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Optimization of Enzymatic Production of Biodiesel from Castor Oil in Organic Solvent Medium

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Abstract

We studied the production of fatty acid ethyl esters from castor oil using *n*-hexane as solvent and two commercial lipases, Novozym 435 and Lipozyme IM, as catalysts. For this purpose, a Taguchi experimental design was adopted considering the following variables: temperature (35–65°C), water (0–10 wt/wt%), and enzyme (5–20 wt/wt%) concentrations and oil-to-ethanol molar ratio (1:3 to 1:10). An empirical model was then built so as to assess the main and cross-variable effects on the reaction conversion and also to maximize biodiesel production for each enzyme. For the system containing Novozym 435 as catalyst the maximum conversion obtained was 81.4% at 65°C, enzyme concentration of 20 wt/wt%, water concentration of 0 wt/wt%, and oil-to-ethanol molar ratio of 1:10. When the catalyst was Lipozyme IM, a conversion as high as 98% was obtained at 65°C, enzyme concentration of 20 wt/wt%, water concentration of 0 wt/wt%, and oil-to-ethanol molar ratio of 1:3.

Index Entries: Alcoholysis; vegetable oils; lipases; reaction kinetics; biodiesel.

Introduction

Biotransformation of vegetable oils through the use of enzymes as catalysts has been a matter of intense investigation recently. The possibility

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of using biodiesel as an additive to mineral diesel to result in a sulfur-free, higher-cetane-number fuel from a renewable resource has motivated efforts in the biomodification of vegetable oils in order to reduce environmental costs and import needs.

Lipases have been extensively used in triglyceride technology, mainly for the biotransformation of oils and fats. Among several important processes for lipid modification are the hydrolysis reactions, synthesis of esters, and transesterification of these materials in the presence of 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.

Esters obtained from alcohols and fatty acids have many remarkable applications. Those from long chain acids (12–20 carbon atoms) and short chain alcohols (3–8 carbon atoms) have been widely employed in the food, cosmetic, and pharmaceutical industries *(1).* Natural esters such as those from jojoba oil, carnauba wax, and whale oil have been used. However, these oils are expensive and are not usually available in large amounts. Therefore, it is desirable to develop methods for the production of such esters using cheaper and more plentiful raw materials *(2).*

The establishment of the Brazilian National Program on Biodiesel and the expectation of commercial availability of the product within 2 yr throughout Brazil have prompted several studies on biodiesel production using different techniques and a variety of vegetable and animal sources. Among several raw materials available, castor oil is one of the most prominent. Besides the advantage of being a native plant in Brazil, the castor plant is versatile concerning climate and ground types, with a very high yield per hectare and high content of oil (¬^{a}50 wt%), affording a biofuel with a much higher cetane number (>60) than mineral diesel and has a good lubricity index. Of course, it should also be remembered that Brazil is one of the world's leading castor oil producers.

Chemical esterification methods use an alcohol and a carboxylic acid in the presence of a mineral acid as catalyst. Sulfuric acid, which is commonly used, leads to the formation of undesirable byproducts, requiring a difficult separation step *(3).* Moreover, in this case, the starting material is a high-value component (fatty acid). Consequently, researchers are interested in the alcoholysis reaction using a vegetable oil with low cost and largely produced in Brazil as a raw material for ester synthesis.

This reaction offers several advantages when compared to esterification of fatty acids, mainly owing to the possibility of using vegetable oils as substrates. In this case, a vegetable oil and an alcohol are used as substrates in the production of glycerol and fatty acid alkyl esters. Conventionally, acid and basic catalysts have been used *(4,5).* However, the use of acid catalysts usually results in low conversions. The use of basic catalysts require the utilization of vegetable oils with low free fatty acid con-

tent (3 mg of KOH/g). In spite of the high yields obtained (90%) when using pretreated acid oils, a decrease in conversion is observed owing to the soap-removing step. The use of enzymes minimizes this problem, since oils with a high acid content can also be used without a pretreatment and no enzymatic activity loss is observed.

Several researches have reported an alternative method to produce esters through enzymatic reactions using lipases as catalysts *(6–12)*. 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 *(13)*.

