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Lipase supported on mesoporous materials as a catalyst in the synthesis of biodiesel from *Persea americana mill oil*

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ABSTRACT

Fatty acid esters (biodiesel) were produced from the enzymatic transesterification of *Persea americana mill oil* (in South America known as Aguacate) with methanol, ethanol, propanol, 2,2-dimethyl-1-propanol, 2-methyl-1-butanol, and 3-methyl-1-butanol were studied. *Candida antarctica B* lipase was immobilised on mesoporous foam synthesised using nonylphenol ethoxylated with $n = 4$ (FC-4), and the enzyme was supported on foam carbon with ethoxylated nonylphenol $n = 6$ (FC-6) for obtaining the biocatalyst. In the conversion of *Persea americana mill oil* to alkyl esters using (FC-6) as a support, 3-methyl-1-butanol gave the highest conversion of 100%, 2-methyl-1-butanol 92%, 2,2-dimethyl-1-propanol 81%, propanol 70% and ethanol 60%, and 48% methyl ester was observed with methanol. With FC-4 used to support the enzyme, 3-methyl-1-butanol gave the highest conversion of 70%, 2-methyl-1-butanol 65%, 2,2-dimethyl-1-propanol 58%, propanol 50% and ethanol 45%, and 37% methyl ester was observed with methanol.

1. Introduction

Biodiesel is composed of a mixture of fatty acid alkyl esters. It is a natural substitute for petroleum-derived diesel fuel and has similar or better specifications concerning density, viscosity, cetane number and flash point, among others. Because biodiesel is formed from renewable resources such as plant oils, it is considered CO_2-neutral, biodegradable and will help conserve fossil fuels. Compared to traditional diesel fuels, its combustion leads to a substantial reduction in polluting emissions [1–4]. Industrially, biodiesel can be produced by the transesterification of vegetable oils and short chain alcohols, usually methanol, with alkaline or acid catalysts. The reaction products are a mixture of the desired esters, mono and diglycerides, glycerol, water and the catalysts. Compared to the process mediated by enzymes, this process is more energy consuming. Due to the presence of soap byproducts, separation and purification of the chemically produced biodiesel requires somewhat more complex steps than enzymatically produced biodiesel. Therefore, the use of biocatalysts could be an interesting alternative because it is more environmentally attractive because biodiesel synthesised enzymatically can be used directly without purification [3–9]. Lipase-catalysed transesterification of vegetable oils has been investigated by many researchers in the last few years [4–12]. For cost reasons, methanol is the alcohol most frequently used for triglyceride transesterification. Nevertheless, other alcohols are also used. In Brazil, one the biggest world plant oil producers, biodiesel is obtained by ethanolysis of triglycerides, since ethanol is a cheap and abundant commodity produced from the fermentation of sucrose from sugarcane. Alternatively, either propanol or butanol can also be used in this process, especially because these two alcohols promote a better miscibility between the alcohol and the oil phases [7]. The use of a triglyceride feedstock for biodiesel production depends on regional availability and economics and many vegetable oils can be used, such as soybean [3,13–15], sunflower [16–18], and rapeseed [3]. The main differences among these oils are their fatty acid compositions, which strongly affects some important properties of the biodiesel (cetane number, heat of combustion, melting point and viscosity) [1,2]. Oxidation of biodiesel is a common problem, depending on the source of vegetable oil.

A large number of heterogeneous catalysts have been reported in the literature, including enzymes, zeolites, clays, guanidines heterogenised 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 optimising the reaction conditions (temperature, alcohol/oil molar

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ratio, type of microorganism which generates the enzyme, enzyme amount and time, among others) to establish the characteristics for industrial applications [19–22]. 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, enzymes seem to prefer ethanol. In the case of chemical catalysis, the high temperature necessary 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 [23–25]. Methanol is also known to cause enzyme inactivation more than ethanol [24,25]. Hence, ethanol is generally preferred for carrying out lipase-catalysed transesterification for the preparation of biodiesel [19,24,25]. 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 [26], sunflower [20,26–28], cottonseed [29], rapeseed [19,30], palm oil [31,32], mango kernel [33], Jatropha oil [22] and beef tallow [32] (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 [33,34]. 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 [35,36] reflects 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 immobilised on mesoporous carbon foams using various non-ionic surfactants from the biocatalyst, of *Persea americana mill oil*. 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

