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Lipase supported on granular activated carbon and activated carbon cloth as a catalyst in the synthesis of biodiesel fuel

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ABSTRACT

Fatty acid esters (biodiesel) were produced from the enzymatic transesterification of palm oil with methanol, ethanol, 1-propanol and 1-butanol. Isopropanol and isobutanol were also studied. *Candida antarctica B* lipase was immobilized on granular activated carbon (ACG-E) and activated carbon cloth (ACC-E) and used as a catalyst (biocatalyst). In the conversion of palm oil to alkyl esters using granular activated carbon as a support, isobutanol gave the highest conversion of 100%, isopropanol 86%, 1-butanol 77%, 1-propanol 68% and ethanol 57%, while only 48% methyl ester was observed with methanol. With activated carbon cloth used to support the enzyme, isobutanol gave the highest conversion of 82%, isobutanol 72%, isopropanol 59%, 1-butanol 45% and propanol 40%, while only 28% methyl ester was observed with methanol.

1. Introduction

The limited reserves of fossil fuels, the increasing prices of crude oils, and environmental concerns have spurred the development of alternative renewable energy sources. Biodiesel, which is a mixture of monoalkyl ester produced by transesterification of vegetable oils, has attracted considerable attention in the recent past as a renewable, biodegradable, and nonpolluting fuel. For industrial biodiesel production, homogeneous basic catalysts, including potassium hydroxide, sodium hydroxide, as well as potassium and sodium alkoxides, are commonly used for the transesterification of vegetable oils with methanol to produce fatty acid methyl esters [1,2]. In addition, biodiesel is better than diesel fuel in terms of sulphur content, flash point, aromatic content and biodegradability [2]. Several types of vegetable oils (soybean, rapeseed, sunflower and palm oils are the most studied), with diverse compositions of fatty acids, can be used for the preparation of biodiesel. Among the raw materials with potential for obtaining biodiesel, palm oil stands out for being the second most abundant oil in the world, as well as for the palm being characterized as having superior productivity among all other crops [3]. The conventional chemical route has several drawbacks: it is energy intensive, recovery of glycerol is difficult, the alkaline catalyst must be removed from the product, alkaline wastewater requires treatment and free fatty acids and water interfere with the reaction [4]. To minimize homogeneous process problems, attempts have been made to use heterogeneous catalyst systems in the alcoholysis of triglycerides [5–8]. These catalysts greatly simplify the post-treatment of the products (separation and purification). They can be easily separated from the system at the end of the reaction and may also be reused. Besides, the use of heterogeneous catalysts does not produce soaps through free fatty acid neutralization or triglyceride saponification. A large number of heterogeneous catalysts have been reported in the literature, including enzymes, zeolites, clays, guanidines heterogenized on organic polymers, ion-exchange resins and oxides, among others. Although the enzymatic process is still not commercially developed, a number of articles have shown that enzymes hold promise as catalysts. These studies mainly consist of optimizing the reaction conditions (temperature, alcohol/oil molar ratio, type of microorganism which generates the enzyme, enzyme amount and time, among others) to establish the characteristics for industrial applications [7–9]. The reaction is carried out under moderate temperatures, thus the catalyst and process temperature do not degrade the reactor material. Also, unlike chemical catalysis which works better with methanol [10], enzymes seem to prefer ethanol. In the case of chemical catalysis, the high temperature necessary

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in the process improves the miscibility between oil and methanol, while in the case of biocatalysis, the reaction is carried out at lower temperatures at which the miscibility of methanol in oil is very poor [1,11–13]. Methanol is also known to cause enzyme inactivation more than ethanol [12,13]. Hence, ethanol is generally preferred for carrying out lipase-catalysed transesterification for the preparation of biodiesel [7,12,13]. Ethanol as such is a renewable starting material for plant feedstock. Thus, an enzyme-based route fits better as a part of developing sustainable technology for biofuels. All of this has generated immense interest in using lipase for the production of biodiesel from a variety of oils/fats such as soybean [14], sunflower [8,15,16], cottonseed [17], rapeseed [7,18], palm oil [19,20], mango kernel [21], Jatropha oil [9] and beef tallow [22] (lipase-catalysed transesterification of mahua oil has not been attempted thus far). This interest exists despite the current high cost of the biocatalyst. It is hoped that efficient downstream processing techniques would make enzyme production costs much cheaper [23,24]. Also, if the enzyme-based transesterification is adopted on a large scale, a high demand would induce large-scale production of the enzyme and would result in the lowering of the market price of lipases. The existing usage of enzymes in several areas such as detergents, dairy products and textile and leather processing [25,26] reflect the validity of such a strategic approach. The present work shows the effect of different alcohols on biodiesel production when *Candida antarctica B* lipase was immobilized on granular activated carbon and activated carbon cloth to form the biocatalyst. The results were compared with systems that use enzymes without support. Additionally, different alcohols were used to study their influence in the production of biodiesel.

