Biodiesel Synthesis: Use of Activated Carbon as Support of the Catalysts

Aida Mireya Ramírez-Arias, Liliana Giraldo and Juan Carlos Moreno-Piraján

Abstract Biodiesel synthesis was performed by means of a transesterification reaction, for which soybean oil and palm oil were used as raw materials. Homogeneous processes, heterogeneous catalysis, and biocatalysis, using *Lipase type II from porcine pancreas* as a biocatalyst, as alcohol, methanol, were used to carry out the reaction. With regard to the heterogeneous catalysts, it is used activated coal and gamma alumina like support. Once the process was finished and biodiesel was purified, the chemical and physical properties like: density, humidity, acid value, saponification, cetane index, and calorific value were determined according to ASTM (American Society for Testing and Materials) standards and the methyl esters were quantified by gas chromatography, where it was in more percentage C18 and C16. The best conditions found for each transesterificación were: 0.7% KOH, at 60 °C, 100 rpm, a molar ratio of alcohol: oil of 6:1 and 90 min for homogeneous catalysis. Heterogeneous catalysis 3.0 wt% catalyst, a molar ratio of alcohol: oil of 9:1, the temperature of 60 °C, at 100 rpm for 90 min, the catalyst that presented higher yields of biodiesel 95 and 96% soybean oil (SO) and crude palm oil (OP) was when pelleted activated carbon (PAC) was used as support. In the biocatalysis conditions were 40 °C for 6 h, 6.0% lipase and 300 rpm agitation.

Finally, it is concluded that the biodiesel yields have a direct relation with the surface area of the catalyst, being a greater area in PAC and with this also the higher yields of biodiesel in each one of the different catalysis. Activated carbon is a material that produced good yields in the synthesis of biodiesel shows potential for transesterification of FFA. The synthesis of biodiesel from the transesterification of soybean oil and palm oil complies with the standards stipulated by ASTM to be used as fuel and thereby generate alternative sources of energy.

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1 Introduction

In this century, the human population must confront the problems generated by the increase in energy demand, the depletion of fossil fuels, rising oil prices, and environmental pollution by greenhouse gases produced largely by oil use. These gases are responsible for much of the climate change around the globe, with adverse effects seen on human health. It is therefore necessary to look for alternative energy sources based on renewable processes. From this perspective, research into the production of biofuels is very important (Banerjee and Chakraborty 2009; Banerjee et al. 2002; Björkling et al. 1991; Bommarius and Riebel 2004). One of the sectors in the industry with increased demand for liquid fuels such as gasoline and diesel is the automotive sector. This unit produces two disadvantages: oil shortage and increased emission of pollutants generated in the incomplete combustion of these liquids (Gomez 2007).

The approval of law 693 marked the entry of Colombia into the new era of biofuels, used for many decades, especially bioethanol, due to the economic attractiveness, according to the Kyoto Protocol, and the dynamic oil prices. Preparation processes of biofuels are intended to diversify the energy mix using new energy alternatives that create a positive impact on economic, social, and environmental levels (Gomez 2007; Hossain et al. 2008).

Biofuels are substances that are produced from biomass, and serve as a source of renewable energy, reducing CO_2 production, closing the carbon cycle, through photosynthesis, transforming in carbohydrates, like sugars and starches (Diaz and Balkus 1996). The authors of the present work show recent results obtained in your laboratory which involved preparing biodiesel, from soybean oil and palm oil by heterogeneous and homogeneous catalysis, where activated carbon and gamma alumina was used as a support for catalysts in transesterification reactions. The activated carbon used is obtained from coconut shell, in granular and pelletised form, with a good surface area (A_{BET} 842–1131 $m^2\ g^{-1}$), a residue that is not given an adequate use.

The biodiesel obtained is characterized, taking into account its physical and chemical properties and the percentage of methyl esters, where it is a biofuel and must comply with certain standards already established by ASTM for its commercialization. Results of three types of catalysis (homogeneous, heterogeneous, and biocatalysis) are presented, in order to determine which process is more viable for biofuel production, taking into account the type of catalyst and the price factor.

