the experimental data. Indeed, comparison of the variance of the estimation procedure with the experimental one through the application of the F test at a confidence level of 95% showed that the model is able to represent the experimental data within the range studied.

Conclusions

In this work, Lipozyme IM and Novozym 435 were used as catalysts in the ethanolysis of palm kernel oil in $SCCO_2$. The use of an experimental design for the production of esters from enzymatic reactions of vegetable oils is proven to be a rational basis to assess the influence of process variables on the reaction conversion. From this study we have observed that, depending on the enzyme, different reaction conversion results can be obtained. From these results an investigation concerning the reaction kinetics in supercritical media has been performed. The performed kinetic study revealed interesting features with respect to the system pressure and the ratio of substrates. Though the conversion−time results cannot easily be interpreted based only on a kinetic mechanism, the approach presented here led to a good agreement between experiment and theory.

Acknowledgment

The authors thank FAPERJ and CNPq for their financial support.

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Received for review November 29, 1999
Revised manuscript received March 17, 2000
Accepted June 8, 2000

IE990865P