2. Materials and methods

The commercial *Candida antarctica B* lipase (Novozym 435®) was immobilized on the granular activated carbon, synthesized in our laboratory from coconut shell, and on the commercial activated carbon cloth (Zorflex®). Methanol, ethanol, 1-propanol, 1-butanol, isopropanol and isobutanol from Merck (purity > 99.8%, Merck, Germany) were used as reactants in the enzymatic reaction. Methyl myristate was purchased from Sigma Aldrich (St. Louis, US) and used as an internal standard. All other chemicals were reagent grade. The biocatalysts were labelled as AGG-E for the enzyme supported on granular activated carbon and ACC-E for the enzyme supported on activated carbon cloth.

2.1. Characterization of the prepared activated carbons used as supports

Coconut shell was cut into small pieces (1.0–2.0 mm), washed with distilled water and oven dried at 120 °C overnight. A series of 10 g of the sample were mixed with 100 ml of $ZnCl_2$ solution of various concentrations (5–40%, w/w). The impregnation process was performed at 80 °C in an oil bath until the excess water had evaporated. The impregnated sample was dried in an oven at 150 °C overnight. The sample was placed inside a quartz tube and inserted horizontally into the middle of a tubular electric furnace. The carbonization and activation were carried out at 800 °C under N_2 gas flow for 5 h followed by CO_2 gas flow for 1 h. The resulting activated carbon was washed with 0.05 M HCl followed by distilled water until traces of chloride ions were no longer detected. The textural characteristics of the resulting activated carbons were determined by nitrogen adsorption at 77 K using an automatic adsorption instrument (Quantachrome, Autosorb-3B). Prior to gas adsorption measurements, the samples were degassed at 300 °C in a vacuum condition for 3 h. Adsorption data were obtained over a relative pressure, P/P_0, ranging from approximately 10^{-6} to 1. The surface area, pore volume and pore size distribution of the activated carbons were determined by the application of the Brunauer–Emmett–Teller (BET) and *t*-plot analysis software available with the instrument, respectively. The BET surface areas were assessed by applying relative pressures ranging from 0.01 to 0.15. The total pore volumes (V_t, cm^3/g) were estimated to be the liquid volumes of N_2 at a high relative pressure near unity (~0.99). The *t*-plot method was applied to the micropore volume and mesopore surface area, and the mesopore volume was obtained by deducting the micropore volume from the total pore volume. Pore size distribution of the activated carbons was obtained by applying the micromeritics density functional theory (DFT) method to the nitrogen adsorption isotherms using the software supplied by Autosorb-3B. The microstructures of activated carbons produced from coconut shell and commercial cloth with the enzyme were examined using scanning electron microscopy (JEOL JSM-5600 LV Model SEM).

2.2. Preparation of pH-tuned enzyme

Lipase (50 mg) from *P. cepacia* was dissolved in 0.5 ml of 0.05 M phosphate buffer at pH 7.0 (this was the optimum pH for the lipases as reported by the vendors and other workers [5–8]). The enzyme solution was immediately frozen at −20 °C and lyophilized for 24 h [26]. These were referred to as "pH-tuned" enzyme preparations.

2.3. Enzyme immobilization

The activated carbon and activated carbon cloth (50 mg) were placed in 5 ml capped vials and moistened with 150 μl of 95% methanol, ethanol, 1-propanol, 1-butanol, isopropanol and isobutanol. This was followed by the addition of 4 ml of *Candida antarctica B* lipase solutions in 20 mM potassium phosphate buffer at pH 7.0. The vials were incubated at 25 °C with constant shaking at 300 rpm overnight. The solutions were then withdrawn from each vial and stored, while the solid porous particles were washed twice with 1 ml of phosphate buffer. The lipase activity and protein were determined in immobilization solution and washings. The immobilized lipase preparations were dried using a speed vacuum system (UVS4004 Universal Vacuum system, Thermo Savant). In this study was fixed of pH and temperature for study of activity of enzyme on alcohols.