The main objective of the present work was the production of ethyl esters from the enzymatic alcoholysis of castor oil using *n*-hexane as solvent. Two commercial lipases, Novozym 435 and Lipozyme IM, were compared. The variables in these experiments were temperature, water and enzyme concentrations in the reaction medium, and the oil:ethanol molar ratio. An empirical model was built to evaluate the effects of process variables on the conversion, and thus to determine the operating conditions that maximize the production of esters for each enzyme.

Materials and Methods

Castor Oil

A commercial castor oil (Delaware-Brazil) was used as purchased without any pretreatment. The fatty acid composition was determined using a gas chromatograph (HP 5890) with a flame ionization detector. The following instrumentation and conditions were used: H_2 as carrier gas, modified polyethylene glycol column (FFAP 2 – 25 m × 0.20 mm id × 0.30-mm film), column temperature of 180–210°C (2°C/min), injector temperature of 250°C, and detector temperature of 280°C. Using this procedure, the approximate fatty acid composition in castor oil is 92 wt% ricinoleic acid and 8 wt% other acids. Ethyl alcohol (95 v/v%) (Merck) and *n*-hexane PA (Merck) were used as substrate and solvent, respectively.

Enzymes

Two commercial immobilized lipases were kindly supplied by Novozymes Brazil (Araucária, PR, Brazil): *Mucor miehei* (Lipozyme IM) immobilized on a macroporous anion-exchange resin (0.15 U/g and 4 wt% water) and *Candida antarctica* (Novozym 435) immobilized on a macroporous anionic resin (0.12 U/g and 1.4 wt% water).

Analytical Method

Samples of fatty acid esters were analyzed with a gas chromatograph interfaced with a mass selective detector (Model QP 5050A; Shimadzu) using a capillary column PE-5 (20 m × 0.18 mm × 0.25 mm). The following

Table 1
Range of Variables of Enzymatic Alcoholysis of Castor Oil

Variable	Range
[*T*] (°C)	35–65
[*W*] (wt/wt%)	0–10
[*E*] (wt/wt%)	5–20
[*R*]	1:3–1:10

column temperature gradient programming was adopted: 60–200ºC (3°C/min) and 200–300ºC (5°C/min). Identification and quantification of the samples (0.5 mL and 10 mL of heptane) were accomplished using ethyl ricinoleate (25 mg/mL) as internal standard. Helium was used as carrier gas, and the injection and detector temperatures were 280 and 320ºC, respectively. The injection of the internal standard with a known concentration allowed determination of the response factor according to the relation $C = A \times RF$, in which A is the peak area, RF is the response factor, and C is the concentration.

Experimental Procedure and Statistical Analysis

The experiments were performed in stoppered 300-mL Erlenmeyers flasks. Lipase was added to the mixture of oil-ethanol-solvent and the flasks were agitated at 200 rpm for 8 h in a controlled-temperature shaker. Samples were taken at each hour in order to follow the course of the reaction. The solvent was used with the purpose of reducing mass transfer limitations, thus promoting efficient contact between substrates (oil and ethanol). Based on previous works *(14,15)*, *n*-hexane was used as solvent medium in a fixed amount of 40 mL. A Taguchi experimental design with two levels and four variables (temperature [*T*], water [*W*] and enzyme [*E*] concentrations, and oil:ethanol molar ratio [*R*]) was adopted. The variable ranges adopted, as presented in Table 1, were based on previous results for a similar system and were chosen to cover the intervals commonly used *(14–16)*. The experimental runs were executed randomly, and duplicate runs were carried out for all experimental conditions providing an average SD of about 5%. The process conversion was then modeled by a polynomial model. The kinetic data, which demonstrated that for almost all experimental conditions the maximum conversions were obtained in 6 h, were used this time for evaluating the influence of the variables and process optimization.

Results and Discussion

The experimental results for Lipozyme IM, which exhibits specificity in the 1,3 positions, and Novozym 435, a nonspecific lipase, are presented

Table 2
Experimental Design and Conversions in Enzymatic Alcoholysis of Castor Oil

		Experimental Conditions (wt/wt %)			Maximum Conversion (%)	
Run	*T* (°C)	[*E*]	[*W*]	*R*	Lipozyme IM	Novozym 435
1	35	5	0	1:3	42.5	47.5
2	35	5	10	1:10	0.65	1.6
3	35	20	0	1:10	70.4	67.0
4	35	20	10	1:3	85.0	34.0
5	65	5	0	1:10	19.7	52.0
6	65	5	10	1:3	33.0	16.5
7	65	20	0	1:3	98.0	60.5
8	65	20	10	1:10	40.6	73.0
9	50	12.5	5	1:6.5	60.6	23.0