2 Theoretical Basis

2.1 Biodiesel

The biodiesel Fatty Acid Methyl Esters (FAME) is a synthetic, clean, nontoxic, and high-quality biofuel, comprising a mixture of monoalkyl esters of long chain fatty acids derived from sources of triacylglycerides such as vegetable oil residues from the food industry and animal fats. For those produced from plant materials, emissions of carbon dioxide (CO_2) are lower than those produced by fossil fuels, in the combustion process. Biodiesel properties vary according to the raw material; among the most outstanding and attractive are its biodegradability and non-toxicity (Boz and Kara 2009; Cadena agroindustrial Etanol 2004).

In contrast to diesel, biodiesel has several advantages: (1) it is a renewable and biodegradable energy (degrades four times faster than diesel); (2) during combustion, it produces fewer emissions of pollutants (carbon dioxide, sulfur oxides, nitrogen, and metals) because of its oxygenated state (Bommarius and Riebel 2004); (3) it has lubricating properties that minimize engine wear; and (4) it is safe for storage and handling due to its low volatility and high flash point (100–170 °C) (Bommarius and Riebel 2004).

The technology for the production of biodiesel has been known for more than 50 years in the United States and it has been primarily made from soy oil. Other sources of biodiesel in the world are canola oil, animal fat, palm oil, corn oil, canola oil, Jatropha oil, coconut oil, waste cooking oil, and algae oil, which have potential to displace diesel, taking into account their oil yield and surface area required (Table 1) (Chisti 2007; Idris and Bukhari 2012; Meher et al. 2006).

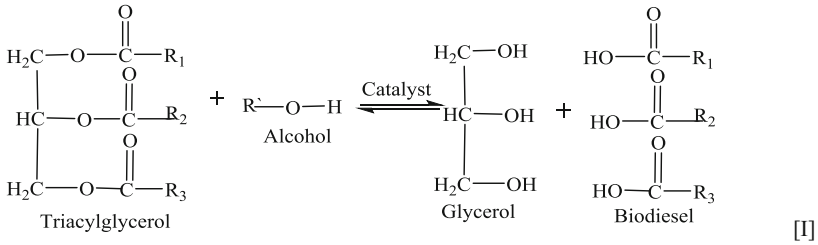
Biodiesel is produced from a transesterification reaction, based on the transformation of a triacylglycerides in Fatty acid methyl esters (FAMES) in the presence of an alcohol (methanol or ethanol) and an acid, with the most common basic catalyst, glycerol, being obtained as a by-product (see Fig. 1) (Demirbas and Demirbas 2011).

In the first reaction [II], diacylglyceride is obtained from triacylglycerides, then monoacylglycerides [III], and finally glycerine [IV]. During the process, three

Table 1 Comparison of some sources of biodiesel (Meher et al. 2006)

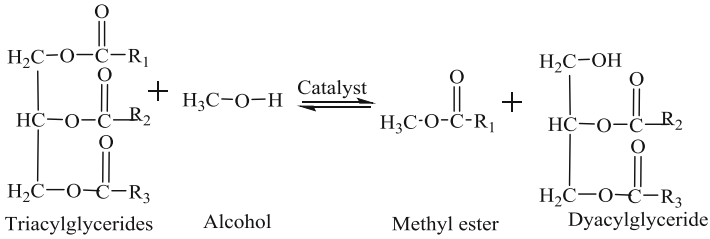
Cultivation	Oil yield (L ha^{-1})	Surface area required (m ha)[a]
Corn	172	1540
Soy	446	594
Canola	1190	223
Jatropha	1892	140
Coconut	2689	99
Palm oil	5950	45

[a]To fulfill 50% of all transport fuel necessary for the United States

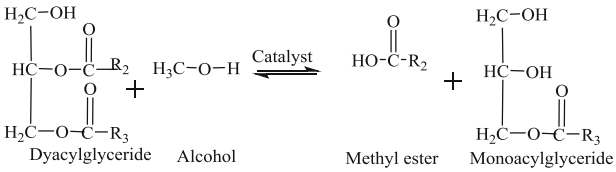


[I]