2.4. Enzymatic transesterification reaction

Palm oil (0.5 g) with methanol, ethanol, 1-propanol, 1-butanol, isopropanol or isobutanol were each placed into different screw-capped vials at a molar ratio of 1:6. The pH-tuned lipase preparations (50 mg) were added to these vials and the mixtures were incubated at 40 °C with a constant shaking at 300 rpm [9]. The progress of the reaction was monitored using aliquots (40 μl) removed at various time intervals. The alkyl esters formed were analyzed using Gas Chromatograph (GC).

2.5. GC Analysis

At the end of the reaction, the enzyme was separated out by filtration and the filtrate was washed with distilled water and hexane after transferring it to a separating funnel. The ethyl esters phase, diluted with hexane, was mixed with methyl myristate, which served as the internal standard. The ethyl ester content in the reaction mixture was quantified by gas-chromatography using a GS Varian 3400, equipped with a fused silica capillary column (30 m × 0.32 mm × 0.1 μm). The column temperature was held at 150 °C for 2 min, then heated to 190 °C at 4 °C/min and held at that temperature for 3 min, heated again to 250 °C at 5 °C/min and

Table 1
Physical properties of carbons used as support for the enzyme.

Sample	S_{BET} (m^2/g)	Method DR		D_p (nm)
		$S_{\mu p}$ ($m^2\ g^{-1}$)	$V_{\mu p}$ ($cm^3\ g^{-1}$)	
ACG	1867	1854	0.52	2.6
ACC	1562	1520	0.45	2.3
ACG-E	1674	1648	0.42	2.0
ACC-E	1387	1325	0.40	1.9

ACG, activated carbon granular; ACC, activated carbon cloth; ACG-E, activated carbon with enzyme; ACC-E, activated carbon cloth with enzyme.

held at that temperature for 5 min, before being raised to 300 °C at 4 °C/min, where this temperature was maintained for 2 min. The temperatures of the injector and detector were set at 320 °C and 330 °C, respectively.

3. Results and discussion

Table 1 shows the textural properties of the carbons used as supports for the enzyme, where: S_{BET} corresponding to surface calculated by Brunauer, Emmet and Teller method, $S_{\mu p}$ is of micropore surface calculated by Dubinin–Raduskevich method, $V_{\mu p}$ is of volume of micropore calculated by Dubinin–Raduskevich method and D_p correspond to pore diameter. The ACG have major superficial areas which diminish when the enzyme is fixed due to the fact that the pores are covered during this process. The volume of pores and the diameter also diminish. The same behavior is observed for the ACC.

In additional experiments, the quantity of lipase immobilized on the carbons was evaluated. These results showed that up to 95% in weight of the lipase was immobilized. Fig. 1 illustrates a scanning electronic microphotograph in which the lipase adsorbed on the activated carbon cloth can be observed.

Methanol is the most commonly used alcohol in biodiesel production. Since any excess of methanol, existing as drops in the oil, could cause enzyme inactivation, a multistep addition of methanol has been developed. Granados et al. achieved conversion of over 90% in a three-step methanolysis system with immobilized *Candida antarctica* lipase [4]. Similar methods have been developed by several other researchers and high yields have been achieved. In agreement with the literature, the best results with methanol in this study were achieved with 6:1 methanol to oil molar ratio [27]. The quantity of biodiesel obtained was evaluated by gas chromatography. Figs. 2 and 3 show the yield of biodiesel synthesized in the transesterification reaction, expressed as percentage yield for the different alcohols studied over 40 h of reaction time, using the biocatalysts synthesized in this investigation, ACG-E and ACC-E. The maximum yield of biodiesel production was achieved with

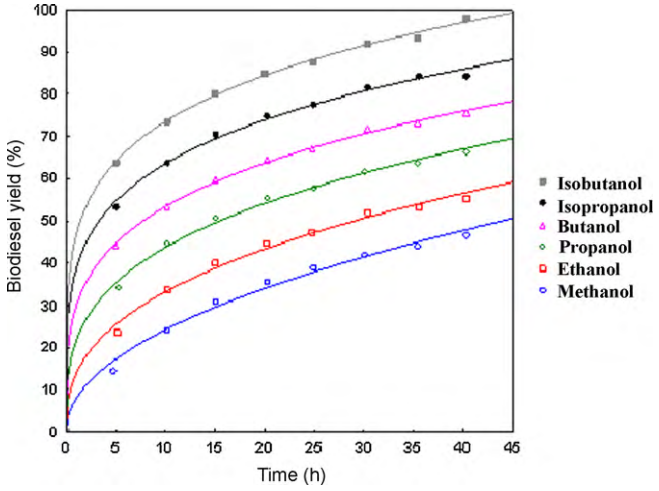

Fig. 2. Alcohols effect in biodiesel production using biocatalyst ACG-E. Reactions parameters: 40 °C, 3% enzyme on oil weight, 40 h of reaction, molar ratio 6:1 alcohol to oil ratio.