2.1. Samples preparation

Activated carbon foam was successfully prepared from phenolic resin, which was synthesised with phenol and formaldehyde under alkaline conditions. The surfactant template synthesis route used to produce mesoporous carbon foam (STn-CF) is illustrated in Fig. 1, where n is the number of ethylene oxide residues per mole of nonylphenol. Typically, phenol and formaldehyde were dissolved in ethanol (PFE). Nonylphenol ethoxylate was then added to the above solution under stirring. The molar composition of phenol/formaldehyde/ethanol/in the nonylphenol ethoxylate solution was 1:2:60:0.38. NaOH was added to the mixture to initiate polymerisation of formaldehyde and resorcinol. Residual NaOH was then removed from the polymerised phenolic resin by repeated immersions in 70 °C water. Finally, the dried polymer precursor was carbonised at 680 °C, using a heating rate of 1 °C/min under purified nitrogen flow, to decompose the surfactant template, giving mesoporous carbon foams. The carbon foam samples produced in the present study were labelled FC-4 and FC-6, corresponding to carbon foams synthesised using nonylphenol ethoxylated surfactant templates with $n = 4$ and 6 ethylene oxide residues per mole of nonylphenol, respectively.

The commercial *Candida antarctica B* lipase (Novozym 435™) was immobilised on the foams carbon (FC), synthesised in our laboratory, as describe before. Methanol, ethanol, propanol,

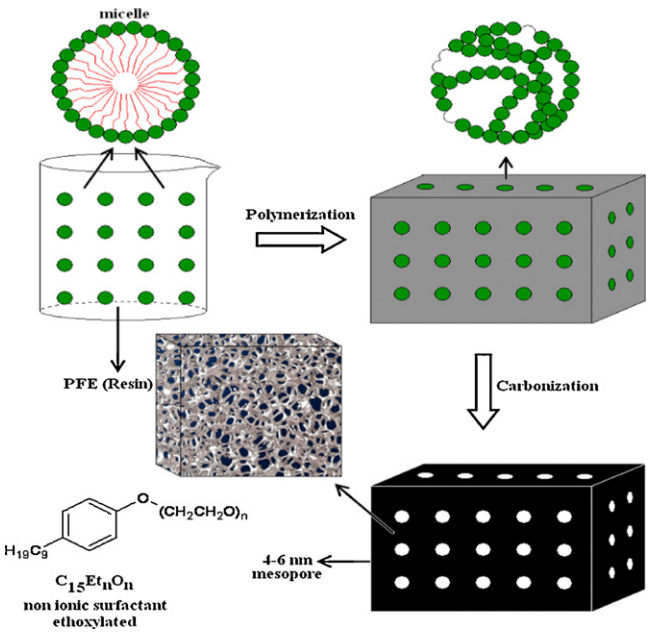

Fig. 1. Preparation of mesoporous carbon foam using a non-ionic surfactant template.

2,2-dimethyl-1-propanol, 2-methyl-1-butanol, and 3-methyl-1-butanol 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 FC-4 and for the enzyme supported on foam carbon synthesised with nonylphenol ethoxylated with $n = 4$, and FC-6 for the enzyme supported on foam carbon with nonylphenol ethoxylated $n = 6$.

2.2. Sample characterisation

The pore structures of the carbon foams synthesised in the present study were examined by SEM. Pore size, porosity, bulk density, and compressive strength were measured by using standard methods. Nitrogen adsorption isotherms were determined at −196 °C using an Autosorb 3B (Quantachrome, Boynton Beach, Miami, FL, US) system in static measurement mode. Prior to sorption experiments, samples were outgassed at 400 °C for 10 h. Pore size distributions were calculated by analysing the desorption branches of the isotherm using the Barrett–Joyner–Halenda (BJH) method [37].

2.3. Preparation of pH-tuned enzyme

Lipase (50 mg) from *Pseudomona Cepacia* was dissolved in 0.5 ml of 0.01 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 lyophilised for 24 h [26]. These were referred to as "pH-tuned" enzyme preparations.

2.4. Enzyme immobilisation

The foams carbon (25 mg) were placed in 5 ml capped vials and moistened with 100 μl of 95% methanol, ethanol, 1-propanol, 1-butanol, isopropanol and isobutanol. This was followed by the addition of 5 ml of *Candida antarctica B* lipase solutions in 10 mM potassium phosphate buffer at pH 7.0. The vials were incubated at

25 °C with constant shaking at 250 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 immobilisation solution and washings. The immobilised lipase preparations were dried using a speed vacuum system (UVS4004 Universal Vacuum System, Thermo Savant). In this study pH and temperature was fixed for the study of activity of enzyme on alcohols [37].