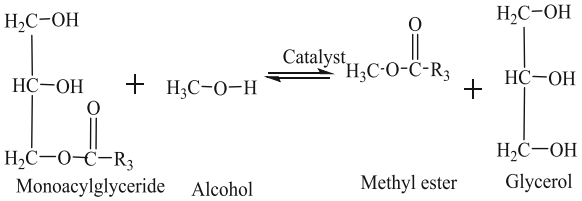
The global process involves a sequence of three reversible reactions:



[II]



[III]



[IV]

Fig. 1 Process for the synthesis of biodiesel

molecules of methyl esters are produced from an excess of alcohol to displace the reaction towards the production of methyl esters (Ebert 2008).

In transesterification, the variables that have the greatest influence are: purity and quality of the reagents, alcohol/oil molar ratio, alcohol type, catalyst type and amount, temperature, agitation and time. For this reason, it is essential to control these variables (Ebert 2008).

2.1.1 Synthesis of Biodiesel

Transesterification Basic Medium

The base-catalyzed transesterification is a classic example of a nucleophilic acyl substitution by an addition–elimination mechanism. The alkoxide ion is highly nucleophilic, attacking the ester carbonyl and subsequently the alkoxide ion which the ester was traveling to, and thus the transesterification occurs (see Fig. 2).

This transesterification is about 4000 times faster than the acid-catalyzed reaction (Fukuda et al. 2001). It commonly uses sodium hydroxide and potassium at a concentration of 1% by weight of oil as catalysts. Alkoxides such as sodium methoxide are even better catalysts. The transesterification is carried out at 60 °C, at atmospheric pressure and takes approximately 90 min to complete (Fukuda et al. 2001).

Transesterification in Acidic

The acid-catalyzed reaction is similar to the above mechanism, only the proton alcohol group, being less nucleophilic, facilitates further transfer given protonation of the oxygen carbonyl group, which behaves as a presented Lewis base due to its lone pairs. The protonation of oxygen gives more character to the carbon electrophile attack alcohol, resulting in the formation of a tetrahedral intermediate which is a deprotonated alcohol group of another molecule. A subsequent protonation provides adequate oxygen, making this group a better leaving group than the unprotonated form; when oxygen enters the electron pair, a molecule of alcohol is ejected, leaving a protonated version of the final product (see Fig. 3) (Fukuda et al. 2001).

2.2 Homogeneous Catalysis

2.2.1 Homogeneous Alkaline Catalysis

Homogeneous catalysis is the best known method for the synthesis of biodiesel, with the following being commonly used as catalysts: sodium hydroxide (NaOH),