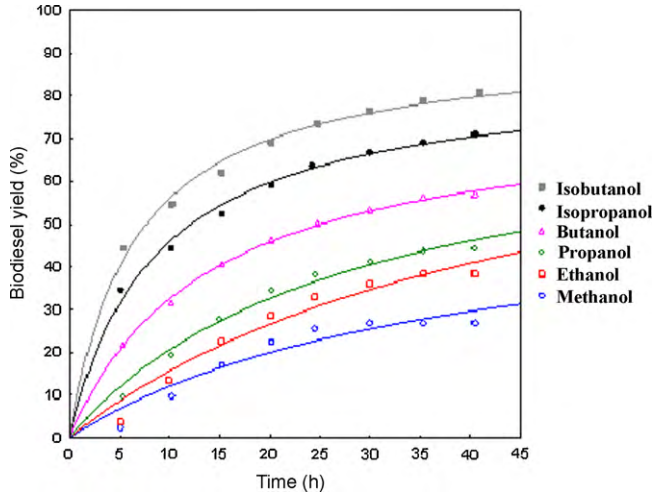

Fig. 3. Effect of alcohols different in biodiesel production with the biocatalyst ACC-E. Reactions parameters: 40 °C, 3% enzyme on oil weight, 40 h of reaction, molar ratio 6:1 alcohol to oil ratio.

the biocatalyst ACG-E versus the ACC-E; this is associated with the textural properties of the supports used in the biocatalysts. The superficial area of the granular activated carbon is larger than that of the commercial activated carbon cloth, which allows a larger amount of lipase to be deposited upon it. An important result of

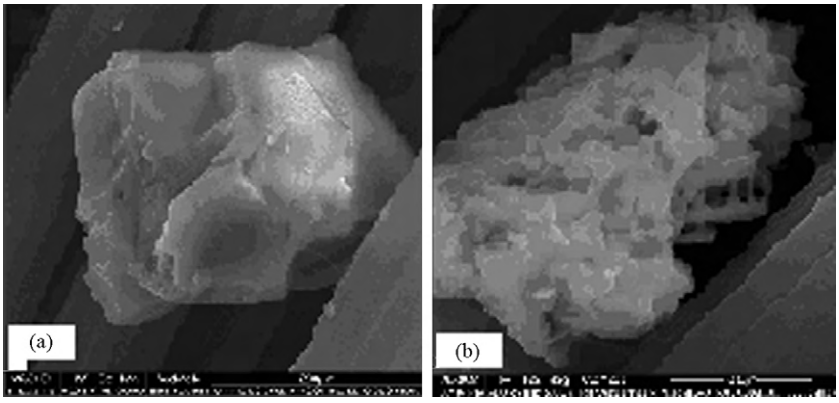

Fig. 1. Microphotography of immobilized lipase on activated carbon cloth.

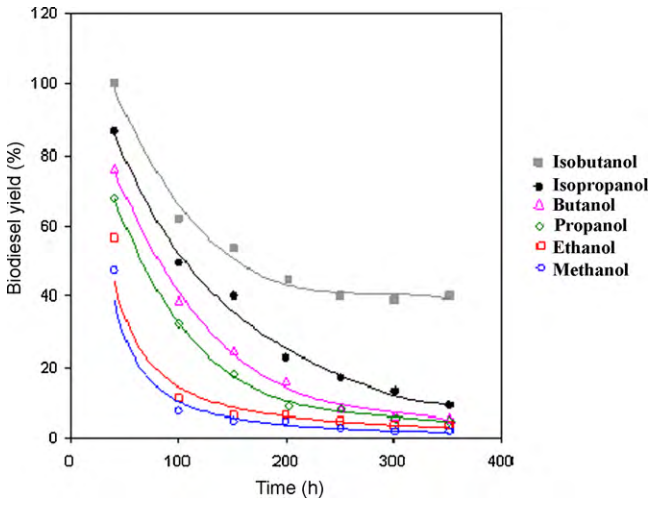

Fig. 4. Operational stability of lipase using biocatalyst ACC-E. Reactions parameters: 40 °C, 3% enzyme on oil weight, 40 h of reaction, molar ratio 6:1 alcohol to oil ratio.