2.5. Enzymatic transesterification reaction

Persea americana mill oil (0.25 g) with methanol, ethanol, propanol, 2,2-dimethyl-1-propanol, 2-methyl-1-butanol, and 3-methyl-1-butanol were each placed into different screw-capped vials at a molar ratio of 1:10. The pH-tuned lipase preparations (25 mg) were added to these vials and the mixtures were incubated at 45 °C with a constant shaking at 250 rpm [9,37]. The progress of the reaction was monitored using aliquots (30 μl) removed at various time intervals. The alkyl esters formed were analysed using a gas chromatograph (GC).

2.6. 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 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 [37].

3. Results and discussion

Fig. 2 depicts the nitrogen adsorption–desorption isotherm and the pore size distributions of the obtained materials. A IV type nitrogen isotherm, characteristic of mesoporous compounds according to the BDDT classification, was observed for all the synthesised samples. A sharp increase in the adsorbed volume of nitrogen due to the capillary condensation was noted for all materials at very high P/P_o values (than 0.5).

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

$C_{15}EtO_n$/PFE	S_{BET} ($m^2\ g^{-1}$)	Method DR		D_p (nm)
n		Sμp ($m^2\ g^{-1}$)	Vμp ($cm^3\ g^{-1}$)	
4	704	687	0.45	3.0
6	756	740	0.48	4.0

This indicated that the pore size of these materials should be rather large since the P/P_o position of the inflection point is related to pore size according to the Kelvin equation. However, it is quite interesting to note that for values samples produced from nonylphenol ethoxylated used, capillary condensation occurs at the same P/P_o, around 0.5. This could suggest that carbon foams obtained from this nonylphenol ethoxylated affect the pore size and the other textural properties of carbon foams.

The above analysis was confirmed by the pore size distribution and average pore diameter of carbon foams obtained with different types of ethoxylated nonylphenol, which were determined by the BJH method using the adsorption branch of the isotherms which are depicted in Fig. 2. It is clear that the two carbon foam samples had a very high surface area around 756 $m^2\ g^{-1}$. Also, the pore size changed from 3.0 to 4.0 nm when the surfactant used was $n = 4$ and 6, respectively. This phenomenon can be explained as follows: when the phenol/formaldehyde/ethanol (PFE) resin was loading, parts of these molecules were solubilised in the inner hydrophobic core of the micelles and PFE acted as a swelling agent. Thus, carbon foams with higher pore diameter were obtained.

The textural properties of the synthesised carbon foams for comparison are summarised in Table 1. Both samples had a high specific surface area (superior to 600 $m^2\ g^{-1}$). For the mixture used in this research, when the surfactant/PFE ratio was constant, it is probable that the amount of PFE added was not sufficient to interact with all the formed micelles, and that a part of the "free" surfactant molecules could very likely have been encapsulated into the formed micelles in interaction with PFE via hydrogen bonds. Thus, in the present case, these "free" surfactant molecules acted as a swelling agent.

The results observed (Table 1) are quite complicated compared to the theoretical expectation that a surfactant with a higher number of oxyethylene units such as $n = 6$ should form the biggest micelles. Consequently, the pore size should decrease from $n = 6$ to $n = 4$ under similar synthesis conditions, as mentioned in the previous paragraph. The general observed trend was that, under the same conditions of surfactant/PFE ratio molar composition, the synthesised carbon foams with ethoxylated nonylphenol $n = 6$ gave