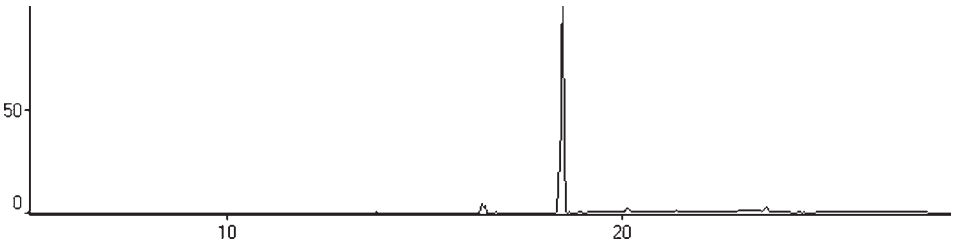

Fig. 1. Chromatogram obtained for biodiesel from castor oil. Experiment 3: time, 2h. The peak was identified as ethyl ricinoleate.

in Table 2. One can observe that the higher yield was achieved in the system with Lipozyme IM when compared with Novozym 435 (98 and 73%, respectively). Note that the enzymes exhibited different behavior probably because lipases generally have optimum working ranges and are affected mainly by the system temperature and water added to the reaction medium. Also note that the oil:ethanol molar ratio directly affected the process conversion and that the enzyme concentration had a positive effect on ester production. The results obtained here are similar to those from Oliveira and Alves (14), who performed lipase-catalyzed reactions using palm and palm kernel oil.

For the sake of brevity, Fig. 1 presents a typical chromatogram found for the biodiesel produced from castor oil using Lipozyme IM (experimental condition 3). Note the presence of a major peak referred to ethyl ricinoleate, which is the compound obtained from the alcoholysis of ricinoleic acid, the main component of castor oil.

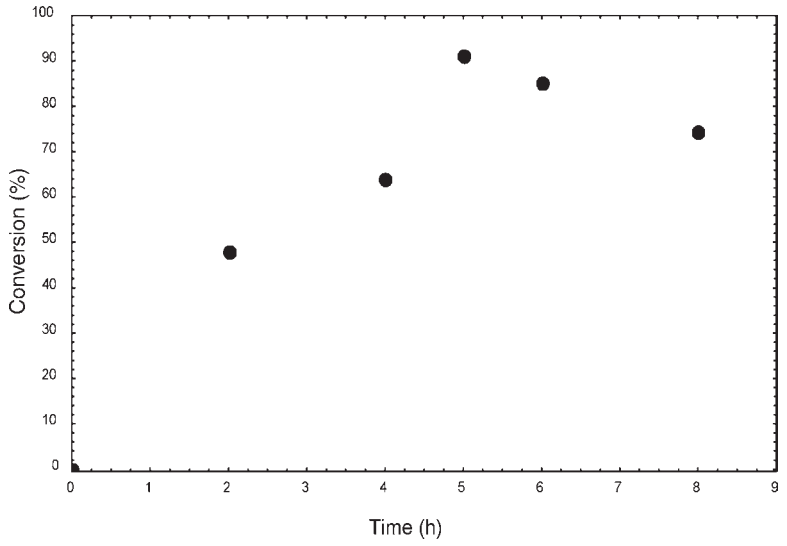

Fig. 2. Kinetics of lipase alcoholysis of castor oil in *n*-hexane for run 4 using Lipozyme IM.