this investigation was the yield of biodiesel achieved by the biocatalysts: in the case of iso-butanol with ACG-E there was a 100% yield of biodiesel and with ACC-E there was an 82% yield. These values are much higher than those reported in the literature using these supports, where the synthesis of biodiesel via *Candida antarctica B* lipase was between 60% and 70% with this same type of oil [3,28,29]. Biodiesel production using branched and lineal chain alcohols such as isopropanol, isobutanol, methanol, ethanol, 1-propanol and 1-butanol were also tested. The results show that the yields of biodiesel produced using the biocatalysts ACG-E and ACC-E were in the following order: isobutanol > isopropanol > 1-butanol > 1-propanol > ethanol > methanol. Additionally, the results showed a decrease in the number of carbon atoms and of the cetane number and an increase in the heat content of the fuel (results not shown here). Also, fatty acid esters of secondary or branched-chain alcohols can be used as fuel additives since they decrease the solidification point and, consequently, the high cloud point and pour point [1,12]. The poor performance of methanol in the production of alkyl esters from palm oil is in agreement with the work of Kalam and Masjuki [5], who found only traces of methyl esters when using methanol in the lipase-catalysed alcoholysis of sunflower oil. This low yield of alkyl esters with methanol could be attributed to the unfavourable viscosity conditions, which affect the intimate mixing of substrates with lipase [8]. This behaviour is associated with surface characteristics of the supports employed, and of the alcohols. The synthesized granular activated carbon has a hydrophobic nature which allows greater retention of branched chain alcohols, forming a system that can react with palm oil. Thus the most effective system was ACG-E isobutanol. Operational stability of the lipase was investigated in consecutive additions of alcohol, in a solvent-free system. The reaction time was 40 h, after which the enzyme was recycled and reused (Fig. 4). Immobilized enzyme have the advantage that they can be reused several times, but their activity eventually decreases due to many factors, such as desorption, substrate deactivation, and product inhibition. Therefore, we tried to improve the stability of the immobilized lipase after each use. After each transesterification reaction, the lipase were recovered by filtration and washed with in all cases, washing with n-hexane, a non-polar solvent tested, that cause greater retention of lipase activity than that obtained when washing with the polar solvents. With all six alcohols that work as acyl acceptors, a high initial yield was achieved. However, the lipase exhibited poor activity during the repeated experiments. In the reaction with methanol, production of alkyl esters was 3% (determined using internal standards in GC); nevertheless is of highlighting that was detected production of biodiesel. The same experimental conditions in the literature have not reported production of biodiesel. This shows the effect of the support in that the large superficial area and pore volume allowed a large quantity of enzyme to be deposited. The operational stability of lipase in the transesterification reaction with isobutanol, isopropanol, 1-butanol, 1-propanol, ethanol, methanol decreased with time and the production of biodiesel decreased to between 40% and 5%.

This decrease in operational stability and diesel production might be due to the inactivation effect caused by the alcohols and the negative effect caused by the glycerol by-product. The glycerol by-product is hydrophobic and insoluble in oil so it is easily adsorbed onto the surface of the immobilized lipase, creating a negative effect on lipase activity and operational stability [15].

Also, decrease in activity may also be due to desorption of enzyme; the adsorption of lipases onto porous solid materials depends on factors such as pH, ionic strength, isoelectric point of the lipase, surface and protein properties, as well as the history dependence of lipase-adsorption kinetics. Most supports usually bind from 2 mg to 50 mg protein per gram of support. While some supports are claimed to bind as high as 170 mg protein per gram of support, such high binding capacity may result in steric interference problems and loss of enzyme activity. In general, the maximum adsorption is observed at pHs close to the isoelectric point of the lipase. In addition, porous particulate supports are superior to nonporous supports for immobilization of lipases due to their greater surface area. However, porous supports can have an internal morphology that allows not only the lipase binding but also an easy accessibility to substrate molecules in order to minimize diffusional limitation. It appears that pore sizes best suited for lipase adsorption are at least 100 nm in diameter. Smaller pore sizes can result in diminished availability of lipase molecules within the pores and in restricted diffusing substrate molecules. Such limitations lead to a lowered efficiency [15]. The affinity of a lipase for an adsorbent generally increases with the hydrophobicity of the surface, and lipases desorbs more easily from hydrophilic than from hydrophobic surfaces.