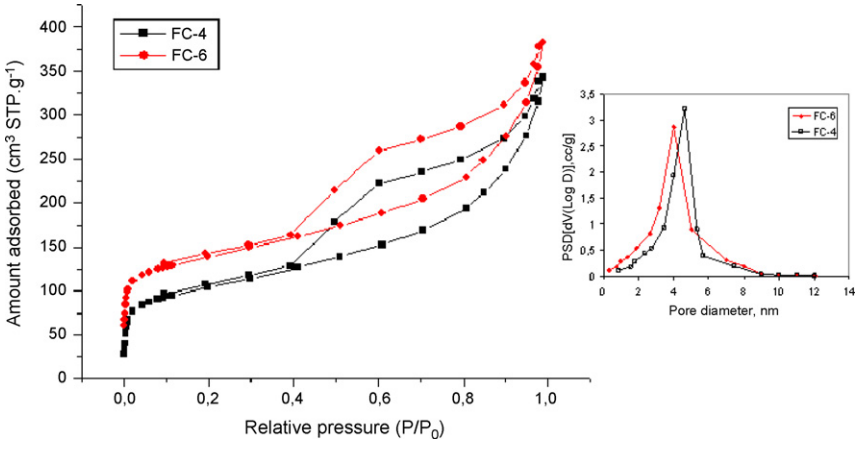

Fig. 2. Nitrogen adsorption isotherms measured at 77 K for the FC-4 and FC-6 and pore size distribution for the materials.

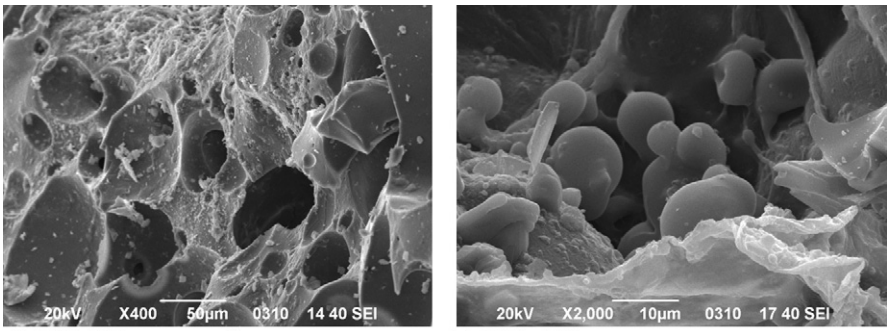

Fig. 3. Microphotography of immobilised lipase on foam carbon (FC-6). (a) Foam carbon without lipase. (b) The biocatalyst (with lipase).

the highest pore diameter compared to those with ethoxylated nonylphenol $n = 4$, which showed the smallest pore aperture. The pore diameter of the materials obtained for a surfactant/PFE ratio molar composition varied with the number of oxyethylene units and was in accordance with the theoretical expectation. Different factors should be taken into account to explain this observation. The first is the possible swelling effect of PFE for a surfactant/PFE ratio at the molar ratios used in this research. However, we also have to look at the cloud point, which arises from a balance of intermolecular van der Waals attractions and hydration repulsion. The cloud point values are not available in the literature for $C_{15}(EtO)_n$ but are available for $C_{15}(EtO)_3$, so we can assume that the surfactants $C_{15}(EtO)_4$ and $C_{15}(EtO)_6$ have a similar cloud point as $C_{15}(EtO_3)$, being 48 °C and 77 °C, respectively for $C_{15}(EtO)_n$ ($n = 4$, 6) surfactants. As for longer ethoxy groups, the hydration repulsion is increased, and a higher temperature is required before attraction and repulsion are balanced, so $C_{15}(EtO)_6$ should have the highest cloud point, i.e. a cloud point that is greater than 77 °C. Second, it is possible that it is more likely that a longer ethylene oxide chain penetrated deeper into the PFE resin hydrophilic phase, can be produced pores after the surfactant is burnt out.

On the other hand, in additional experiments, the quantity of lipase immobilised on the foams carbons was evaluated. These results showed that up to 90% in weight of the lipase was immobilised; this is slightly lower than other reported works [37], but this did not influence the performance of biodiesel production. Fig. 3 illustrates a scanning electronic microphotograph in which the lipase adsorbed on the foam carbon FC-6 can be observed. Fig. 3a shows the foam carbon without lipase and Fig. 3b shows the biocatalyst as such.

Fig. 3a shows the porosity developed in the synthesised foams. Once the process was carried out fixing the lipase is the SEM was taken once again and is shown in Fig. 3b, in which are clearly visible deposits of lipases. Similar results are reported in the literature [37]. The micrograph shows that the lipase adhere on the surface of catalyst. Interestingly, these micrographs show it checks the setting of lipases.

