BIODIESEL PRODUCTION VIA ESTERIFICATION REACTIONS CATALYZED BY LIPASE

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***Abstract*— The synthesis of ethyl hexadecanoate was carried out by esterification of palmitic acid with ethanol in a solvent-free system. A commercial immobilized lipase (Lipozyme RM-IM) was mixed with the reagents, in a 15 mL closed batch reactor with constant stirring and coupled with a condenser. The effects of palmitic acid/ethanol molar ratio (0.16 to 1.84), reaction temperature (65 to 75°C) and enzyme concentration (0.48 to 5.52% w/w) on the initial reaction rate of ethyl hexadecanoate were determined using central composite design 2^3 with six central points. Statistical analysis indicated that the enzyme concentration and palmitic acid/ethanol molar ratio had been found to be the most significant variables affecting the initial reaction rate. The best result was obtained under the following experimental conditions: palmitic acid/ethanol molar ratio of 0.50, temperature of 67°C, and enzyme concentration of 4.50% (w/w).**

***Keywords*— Biodiesel, Esterification, Lipase.**

I. INTRODUCTION

Biodiesel has been defined as the monoalkyl esters of long-chain fatty acids, preferentially methyl and ethyl esters, derived from renewable feedstocks, such as vegetable oils or animal fats. Its properties are close to diesel fuels, and therefore biodiesel becomes a strong candidate to replace the diesel fuels (Srivastava and Prasad, 2000). Recently, because of increases in crude oil prices, limited resources of fossil oil, environmental concerns, population increase and hence, higher energy demand, biodiesel represents a promising alternative fuel for use in compression ignition (diesel) engines.

The biodiesel advantages over conventional fuels are its lower toxicity, high biodegradability, substantial reduction in SO_x emissions, considerable reduction in carbon monoxide (CO), polyaromatics hydrocarbons, smoke and particulate matter. It is obtained from renewable resources (vegetable oils) consuming more carbon dioxide from the atmosphere during the production than is added to it by their later combustion. Therefore, it reduces the carbon dioxide content of the atmosphere and hence, reduces the greenhouse effect. Furthermore, the sulphur contents of vegetal oils are close to zero and consequently, the environmental damage caused by sulphuric acid is reduced (Vicente *et al.*, 1998; Srivastava and Prasad, 2000; Fukuda *et al*., 2001; Soumanou and Bornscheuer, 2003).

The industrial process of biodiesel production is usually carried out by heating an excess of alcohol usually methanol or ethanol with vegetable oils under different conditions in the presence of an inorganic catalyst (Mittelbach, 1990). This chemical process is called by transesterification or alcoholysis. The most commonly used catalysts are alkali hydroxides (NaOH, KOH), carbonates and corresponding sodium and potassium alkoxides. A disadvantage of alkali-catalyzed procedures is that the homogenous catalysts are removed with the glycerol layer after the reaction and cannot be reused. Furthermore, neutralization to prevent toxic wastes is necessary and the purification of glycerol is more difficult when large amounts of catalyst have to be removed. Another disadvantage is the partial saponification reaction, which produces soap. The soap lowers the yield of esters and makes the separation of ester and glycerol difficult (Ma and Hanna, 1999; Fukuda *et al*., 2001; Köse *et al.*, 2002). Besides, the use of more expensive refined oils is necessary because oils should have low free fatty acids content (inferior to 1%). Thus, the cost associated to oils and fats is relatively high constituting about 80% of the total cost of the biodiesel production (Bender, 1999; Faria *et al*., 2003).

So, the production of biodiesel by direct esterification of fatty acids and alcohols (methanol or ethanol) catalyzed by lipase is an interesting alternative to decrease the operating costs associated to the conventional process as well as to overcome the above mentioned problems. In addition, it is possible the use of fatty acid mixtures in this process, usually obtained from the vegetable oil refining with a lower cost than that of the triglycerides. Normally, methanol is used as it is the cheapest alcohol in most countries. However, in Brazil it is advantageous to use anhydrous ethanol, which is already produced in large quantities for blending with gasoline. Furthermore, the environmental concern should also be emphasized, since ethanol is obtained from biomass and hence, it is not contributing for the greenhouse effect; unlike methanol, which is mainly obtained from petroleum resources (Schuchardt *et al*., 1998; Júnior *et al.*, 2003).