The alcohols used in the transesterification process according to its structure behave in different ways, e.g. methanol and ethanol are not missible in triglycerides at room temperature and is required to do a mechanical agitation to facilitate mass transfer, however, in the course of the reaction there is the emulsion formation, which in the case of the meta-analysis, are easily and rapidly dissolved to form an inner layer rich in glycerol and another one in the top, rich in methyl esters. In the ethanolysis case, these emulsions are more stable, making the process of separation and purification of methyl esters into something more complex.

The transesterification reaction, involves the displacement of alcohol from an ester by another, in a similar process to hydrolysis, except that alcohol is used instead of water. This process is used to reduce the high viscosity of triglycerides. Fig. 2 represents the general equation of the transesterification reaction:

$$\underset{\text{Ester}}{\text{RCOOR}^1} + \underset{\text{Alcohol}}{\text{R}^2\text{OH}} \overset{\text{Catalyst}}{\rightleftharpoons} \underset{\text{Ester}}{\text{RCOOR}^2} + \underset{\text{Alcohol}}{\text{R}^1\text{OH}}$$

The transesterification reaction is reversible and an excess of alcohol is used to move the equilibrium towards the formation of esters. The kinetics model of reaction that has the best fits is the pesudo-second order, in the initial stage of reaction, followed by a first or zero order. Chemically, transesterification mechanism consists of three consecutive reversible reactions; the triglyceride is converted sequentially into diglycerides, monoglycerides and glycerol (plus the methyl esters) (Fig. 5).

On the other hand is necessary to consider that alcohol plays an important role in the mass transfer, for example in the case of the

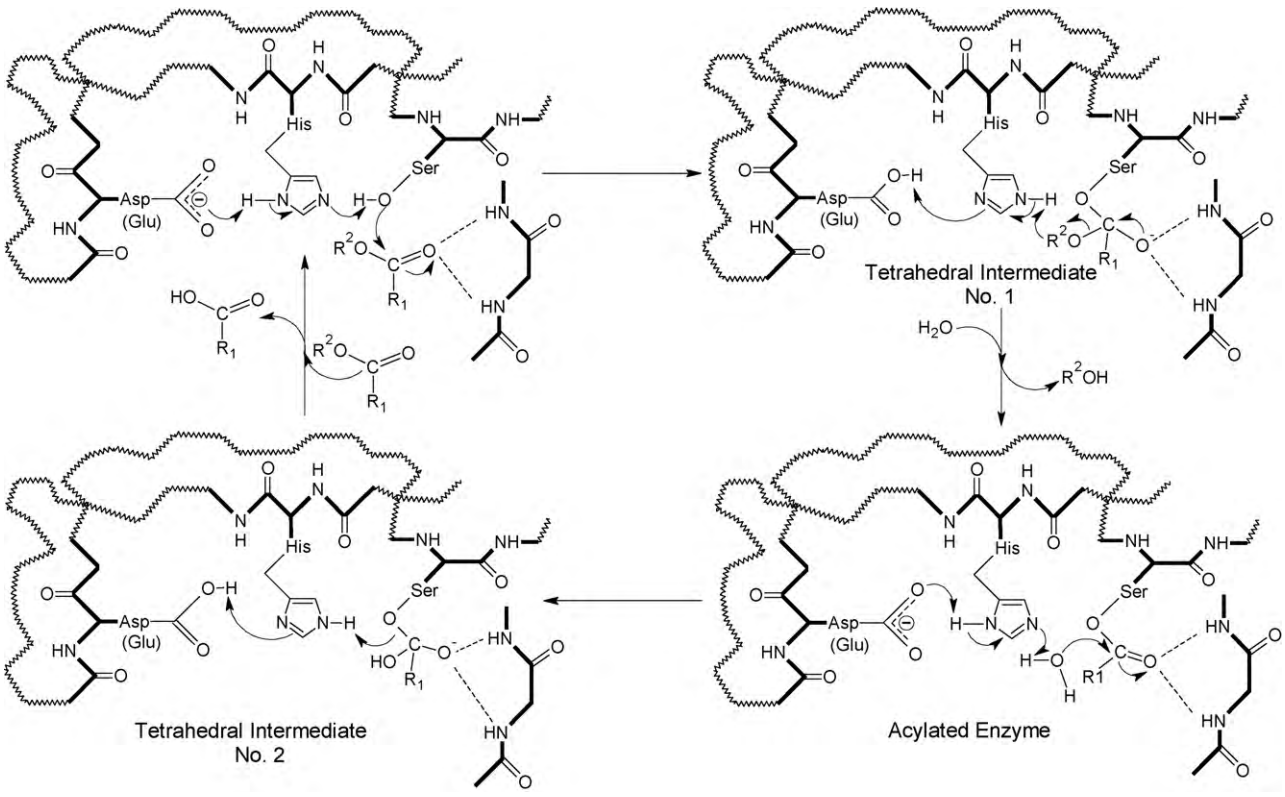

Fig. 5. Reactions between alcohol, palm oil and lipase for production biodiesel.