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 immobilised *Candida antarctica* lipase [38]. 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 10:1 methanol to oil molar ratio [31–33]. The quantity of biodiesel obtained was evaluated by gas chromatography. Figs. 4 and 5 show the yield of biodiesel synthesised in the transesterification reaction, expressed as a percentage yield for the different alcohols studied over 32 h of reaction time, using the biocatalysts synthesised in this investigation, FC-6 and FC-4. The maximum yield of biodiesel production was achieved with the biocatalyst FC-6 versus FC-4; this is associated with the textural properties of the supports used in the biocatalysts as we already had established in earlier work [37]. The superficial area of the FC-6 is larger than that of the biocatalyst FC-4, which allows

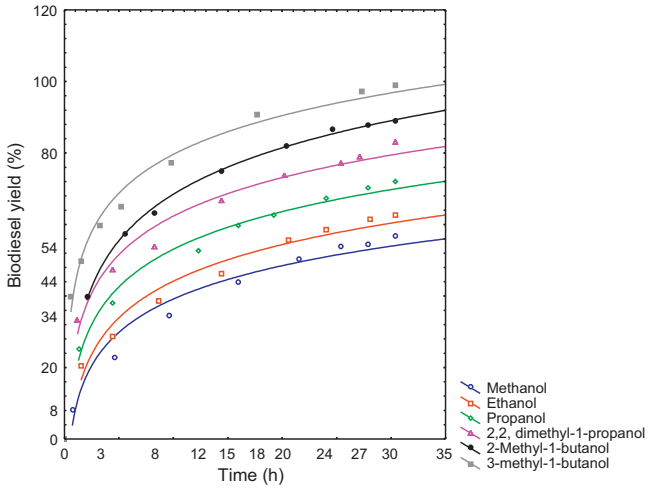

Fig. 4. Alcohols effect in biodiesel production using biocatalyst FC-6. Reactions parameters: 45 °C, 1% enzyme on oil weight, 32 h of reaction, molar ratio 10:1 alcohol to oil ratio.

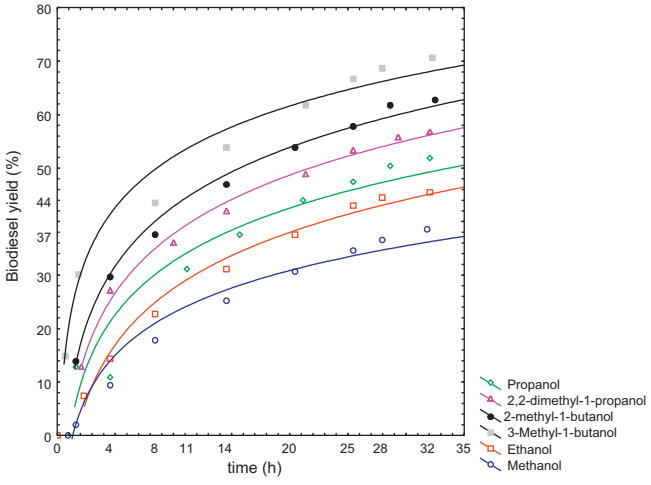

Fig. 5. Alcohols effect in biodiesel production using biocatalyst FC-4. Reactions parameters: 45 °C, 1% enzyme on oil weight, 32 h of reaction, molar ratio 10:1 alcohol to oil ratio.

a larger amount of lipase to be deposited upon it. An important result of this investigation was the yield of biodiesel achieved by the biocatalysts: in the case of 3-methyl-1-butanol with FC-6 there was a 100% yield of biodiesel and with FC-4 there was a 70% yield. This decrease is due to textural changes caused by synthesising nonylphenol ethoxylated with $n = 4$ and $n = 6$, since $n = 6$ generated a support with a more accessible area and therefore a higher yield in the reaction. 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 [19,31–34]. Biodiesel production using branched and lineal chain alcohols such as methanol, ethanol, propanol, 2,2-dimethyl-1-propanol and 2-methyl-1-butanol, were also tested. The results show that the yields of biodiesel produced using the biocatalysts FC-4 and FC-6 were in the following order: 3-methyl-1-butanol > 2-methyl-1-butanol > 2,2-dimethyl-1-propanol > 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 [19,24]. The cloud point is an important property of biodiesel, controlling its low temperature behaviour, especially the fluidity of the fuel. Although biodiesel is an interesting renewable energy source, data for the melting/cloud point of fatty acid esters of secondary or branched-chain alcohols area are still scare in the literature, particularly for these compounds, so this result is an interesting contribution of this work. In this research, we also found a low production of alkyl esters that was published [37]. Although no explanation is reported in the literature for this phenomenon, we believe the explanation is similar to when using palm oil. So, according to the scientific literature the poor performance of methanol in the production of alkyl esters from palm oil is in agreement with the work of Kalam and Masjuki [39], 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 synthesised foam carbon has a hydrophobic nature which allows greater retention of branched chain alcohols, forming a system that can react vigourously with *Persea americana mill oil*. Thus the most effective system was FC-6 with 3-methyl-1-butanol. Is important to note that our results show there is a relation between the yield of biodiesel obtained, addition to the textural and chemical characteristics of the biocatalyst with the properties of alcohols, and particularly used are the number of carbon atoms and if they are primary or secondary. According to the results obtained in this investigation, we can see that the production of biodiesel could increase with respect to the lipase/mesoporous activated carbon, this due to the higher mesoporosity develops the greatest amount of carbon deposits and lipase both the performance of biodiesel production will be higher.