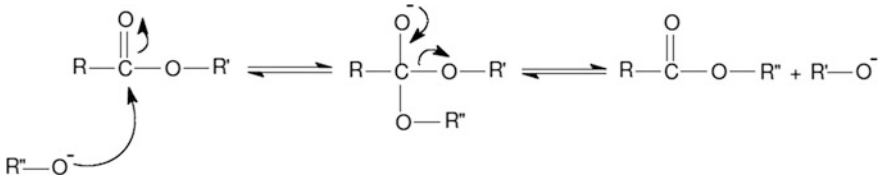


Fig. 2 Transesterification in a basic medium

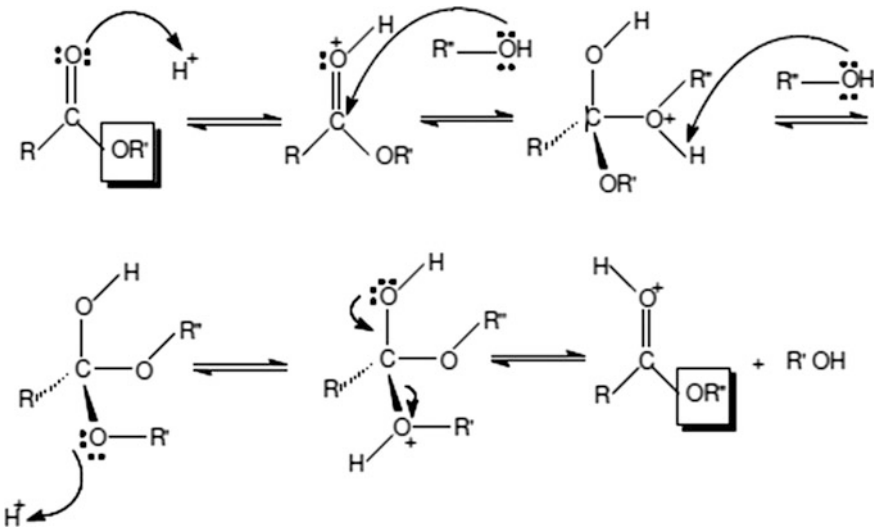


Fig. 3 Acid catalyzed transesterification

potassium hydroxide (KOH), and sodium methoxide ($NaOCH_3$); these soluble catalysts are inexpensive and generate the transesterification reaction at short times, using a lower temperature, moderate pressure and a 3:1 oil:alcohol ratio for the production of methoxide to react with the oil to produce biodiesel and glycerol. Despite these advantages, the preparation and catalyst recovery is tedious (Kok et al. 2011), since it is not easy to separate the catalyst from the reaction products, which makes it necessary to perform extensive washings with water, producing large quantities of water alkaline waste which has to be treated for proper disposal (Knothe et al. 2005). Also, if the starting material (oil) contains a high proportion of free fatty acids (FFA), or water, soap FFA is formed, which can affect the performance of biodiesel (Kok et al. 2011).

2.2.2 Homogeneous Acid Catalysis

In such catalysis, the most frequently used catalysts are sulphuric acid, hydrochloric acid, and phosphoric acid. In contrast to the basic homogeneous catalysis, this uses a higher temperature, increased reaction time, higher oil:alcohol ratio, and more alcohol. No side reactions occur in the transesterification process, with high percentages of fatty acids (greater than 3%) and water (Kok et al. 2011).

However, homogeneous reactions have several disadvantages that make them economically unattractive, such as the homogeneous phase between the catalyst and products, which makes separation and purification steps more complicated. Alkaline water and oily residues generated in the separation increase the production

costs of biodiesel; in addition, glycerine purification is difficult due to the solubility of methanol (Knothe et al. 2005).

2.3 Heterogeneous Catalysis

The use of heterogeneous catalysts reduces the problems presented by homogeneous catalysts, including avoiding the complex separation procedures required in homogeneous catalysis. Here, the catalyst and products are in different phases; heterogeneous catalysts can also be easily recovered and reused, reducing the cost of the production of biodiesel [16.17]. This catalysis is not affected by the presence of high fatty acid content (greater than 3%) in oil. However, compared with homogeneous catalysis, heterogeneous catalysis proceeds at a slower rate due to the three-phase system: oil, alcohol and solid catalyst (Kok et al. 2011). To avoid the problem of mass transfer, porous supports with catalysts that can provide a relatively high specific surface area are used; the presence of pores can react with triacylglycerides (Kok et al. 2011; Zabeti et al. 2009). Also, ɤ-Al_2O_3 has been widely used as a support in catalysis processes due to the thermal and mechanical stability, specific surface area and pore volume (Zabeti et al. 2009; Yun et al. 2011) (Table 2).

Table 2 Characteristics of heterogeneous acidic and basic catalysis

Heterogeneous basic catalysis (Boz and Kara 2009; Yun et al. 2011)	Heterogeneous acidic catalysis (Zabeti et al. 2009; Yun et al. 2011)
✓ The catalyst is solid and the reactants and products are liquid ✓ The catalyst can be fully recovered and reused ✓ The catalyst is easy to separate and purify, typical of homogeneous processes catalyzed by bases.	
Less explored than basic homogeneous catalysis	The solid acid catalysts in heterogeneous transesterification are useful when the oil has a high content of FFA and water
Requires less temperatures than acid catalysis	Require high temperatures for the reaction to complete the transesterification process
Most of the basic solid catalysts have a higher activity and reaction rate with respect to solid acids in transesterification processes	The catalytic activity of solid acid catalysts, it is necessary modify the surface, to increase the pore size, density of active sites (Boz and Kara 2009; Bengoagorostiza 2012)
Between the basic solid catalysts are: NaOH/ɤ-Al_2O_3, K/ɤ-Al_2O_3, KOH/NaY, KOH/ɤ-Al_2O_3 and zeolites	Among the solid acid catalysts are: SO_4^{2-}/ZrO_2, SO_4^{2-}/SnO_2, sulphonated amorphous carbon, ZrO_2, and WO_3