It is well established that the palmitic acid is the saturated fatty acid of long chain found in larger proportion in vegetables oils. In the same way, considering the possibility to use fatty acid mixtures as raw material for

biodiesel production, this acid is also the main constituent (42.8%) of that mixture (Faria *et al.*, 2003).

In the present work, lipase catalyzed esterification of hexadecanoic acid (palmitic acid) with ethanol in the presence of commercial immobilized lipase from *Mucor meihei* (Lipozyme IM-20) in a solvent-free medium was investigated. The Factorial Design of Experiments technique was used to study the influence of the variables, acid/alcohol molar ratio, reaction temperature and enzyme concentration on the initial rate of reaction.

II. MATERIALS AND METHODS

A. Materials

The catalyst used was Lipozyme RM-IM (EC 3.1.1.3), a commercial *Mucor meihei* lipase immobilized on an ionic resin donated by Novozymes A/S (Bagsvaerd, Denmark). Palmitic acid (98% w/w) and ethanol (98% w/w), with analytical grade, were supplied by Merck (Darmstadt, Germany). Ethyl hexadecanoate (ethyl palmitate) of higher purity was supplied by Sigma Co. (St. Louis, USA). Ethyl acetate, n-hexane, glycerol and lauric acid were supplied by Merck (Darmstadt, Germany). Sodium hydroxide and acetone were supplied by Vetec (Brazil).

B. Determination of Esterification Activity of Lipozyme

Lipozyme´s activity was determined by the consumption of lauric acid in the esterification reaction with glycerol (lauric acid/glycerol molar ratio of 3) at 60°C, with the enzyme concentration of 5% (w/w), by the method described by Langone and Sant'Anna (1999). One esterification unit of Lipozyme (U) was defined as 1 μmole of lauric acid consumed/min under the experimental conditions described herein. The enzyme used in this work had an esterification activity of 20 U/g.

C. Chromatography Analysis

The fatty acid and ester were analyzed by capillary gas chromatography (GC). Each sample of 20 μL was diluted (1000 times) in a 1:1 hexane/ethyl acetate mixture and 2 μL were injected into a Varian CP–3380 gas chromatography with a flame ionization detector (FID). The GC was fitted with a 30m × 0.25mm × 0.25μm CP WAX 52 CB column in a split injection system with ratio of 1:20. The detector and injector temperatures were set at 280ºC and 300ºC, respectively. The column temperature was set at 180ºC and then programmed to rise at 20ºC/min up to 220ºC, where it was maintained constant for 6 minutes. Hydrogen was used as the carrier gas at a flow rate of 2 mL/min and the column pressure was maintained constant at 20 psi.

D. Esterification Experiments

All experiments were carried out in a 15 mL closed batch reactor with constant stirring, using a magnetic stirrer, and coupled with a condenser. The reactor was kept at the desired temperature by a thermostatic water bath. In order to prevent alcohol loss by volatility, the water that circulated inside the condenser was cooled by a thermostatic water bath. The reactor was loaded with palmitic acid, ethanol and immobilized lipase. The starting time of the reaction was the addition of the Lipozyme. The progress of the reaction was followed by withdrawing 20 μL aliquots at various time intervals, and then analyzing them by the GC method described previously.

E. Experimental Design

To optimize the conversion or the initial reaction rate one must identify the most important process' parameters. This can be an exhausting task since it requires changing each one of the operating variables at a time, and frequently only an apparent optimum is obtained, when interactions among variables are ignored. In order to solve this problem, the Factorial Design of Experiments technique was used. Based on simple statistics, experimental design reduces the number of experiments to be performed and thus costs drop, allowing the researcher to establish interactions among variables within the range studied (Garcia *et al.*, 1999a; Montgomery, 2001).

A central composite design 2^3 with six central points was used to determine the effects of some parameters on the palmitic acid initial reaction rate ($-r_{Ao}$). The palmitic acid/ethanol molar ratio (MR), the temperature (T) and the enzyme concentration (E) were the variables investigated. The assay conditions for reaction parameters were taken at zero level (center point) and one level (+1 and -1). The design was extended up to ±α = 1.68 (axial point). The range of values studied for each variable are indicated in Table 1.

Table 1. Levels of variables for central composite design.