The operational stability of the lipase was investigated in consecutive additions of alcohol, in a solvent-free system. The reaction time was 32 h, after which the enzyme was recycled and reused as showed in Fig. 6.

Immobilised enzymes 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 phenomena which has been reported in the literature when using enzymes for the synthesis of biodiesel. Therefore, we tried to improve the stability of the immobilised lipase after each use, and then compared the results obtained in our laboratory.

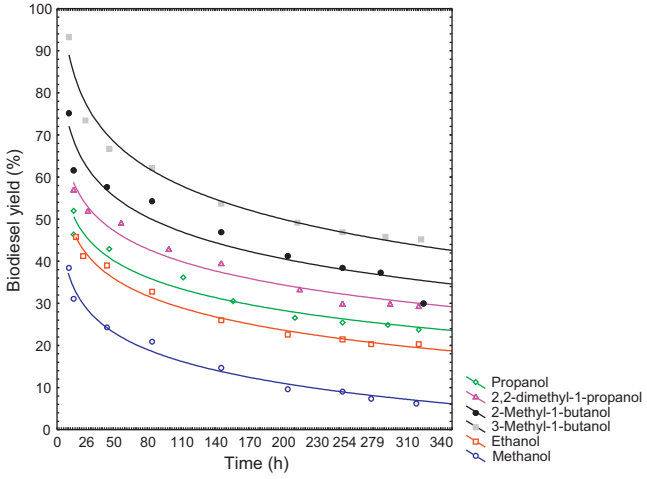

Fig. 6. Operational stability of lipase using biocatalyst FC-6. Reactions parameters: 45 °C, 1% enzyme on oil weight, 32 h of reaction, molar ratio 10:1 alcohol to oil ratio.

After each transesterification reaction, the lipase was recovered by filtration and washed with, in all cases, n-hexane, a tested non-polar solvent, that causes greater retention of lipase activity than that obtained when washing with the polar solvents, as we previously we had already reported using others supports and other alcohols. With all six alcohols that work as acyl acceptors, a high initial yield was achieved. The lipase exhibited better activity during the repeated experiments compared with those in the literature. In the reaction with methanol, the production of alkyl esters was 8% (determined using internal standards in GC); is of highlighting that was detected production of biodiesel, new result obtained with this system and has not been reported in the literature. The same experimental conditions in the literature have not reported production of biodiesel. This shows the effect of the combination support (foam carbon), 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 2,2-dimethyl-1-propanol, propanol, ethanol and methanol decreased with time and the production of biodiesel decreased to between 60–38%, 52–30%, 48–28% and 40–8%, respectively, as shown in Fig. 6. 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, as explained by us previously. The glycerol by-product is hydrophobic and insoluble in oil, so it is easily adsorbed onto the surface of the immobilised lipase, creating a negative effect on lipase activity and operational stability [15]. Additionally, a 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 a high binding capacity may result in steric interference problems and loss of enzyme activity. This shows that could prolong the service time of the immobilised lipase/mesoporous activated carbon by an assembly which continuously removes the production of glycerol to prevent it inactivating the action of lipase. On the other hand, the support (mesoporous activated carbon) can be chemically modified to achieve better fixation of lipase.