meta-analysis, methanol is not soluble in triglycerides or methyl esters of glycerin. However, methanol is soluble in methyl esters and glycerin. Therefore during the first few minutes of reaction, there is a system of two phases, which becomes a homogeneous phase to form methyl esters, but these reappear as soon as they form substantial amounts of glycerine. Additionally, the acidic character of the alcohols used decreases as the chain increases, so the methanol is the alcohol more acidic within those used, because the larger the chain stabilizes more the OH group; the isobutanol is the less acidic, it is clear that this is directly associated with the results in the biodiesel production.

The degree of deactivation was found to be inversely proportional to the number of carbon atoms in the linear lower alcohols. Fig. 4 shows the stability effect of the branched chain alcohols and linear alcohols used on the immobilized lipase. The degree of stability by the branched alcohols was demonstrated to be lower than that by the linear alcohols. The curves for isopropanol and isobutanol in Fig. 4 are less pronounced than in the case of linear chain alcohols. It was also observed that when the immobilized lipase was decreasing its stability by methanol or ethanol, the immobilized lipase particles underwent a conspicuous change in appearance and accompanied by swelling and caking. This work shown that alcohols with more than three carbon atoms were completely miscible with palm oil at a ratio molar researched. The experimental results indicated that one of the main causes of decreased of the lipase was due to the immiscibility between triglycerides and of short chain alcohols (i.e. methanol or ethanol). It is possible that for short linear alcohols employed formed small droplets which attached to the resin particles. As the alcohol was adsorbed to the immobilized enzyme, the entry of triglycerides was blocked, causing the reaction to stop.

In Fig. 2a and b shows the behaviors of the biodiesel yield in function of time in the different alcohols. *Candida antarctica B* lipase was immobilized on granular activated carbon (ACG-E) and activated carbon cloth (ACC-E) and used as a catalyst (biocatalyst) that present a highest yield with of ACG-E.

Table 2a shows the constants when apply the model of Langmuir to experimental data sample, themselves that constants *K* the low values were 0.021 and 0.153, for the samples using ethanol and isobutanol that relates with the yield of biodiesel production with the ACC-E biocatalyst, and the coefficients find in a range between 0.973 and 0.998, that indicate a adjustment for only samples regarding the model chosen, because this reason another model was used to compare results.

Table 2b evidences adjustment from the experimental data using Freundlich's model. The constants of adsorption and the *R*

Table 2a
Parameters using Langmuir's model type.

Sample	Parameter		
	Q	*K*	*R*
Methanol	57.26301	0.026745	0.97277
Isopropanol	88.19869	0.021601	0.98642
1-Propanol	78.35169	0.035722	0.99502
1-Butanol	77.46852	0.072453	0.99935
Isobutanol	77.46852	0.116202	0.99748
Ethanol	92.59196	0.153109	0.99800

Table 2b
Parameters using Freundlich's model type.

Sample	Parameter		
	K_f	*n*	*R*
Methanol	7.842128	2.041763	0.99679
Isopropanol	13.70891	2.602854	0.99776
1-Propanol	21.49625	3.244227	0.99893
1-Butanol	29.62728	3.914238	0.99972
Isobutanol	38.45882	4.586870	0.99935
Ethanol	46.45876	5.014084	0.99977

shows a range more reduced of 0.996–0.999, it indicate that the facts that model this suggests better to the experimental data obtained in this.

The higher values for the maximum yield of biodiesel find for the samples with (ACG-E) biocatalyst impregnated using isobutanol; the constant values of K_f evidence a highest affinity between this biocatalyst and the system of reaction.

4. Conclusions

In this study, the use of different alcohols that work as acyl acceptors in lipase-catalysed biodiesel synthesis was studied. Granular activated carbon and activated carbon cloth were use as supports for lipase. They allowed a large quantity of enzyme to settle, resulting in the generation of a large quantity of biodiesel. The studied alcohols isopropanol, isobutanol, 1-butanol, 1-propanol and methanol have less of a negative effect on lipase stability in comparison to the traditionally used methanol. High yields of biodiesel could be achieved, but only with a stepwise addition of alcohol, as in the case of ethanol that showed a biodiesel yield of 100%. The by-product glycerol is hydrophobic and insoluble in oil, so it was easily adsorbed onto the surface of the immobilized lipase and created a negative effect on lipase activity and operational stability.