Variables	Levels				
	-1.68	-1	0	+1	+1.68
MR	0.16	0.50	1.00	1.50	1.84
T (ºC)	65	67	70	73	75
E (% w/w)	0.48	1.50	3.00	4.50	5.52

The lower temperature level was 65°C, since the melting point of palmitic acid was 63ºC at atmospheric pressure. The upper temperature level, 75ºC, was determined by the boiling point of ethanol.

III. RESULTS AND DISCUSSION

All experimental conditions were generated by the software Statistica for Windows, version 6.0 produced by Statsoft. The initial rates of reaction were obtained by the batch reactor molar balance, which is shown in the Eq. (1).

$$(-r_{A_o}) = C_{A_o}\left(\frac{dX_A}{dt}\right)_0 \quad (1)$$

where X_A is the conversion of palmitic acid, C_{Ao} its initial concentration (mol/g) and t is time. The derivatives dX_A/dt for each experimental condition were obtained by polynomial fitting of data on X_A versus time. For

example, the graph for the experimental conditions MR = 1.00, T = 70ºC and E = 0.48% (w/w) is shown in Fig. 1.

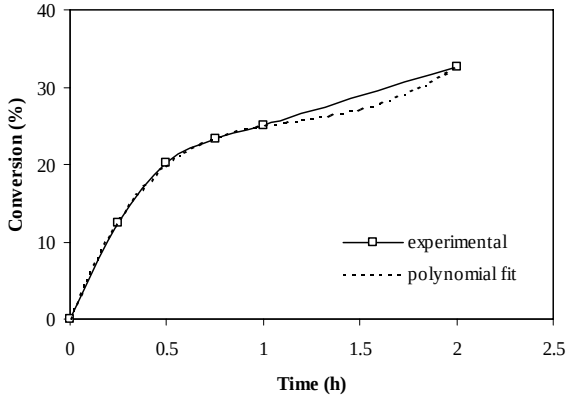

Figure 1. Conversion *versus* time for the esterification reaction between palmitic acid and ethanol using Lipozyme IM-20.

The results are illustrated in Fig. 2. The best conditions for acid conversion (52%) and initial rate of reaction (0.88 mol/g.h) were: 67ºC, acid/alcohol molar ratio of 0.50 and enzyme concentration of 4.50% (w/w).

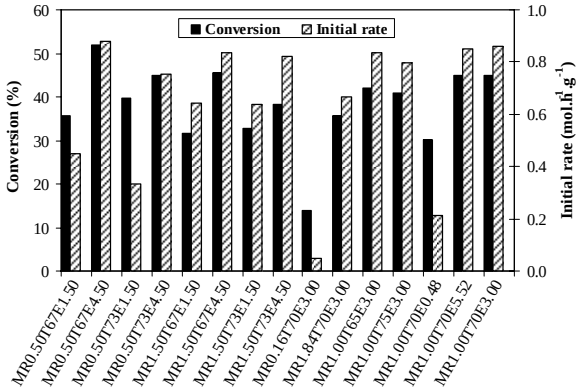

Figure 2. Influence of variables (MR, T and E) on the initial rate and on the conversion after 1 hour.

The values of the coefficients and associated errors of each one of the studied parameters and their interactions in relation to initial rate were determined using the software Statistica. The second order mathematical model is shown in the Eq. (2):

$$(-r_{A_o}) = (0.855 \pm 0.004) + (0.116 \pm 0.003) \times MR - (0.025 \pm 0.003) \times T + (0.167 \pm 0.003) \times E - (0.152 \pm 0.003) \times MR^2 + (0.011 \pm 0.003) \times T^2 - (0.090 \pm 0.003) \times E^2 + (0.026 \pm 0.003) \times MR * T - (0.062 \pm 0.003) \times MR * E + (0.0007 \pm 0.0034) \times T * E \quad (2)$$

The proposed method presented a good coefficient of determination (R^2 = 0.914). The effects of the enzyme concentration (E), acid/alcohol molar ratio (MR), quadratic temperature (T^2), acid/alcohol molar ratio–temperature interaction (MR*T) and temperature–enzyme concentration interaction (T*E) are positive, indicating that initial rate of reaction increases with the increase of these variables. However, the effects of the parameters temperature (T), quadratic acid/alcohol molar ratio (MR^2), quadratic enzyme concentration (E^2) and acid/alcohol molar ratio–enzyme concentration interaction (MR*E) are negative, therefore, an increase of these variables results in a decrease of the initial rate of reaction.