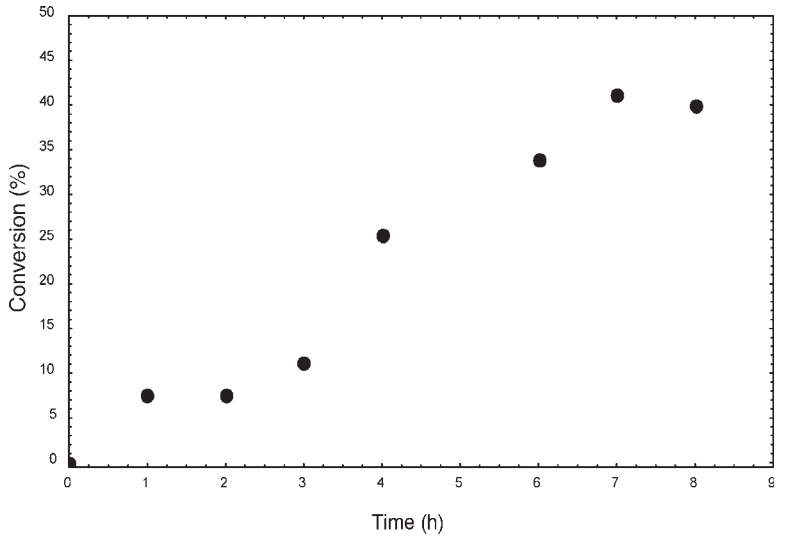

Fig. 3. Kinetics of lipase alcoholysis of castor oil in *n*-hexane for run 4 using Novozym 435.

As an example, Figs. 2–5 present kinetic curves obtained for runs 4 and 7 for each system studied up to 8 h of reaction. Undoubtedly, the knowledge of time evolution of the reaction plays an important role if one takes into account a possible scale-up of a continuous process.

Effects of Process Variables

The influence of temperature, water and enzyme concentrations, and oil:ethanol molar ratio, as well as the cross-interactions, was investigated.

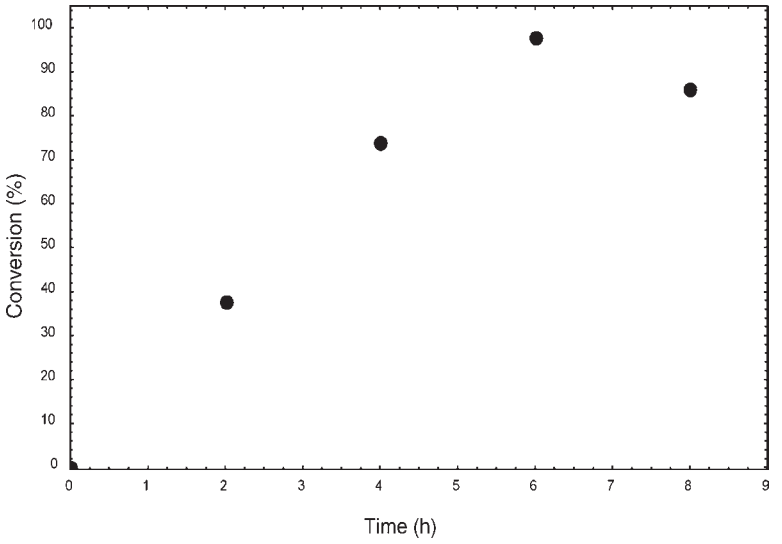

Fig. 4. Kinetics of lipase alcoholysis of castor oil in *n*-hexane for run 7 using Lipozyme IM.

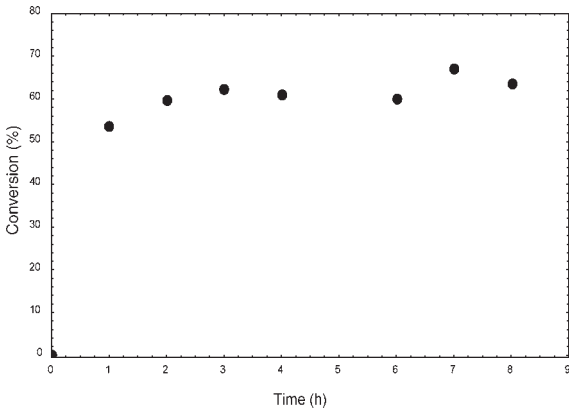

Fig. 5. Kinetics of lipase alcoholysis of castor oil in *n*-hexane for run 7 using Novozym 435.

To allow a direct comparison of each variable effect, the independent variables were normalized in the range of –1 to +1, according to

$$x_i = \frac{2\left(X_i - X_{\max}\right)}{\left(X_{\max} - X_{\min}\right)} - 1 \qquad (1)$$

in which x_i is the normalized value of the variable X at condition i; X_i is the actual value; and $X_{\min}$ and $X_{\max}$ are the lower and upper limit, respectively.

The "–1" level represents the lower limit, while the "+1" level represents the upper limit of each variable. A statistical modeling technique was used to obtain an empirical model able to reproduce the experimental data.