In general, the maximum adsorption is observed at pHs close to the isoelectric point of the lipase [37]. In addition, porous particulate supports are superior to nonporous supports for immobilisation of lipases due to their greater surface area and chemical properties. However, porous supports can have an internal

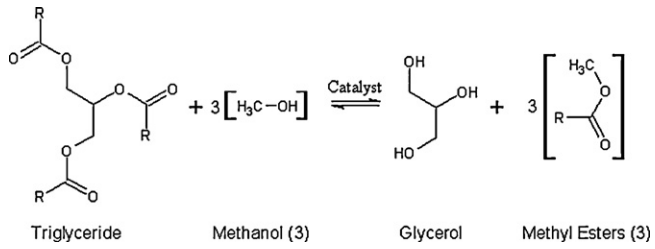

Fig. 7. Equation general of the transesterification reaction of triglycerides.

morphology that allows not only the lipase binding, but also an easy accessibility to substrate molecules in order to minimise diffusional limitation. It appears that pore sizes best suited for lipase adsorption are at least 100 nm 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 [26]. The affinity of a lipase for an adsorbent generally increases with the hydrophobicity of the surface, and lipases desorb 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 miscible in triglycerides at room temperature and mechanical agitation is required to facilitate mass transfer. However, in the course of the reaction there is the formation of an emulsion, which in the case of the meta-analysis, are easily and rapidly dissolved to form an inner layer rich in glycerol and another one on 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 process similar to hydrolysis, except that alcohol is used instead of water. This process is used to reduce the high viscosity of triglycerides. Fig. 7 represents the general equation of the transesterification reaction:

The transesterification reaction is reversible and an excess of alcohol is used to move the equilibrium towards the formation of esters. The kinetic model of reaction that has the best fit is the pseudo second order, in the initial stage of reaction, followed by a first or zero order reaction [37]. Chemically, transesterification mechanism consists of three consecutive reversible reactions; the triglyceride is converted sequentially into diglycerides, monoglycerides and glycerol (plus the methyl esters) (Fig. 8) [37]. On the other hand, it is necessary to consider that alcohol plays an important role in the mass transfer, for example in the case of the meta-analysis, methanol is not soluble in triglycerides or methyl esters of glycerine. However, methanol is soluble in methyl esters and glycerine. Therefore during the first few minutes of the 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, because a larger chain stabilises the OH group; the 3-methyl-1-butanol is the less acidic, it is clear that this is directly associated with the biodiesel production; this research shows that the acidity or basicity of the alcohol used directly influences the production of biodiesel. The degree of deactivation was found to be inversely proportional to the number of carbon atoms in the linear lower alcohols. Fig. 6 shows the stabilising effect of the branched chain alcohols and linear alcohols used on the immobilised lipase. The degree of stability brought out by the branched alcohols was demonstrated to be greater than that by the linear alcohols. The curves for 3-methyl-1-butanol, 2-methyl-1-butanol and 2,2-dimethyl-1-propanol in Fig. 6 are less pronounced than in the case of linear chain alcohols. It was also observed that when the immobilised lipase was decreasing its stability by methanol or ethanol, the immobilised lipase particles underwent a conspicuous change in appearance, accompanied by swelling and caking. This work has shown that alcohols with more than three carbon atoms were completely miscible with palm oil at the molar ratio researched. The experimental results indicated that one of the main causes of the decrease of the lipase was due to the immiscibility between triglycerides and short chain alcohols (i.e. methanol or ethanol). It is possible that the short linear alcohols employed formed small