The Student's t-test was used to establish if calculated effects presented statistical significance. These evaluations are illustrated in Fig. 3 through the Graph of Pareto. This graph also includes a vertical line that indicates the minimum magnitude of the effects that showed statistical significance for a confidence level of 95%. The values accompanying in the horizontal columns are those of the Student's t-test for each factor. An effect that exceeds the vertical line is considered significant.

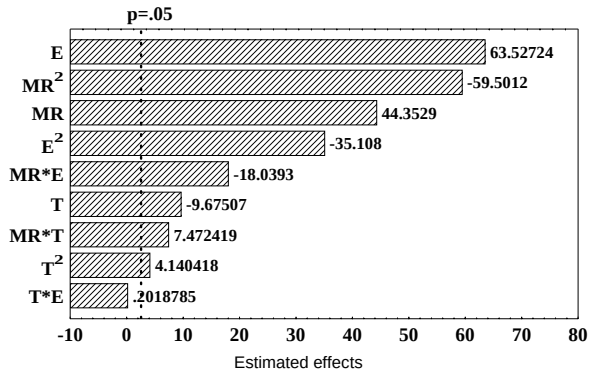

Figure 3 Graph of Pareto for the effects on the initial rate of ethyl hexadecanoate.

In agreement with Fig. 3, all the parameters, except for temperature–enzyme concentration interaction (T*E), showed statistical significance, since their effects exceed the vertical line (p = 0.05). It can be concluded that the enzyme concentration, the acid/alcohol molar ratio and the quadratic acid/alcohol molar ratio are the parameters that have the most significance in the initial rate of reaction.

The second order model can be plotted as a three-dimensional surface representing the response (initial rate of reaction) as a function of two factors for the experimental range considered. The response surface of initial rate as a function of palmitic acid/ethanol molar ratio and enzyme concentration at the temperature of central point (T = 70ºC) is plotted in Fig. 4. This response surface indicates that, for low acid/alcohol molar ratio, the initial rate of reaction increases with increasing enzyme concentration. This is due to the fact that the most significant factor is the enzyme concentration, and its effect is positive. For higher acid/alcohol molar ratio, however, a different behaviour is observed. The initial rate of reaction increases initially, reaching a maximum at intermediate enzyme concentrations, and

then decreases at higher enzyme concentrations. This was probably due to the negative effects of acid/alcohol molar ratio–enzyme concentration interaction, quadratic acid/alcohol molar ratio and quadratic enzyme concentration.

In agreement with Fig. 4, the largest values of initial rate of reaction were obtained in molar ratio of reactants between 0.50 and 1.50 and in enzyme concentrations between 3.0 and 5.5% (w/w). The optimized conditions obtained by Statistica were palmitc acid/ethanol molar ratio of 1.1, temperature of 72ºC and enzyme concentration of 4.2% (w/w).

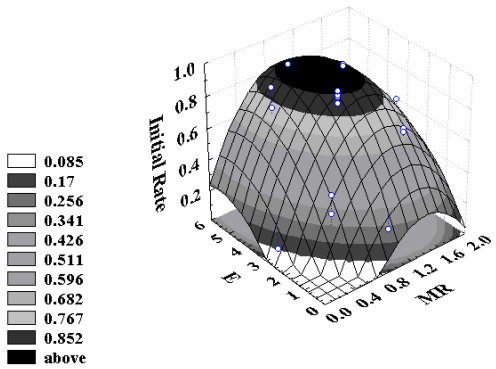

Figure 4. Response surface of the initial rate of reaction as a function of MR and E at 70ºC.

Table 2 shows the results of the Analysis of Variance (ANOVA) which ratifies the conclusions based on the Graph of Pareto (Fig. 3) and confirms that the temperature–enzyme concentration interaction (T*E) does not have statistical significance.

Table 2. ANOVA Table.