Table 3
Regression Results for Lipozyme IM System

$Y = a_0 + a_1R + a_2W + a_3E + a_4R^2 + a_5TE + a_6RE$

Average absolute deviation: 4.9 [a]

Parameter		SD
a_0	60.60	4.09
a_1	–15.79	1.45
a_2	–8.92	1.45
a_3	24.87	1.45
a_4	–11.97	4.34
a_5	–3.39	1.45
a_6	–2.21	1.45

[a]

$$\text{AAD\%} = \frac{1}{N}\sum_{i}^{N}\frac{\left|Y_i^{\text{exp}} - Y_i^{\text{calc}}\right|}{Y_i^{\text{exp}}} \times 100$$

in which the subscripts exp and calc are, respectively, the experimental and calculated values; and the quantity Y is the reaction conversion.

Empirical models were built by assuming that all variable interactions were significant, estimating the parameters related to each variable interaction and main variable effects, and discarding the meaningless parameters considering a confidence level of 95%, by using the student's *t*-test. The parameters were estimated through the maximum likelihood method *(17)*.

Reactions Catalyzed by Lipozyme IM

Regarding the system catalyzed by Lipozyme IM, the data in Table 3 show that the water added to the system, the oil:ethanol molar ratio, and the interactions of temperature–enzyme concentration and oil:ethanol molar ratio–enzyme concentration had a negative effect on the conversion. In addition, an excess of ethanol may inhibit the enzymatic reaction and the addition of water caused a negative effect on the conversion, corroborating the fact that excess water may change the reaction equilibrium, thus decreasing the formation of esters. Also note that the enzyme concentration had a strong positive influence on the biodiesel production. Once the effects of process variables were evaluated, the optimization was carried out. For this system, however, the optimum condition found from the experimental design was the same as observed for run 7 (Table 2), since the conversion value predicted by the empirical model was 99.6%, which agrees very satisfactorily with the experimental one, 98.0%.

Table 4
Regression Results for Novozym 435 System

$Y = a_0 + a_1 T + a_2 R + a_3 W + a_4 E + a_5 R^2 + a_6 TR + a_7 TW$

Average absolute deviation: 4.6[a]

Parameter		SD
a_0	23.01	4.64
a_1	6.48	1.64
a_2	4.39	1.64
a_3	-12.73	1.64
a_4	14.61	1.64
a_5	21.02	4.92
a_6	7.61	1.64
a_7	6.98	1.64

[a]

$$\text{AAD\%} = \frac{1}{N}\sum_{i}^{N}\frac{\left|Y_i^{\text{exp}} - Y_i^{\text{calc}}\right|}{Y_i^{\text{exp}}} \times 100$$

in which the subscripts exp and calc are, respectively, the experimental and calculated values; and the quantity Y is the reaction conversion.

Reactions Catalyzed by Novozym 435

From the results presented in Table 2 one can see that the greatest conversion was obtained at the upper limit of all process variables. Table 4 reveals that, as in the case of Lipozyme IM, the addition of water led to inhibition of the reaction. The enzyme concentration, the temperature, the oil:ethanol molar ratio, and the interactions temperature–oil:ethanol molar ratio and temperature–water addition had a positive effect on the production of biodiesel. Concerning temperature, the result obtained confirms the fact that the optimum temperature for this enzyme is about 70°C. As expected, the enzyme concentration, in the experimental range investigated, had a positive effect on the reaction conversion. Note also that for this system no alcohol inhibition was verified. The optimization for this system led to the following process variables values: T = 65°C, [E] = 20 wt/wt%, [W] = 0 wt/wt%, and R = 1:10, with a predicted maximum conversion of 82% in 6 h. The execution of the experiment resulted in an experimental value at these conditions of 81.4%, which agrees very well with the value predicted from the experimental model.

Conclusion

The use of an experimental design for the production of esters from the enzymatic reactions of vegetable oils proved to be a rational means to

investigate the influence of process variables on the conversion. Empirical models were built to represent experimental data and to allow the determination of process variables that maximize the conversion. The results obtained using Novozym 435 gave lower conversions when compared to the systems using Lipozyme IM. The results obtained here may be useful if one considers that a low-cost raw material, a renewable source, can be used for the production of high-value-added products and/or as a biofuel.

Acknowledgments

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