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References

[1] V. Kumari, S. Shah, M.V. Gupta, Energy Fuels 21 (2007) 368.
[2] A.B.R. Moreira, V.H. Perez, G.M. Zanin, H.F. de Castro, Energy Fuels 21 (2007) 3689.
[3] H. Noureddini, X. Gao, R.S. Philkana, Bioresour. Technol. 96 (2005) 69.
[4] M.L. Granados, M.D. Zafra, D. Martín-Alonso, R.F. Mariscal, R. Cabello-Galisteo, J.S. Moreno-Tost, J.G.L. Fierro, Appl. Catal. B 73 (2007) 317.
[5] M.A. Kalam, H.H. Masjuki, Biomass Bioenerg. 23 (2002) 471.
[6] G. Antolin, F.V. Tinaut, Y. Briceno, V. Castano, C. Perez, A.I. Ramirez, Bioresour. Technol. 83 (2002) 111.
[7] K. Oznur, M. Tuter, H.A. Aksoy, Bioresour. Technol. 83 (2002) 125.
[8] B.K. De, P.K. Bhattacharya, C. Bandhu, J. Am. Oil Chem. Soc. 76 (1999) 451.
[9] R.P. Abigor, P. Vadia, T. Foglia, M. Hass, K. Jones, E. Okefa, J. Obibuzor, M. Bator, Biochem. Soc. Trans. 28 (2000) 979.
[10] E. Crabbe, C. Nolasco-Hipolito, G. Kobayashi, K. Sonomoto, A. Ishizaki, Process. Biochem. 37 (2001) 65.
[11] Y.Y. Linko, M. La Masa, X. Wu, W. Vosukainen, J. Sappala, P. Linko, J. Biotechnol. 66 (1998) 41.
[12] A.-F. Hsu, K. Jones, W.N. Marmer, T.A. Foglia, J. Am. Oil Chem. Soc. 78 (2001) 585.
[13] T.P. Przybycien, N.S. Pujar, L.M. Steele, Curr. Opin. Biotechnol. 15 (2004) 469.
[14] M.N. Gupta, in: M.N. Gupta (Ed.), Methods in Affinity-Based Separations of Enzymes and Proteins;, Brikhauser Verlag, Basel, Switzerland, 2002, pp. 1–15.
[15] M.V. Arbige, W.H. Pitcher, Trends Biotechnol. 7 (1989) 330.
[16] V.T. John, G. Abraham, in: J.S. Dordick (Ed.), Biocatalysts for Industry, vol. 10, Plenum Press, New York, 1990, pp. 193–197.
[17] A. Houde, A. Kademi, D. Leblanc, Appl. Biochem. Biotechnol. 118 (2004) 155.
[18] M.N. Gupta, I. Roy, Eur. J. Biochem. 271 (2004) 2575.
[19] E.P. Hudson, R.K. Koppler, D.S. Clark, Curr. Opin. Biotechnol. 16 (2005) 637.
[20] H. Ishihara, H. Okuyama, H. Ikezawa, S. Tejima, Biochem. Biophys. Acta 388 (1975) 413.
[21] Y. Kojima, M. Yokoe, M.T. Mase, Biosci. Biotechnol. Biochem. 58 (1994) 1564.
[22] Y. Takeda, R. Aono, N. Doukyu, Extremophiles 10 (2006) 1433.
[23] B. Mattiasson, P. Adlercreutz, Trends Biotechnol. 9 (1991) 394.
[24] A. Salis, E. Sanjust, V. Solinas, M. Monduzzi, Biocatal. Biotransform. 23 (2005) 381.
[25] S. Shah, A. Sharma, M.N. Gupta, Anal. Biochem. 18 (2006) 154.
[26] M. Kreiner, B.D. Moore, M.C. Parker, Chem. Commun. 10 (2001) 1096.
[27] O. Kose, M. Tuter, H.A. Aksoy, Bioresour. Technol. 83 (2002) 125.
[28] K.R. Jegannathan, J.Y. Leong, E.S. Chan, P. Ravindra, Renew. Sust. Energy Rev. 1 (2009) 101.
[29] M. Kaieda, T. Samukawa, A. Kondo, H. Fukuda, J. Biosci. Bioeng. 91 (2001) 12.