Variables	SS	df	MS	p
MR	0.1852	1	0.1852	1.10×10^{-7}
T	0.0088	1	0.0088	2.00×10^{-4}
E	0.3799	1	0.3799	1.83×10^{-8}
MR^2	0.3333	1	0.3333	2.54×10^{-8}
T^2	0.0016	1	0.0016	8.99×10^{-3}
E^2	0.1160	1	0.1160	3.53×10^{-7}
MR*T	0.0053	1	0.0053	6.78×10^{-4}
MR*E	0.0306	1	0.0306	9.62×10^{-6}
T*E	3.84×10^{-6}	1	3.84×10^{-6}	0.8480
Lack of fit	0.0972	5	0.0194	8.71×10^{-6}
Pure Error	0.0005	5	9.41×10^{-5}	
Total SS	1.1372	19		

Therefore, the interaction T*E was excluded of the proposed model. The second order mathematical model is given by Eq. (3):

$$(-r_{A_0}) = 0.855 + 0.116\times MR - 0.025\times T + 0.167\times E - 0.152\times MR^2 + 0.011\times T^2 - 0.090\times E^2 + 0.026\times MR*T - 0.062\times MR*E \quad (3)$$

Although the model coefficients obtained are empirical and cannot be associated with the physical chemistry involved, they are very useful to predict results of untested operation conditions.

Influence of acid/alcohol molar ratio (MR)

As shown in Fig. 2, it can be observed that experiments performed with MR = 0.50 (excess of ethanol) presented similar results of initial rate as experiments with MR=1.50 at 67 ºC with 4.5 % w/w of Lipozime. This is probably due to the fact that the enzyme concentration is the most important factor throughout the esterification process for the experimental range studied and has the greatest effect. Fig. 2 also shows that the use of great excess of ethanol (MR = 0.16) resulted in a significant reduction of the initial rate of reaction and the acid conversion.

In the enzymatic catalysis in nonaqueous medium, the nature of the organic solvent influences the activity and the stability of the enzymes significantly. The most harmful solvents to the enzymes are those highly polar and more hydrophilic, because they are capable of solubilizing large amounts of water and to remove the layer of essential water from the enzymes, causing loss of the catalytic activity. Water is essential for the integrity of the three-dimensional structure of the enzyme molecule and, therefore, the lipase activity is a function of the water content (Illanes, 1994; Koskinen and Klibanov, 1996).

In agreement with Fig. 2, for the reactions where a great excess of ethanol was used, the initial rate of reaction and the acid conversion had the lowest values. This result may be explained by the ability of ethanol to affect water–enzyme interactions that stabilizes the enzyme (Páez *et al.*, 2003). Nevertheless, the increasing of palmitic acid/ethanol molar ratio favours the ethyl hexadecanoate as it was suggested by statistical analysis. Those results were confirmed in Figs. 5 and 6. When excess of ethanol was used (MR =0.50), the conversion and initial rate values were smaller than those with stoichiometric molar ratio of reagents.

An important result from Zaidi *et al*. (2002) is the correlation existing between the kinetic parameters and the chain-length of the substrates in the enzymatic esterification of oleic acid with different alcohols. When the number of carbon atoms increased from 1 (methanol) to 18 (cis-9-octadecenol-1), the inhibition coefficient of the alcohol increased from 0.034 to 0.42 mol/L, respectively, indicating the stronger inhibition by the smaller molecules of methanol and ethanol.

Influence of temperature (T)

As shown in Fig. 2, experiments at 67ºC gave the highest acid conversion and initial rate of reaction. The experimental temperature range studied was limited by the

melting point of palmitic acid and the boiling point of ethanol. However, there was not a significant influence in the initial rate of reaction. However, as shown in Fig. 2, the acid conversion and the initial rate of the reactions using an excess of ethanol decreased even for a small increase of temperature (67 to 73ºC). In agreement with Illanes (1994), the thermal stability of the enzymes decreases with the increase of the amount of water in the organic solvent. In other words, hydrophilic solvents, such as ethanol, tend to deactivate the enzymes reducing their thermal stability. Probably, the presence of ethanol excess in the reaction medium caused the deactivation of the enzyme with the increasing temperature.