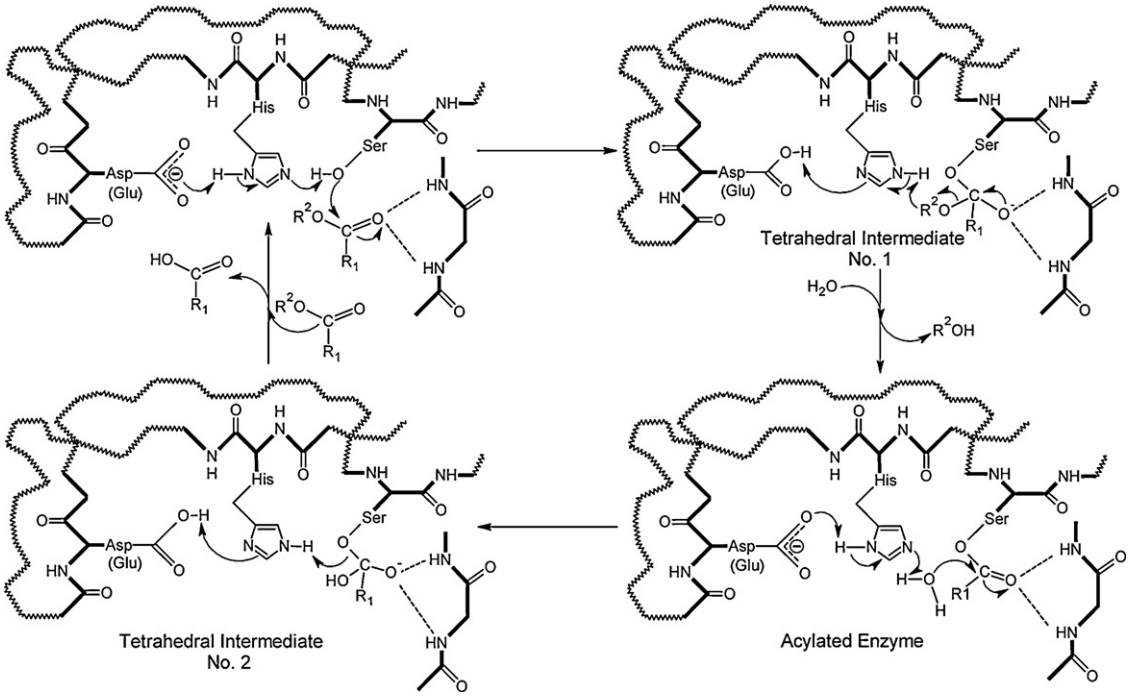

Fig. 8. Reactions between alcohol, *Persea americana mill oil* and lipase for production biodiesel [37].

Table 2
Parameters using Freundlich's and Langmuir' models for the biocatalyst FC-6.

Alcohol	Linear K_D (l/g)	Freundlich model			Langmuir model			
		K_f	$1/n$	R^2	q_{max} (mg/g)	b (l/g)	R_L	R^2
Methanol	5.08	7.45636	0.432	0.99954	67.4532	0.010	0.088	0.98979
Ethanol	7.65	14.7658	0.344	0.99964	53.9876	0.016	0.077	0.97984
Propanol	8.02	23.7547	0.312	0.99989	73.9845	0.012	0.067	0.95976
2,2-Dimethyl-1-propanol	8.98	31.3075	0.243	0.99994	86.9899	0.003	0.056	0.96995
2-Methyl-1-butanol	9.56	40.3599	0.204	0.99996	83.4565	0.005	0.025	0.97976
3-Methyl-1-butanol	10.65	48.8755	0.185	0.99998	89.8769	0.004	0.010	0.97995

droplets which attached to the resin particles. As the alcohol was adsorbed to the immobilised enzyme, the entry of triglycerides was blocked, causing the reaction to stop. Table 2 shows the behaviours of the biodiesel yield as a function of time in the different alcohols. *Candida antarctica B* lipase was immobilised on FC-6 and FC-4 and used as a catalyst (biocatalyst) that present a highest yield with FC-6.

Table 2 shows the constants when the Langmuir model was applied to experimental data sample, and the constants RL have low values were 0.088, 0.077 and 0.067; for the samples using methanol, ethanol and propanol that relates with the yield of biodiesel production when is used FC-6 biocatalyst, and the coefficients find in a range between 0.98979 and 95 976, that indicate a adjustment low for samples, reason by was chosen, another model to compare results. Table 2 evidences adjustment from the experimental data using Freundlich's model. The constants of adsorption and the R value show a more reduced range of 0.99954–0.99998, indicating that this model suggests a better adjustment to the experimental data obtained in this. The higher values for the maximum yield of biodiesel were found for the samples with FC-6 biocatalyst impregnated using isobutanol; the constant values of K_f evidence a highest affinity between this biocatalyst and the reaction system.

It is possible that the system can reuse lipase/mesoporous activated carbon for the synthesis of biodiesel glycerol, preventing accumulation, and that this inactives the lipase. This can be achieved with a dynamic system which constantly removes glycerol.

4. Conclusions

In this study, the use of different alcohols that work as acyl acceptors in lipase-catalysed biodiesel synthesis was studied. Foam carbons were synthesised using nonylphenol ethoxylated with $n = 4$ (FC-4) and $n = 6$ (FC-6), and were used 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, methanol, ethanol, propanol, 2,2-dimethyl-1-propanol, 2-methyl-1-butanol, and 3-methyl-1-butanol, have less of a negative effect on lipase stability in comparison to the traditionally used methanol. High yields of biodiesel could be achieved using *Persea americana mill oil*, but only with a stepwise addition of alcohol, as in the case of 3-methyl-1-butanol, which 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 immobilised lipase and created a negative effect on lipase activity and operational stability.

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