Influence of enzyme concentration (E)

As illustrated in the Graph of Pareto (Fig. 3), the enzyme concentration was the variable that had the highest influence in the ethyl hexadecanoate synthesis. As expected, the increase of the enzyme concentration resulted in the increase of the initial rate of reaction. In agreement with the results presented in the Fig. 2, it can be verified, for instance, that the increase in the enzyme concentration of 1.50 to 4.50% (w/w) using acid/alcohol molar ratio of 0.50 and 1.50 at 67°C, the acid conversion increased about 45% and 30%, respectively. However, a non-significant increase of acid conversion and initial rate with the increase of enzyme concentration of 3.00 to 5.52 % w/w in the reactions with R=1.00 at 70 ºC (Fig. 2) was observed. Therefore, experiments with different enzyme concentrations were proposed at the temperature of 70ºC with acid/alcohol molar ratios of 0.50 and 1.00.

The graph of conversion at 1 hour and initial rate as a function of enzyme concentration under experimental conditions of acid/alcohol molar rate of 0.50 and 1.00 at 70ºC is shown in Figs. 5 and 6. In agreement with this graph the values of enzyme concentration above 3.00% (w/w) didn't show a proportional increase in the acid conversion. The same behaviour can be observed in Fig. 6. After 3.00% (w/w) of enzyme concentration, the initial rate practically did not change with enzyme concentration. It is possible to conclude that the enzyme concentration of 3.00% is already enough to saturate the enzyme–substrate system under tested conditions.

Garcia *et al.* (1999b) investigated the influence of enzyme concentration during the esterification of palmitic acid with isopropyl alcohol using Novozym 435, stoichiometric molar ratio at 70ºC. The studied values of enzyme concentration were 3, 5 and 7% (w/w). The results showed that conversion increased with increasing enzyme concentration. An average increase in conversion of 10% was obtained when the enzyme concentration was increased by 2%.

IV. CONCLUSIONS

In this study, central composite experimental design with central point has been applied to optimize the synthesis of ethyl hexadecanoate. The results indicated that the synthesis of ethyl hexadecanoate is viable in the proposed system. Conversion values larger than 50% after one hour of reaction were obtained using the following experimental conditions: MR = 0.50, T = 67ºC and E = 4.50% (w/w). Besides, the absence of organic solvent is very advantageous for the esterification since it avoids the separation problems and toxicity of the organic solvents, allows the recovery of the product without the necessity of additional steps of purification and decreases the cost of the final product. According to the statistical analysis, the variables that most influenced the initial reaction rate in the tested conditions were the acid/alcohol molar ratio and the enzyme concentration.

The proposed experiments with different enzyme concentrations at 70ºC with acid/alcohol molar ratio of 0.50 and 1.00 showed that above 3.00% (w/w) of enzyme concentration, the acid conversion and the initial rate practically did not change with enzyme concentration because the reaction system was already saturated under tested conditions.

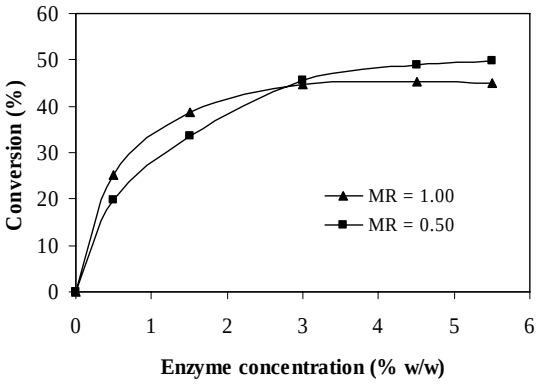

Figure 5. Conversion after 1 hour *versus* enzyme concentration using MR = 0.50 and MR = 1.00 at 70ºC.

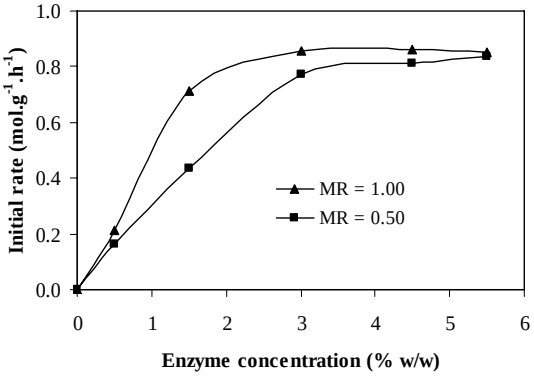

Figure 6. Initial rate *versus* enzyme concentration using MR = 0.50 and MR = 1.00 at 70ºC.

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REFERENCES

Bender, M. "Economic feasibility review for community-scale farmer cooperatives for biodiesel" *Bioresour. Technol*., **70**, 81–87 (1999).

Faria, W. L. S., Carvalho, L. M., Júnior, N. M., Vieira, E. C., Constantino, A. M., Silva, C. M., Aranda, D. A. G. "Esterificação de ácido graxo para produção de biodiesel". *In Anais do 12º Congresso Brasileiro de Catálise*. Angra dos Reis – RJ, Brazil, **2**, 943–946 (2003).

Fukuda, H., Kondo, A., Noda, H. "Biodiesel fuel production by transesterification of oils". *J. Biosci. Bioeng.*, **92**, 405–416 (2001).

Garcia, T., Sanchez, M., Martinez, M., Aracil, J. "Enzymatic synthesis of fatty esters Part II. Optimization studies". *Enzyme Microb. Technol.*, **25**, 591–597 (1999a).

Garcia, T., Sanchez, M., Martinez, M., Aracil, J. "Enzymatic synthesis of fatty esters Part I. Kinetic approach". *Enzyme Microb. Technol.*, **25**, 584–590 (1999b).

Illanes, A. *Biotecnologia de Enzimas*. Chile: Ediciones Universitarias de Valparaíso de la Universidad Católica de Valparaíso (1994).

Júnior, N. M.; Souza, P. H. G.; Pereira, R. E.; Carvalho, L. M.; Faria, W. L. S.; Sales, A. S.; Bom, E. P. S.; Aranda, D. A. G. "Produção de biodiesel etílico utilizando misturas de óleo de fritura e óleo de soja por catálise básica". *In Anais do 12º Congresso Brasileiro de Catálise*. Angra dos Reis – RJ, Brazil, **2**, 947–951 (2003).

Köse, Ö.; Tüter, M.; Aksoy, H. A. "Immobilized *Candida antarctica* lipase-catalyzed alcoholysis of cotton seed oil in a solvent-free medium". *Bioresour. Technol.*, **83**, 125–129 (2002).

Koskinen, A. M. P., Klibanov, A. M. *Enzimatic Reactions in Organic Media*. 1. ed. London: Black Academic & Professional (1996).

Langone, M. A. P.; Sant´anna, G. J. "Enzymatic synthesis of medium-chain triglycerides in a solvent-free system". *Appl. Biochem. Biotechnol.*, **77–79**, 759–770 (1999).

Ma, F., Hanna, M. A. "Biodiesel production: a review[1]". *Bioresour. Technol.*, **70**, 1–15 (1999).

Mittelbach, M. "Lipase catalyzed alcoholysis of sunflower oil. *J. Am. Oil Chem. Soc.*, **67**, 168–170 (1990).

Montgomery, D. C. *Design and Analysis of Experiments*. 5th ed. United States of America. John Wiley & Sons (2001).

Páez, B. C., Medina, A. R., Rubio, F. C., Moreno, P. G., Grima, E. M. "Modeling the effect of free water on enzyme activity in immobilized lipase-catalyzed reaction in organic solvents". *Enzyme Microb. Technol.*, **33**, 845–853 (2003).

Schuchardt, U., Sercheli, R.; Vargas, R. M. "Transesterification of Vegetable Oils: a Review". *J. Braz. Chem. Soc.*, **9**, 199–210 (1998).

Soumanou, M. M., Bornscheuer, U. T. "Improvement in lipase catalyzed synthesis of fatty acid methyl esters from sunflower oil". *Enzyme Microb. Technol.*, **33**, 97–103 (2003).

Srivastava, A., Prasad, R "Triglycerides-based diesel fuels". *Renew. Sust. Energ. Rev.*, **4**, 111–133 (2000).

Vicente, G., Coteron, A., Martinez, M., Aracil, J. "Application of the factorial design of experiments and response surface methodology to optimize biodiesel production". *Ind. Crops Prod.*, **8**, 29–35 (1998).

Zaidi, A.; Gainer, J. L.; Carta, G.; Mrani, A.; Kadiri, T.; Belarbi, Y.; Mir; A. "Esterification of fatty acids using nylon-immobilized lipase in n-hexane: Kinetic parameters and chain length effects". *J. Biotechnol.*, **93**, 209–216 (2002).

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