2.4 Biocatalysis

In recent decades, biotechnology has achieved progress in various areas of science; in particular, enzymes have become important tools for catalysis, reaching a broad spectrum of industrial applications, among which chemical synthesis, the food industry, and the pharmaceutical industry should be mentioned, as these present clear advantages over non-biological conventional catalysts, greater specificity and selectivity, working better under pressure, temperature and moderate pH and reaching speeds similar to the chemical catalyst reactions should be mentioned. Within the wide variety of industrially important enzymes are lipases (acyl ester hydrolases), which catalyze the hydrolysis and formation of lipids, with applications in the oleochemical industry, and fats, as in biodiesel production. This is one of the most interesting factors for the transesterification of oils, because it has the most efficient mechanism, there is no need for pretreatment of the raw material, since the catalyst is selective, and it consumes less power, generating little waste and making it environmentally attractive (Refaat 2010).

2.4.1 Lipases

Lipases (acyl ester hydrolases) are enzymes that catalyse the hydrolysis of triacylglycerols, especially long chain, which give monoacylglycerides, diacylglycerides, free fatty acids, and glycerol in an equilibrium reaction controlled by water content in the reaction medium. They modify the reaction conditions, and are also able to catalyse other reactions such as esterification, transesterification, and interesterification (Naranjo et al. 2010), as illustrated in Fig. 4.

These enzymes are mainly characterized by being water soluble and insoluble substrates and acting on aggregates, which operate together to form organic–aqueous interfaces; these are the only enzymes capable of this, unlike esterases, which hydrolyse soluble esters, working without the need for an interface. Most lipases are esterases, although not all esterases are lipases. The author (Verma et al.

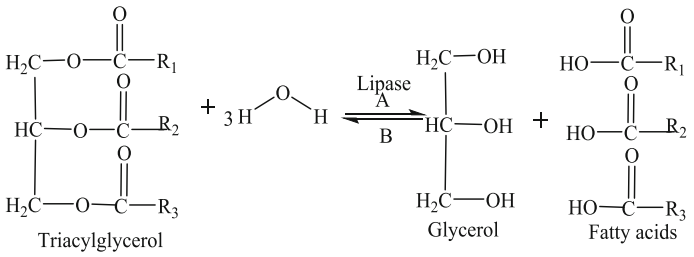


Fig. 4 Catalytic reaction of natural lipase. A triacylglycerol can be hydrolysed to glycerol and fatty acids (A), or the reverse reaction combining glycerol and fatty acids to form triacylglycerol (B)

2012) presented as a fold structure of polypeptides composed of eight β-sheets, connected by six α-spirals. The triad Ser-His-Asp/Glu is completely covered by a lid which must be fully opened to access the substrate; the catalytic triad is embedded in a consensus region Gly-X-Ser-X-Gly. Its mechanism of activation may be open or closed: (1) Open or active: the polypeptide chain is displaced and the active site is exposed to the reaction medium; and (2) Closed: The polypeptide chain closes the active site, forming the top "lid", causing inactivation of the enzyme (Verma et al. 2012).

2.4.2 Transesterification and Lipase Interesterification

The transesterification reaction is a process in which the acyl donor is an ester and can be classified via glycerolysis and alcoholysis, as glycerol or an alcohol, and the acyl receptor (see Fig. 5).

The transesterification reaction is reversible and an excess of alcohol is used to drive equilibrium towards the formation of esters; chemically, the enzymatic transesterification mechanism comprises three consecutive reversible reactions: the triacylglycerides becomes sequentially diacylglycerides, monoacylglycerides, glycerol, and methyl esters (see Fig. 6) (Naranjo et al. 2010).

Interesterification is the reaction between two esters exchanging their acyl groups, while acidolysis is the reaction between an ester and a carboxylic acid, which proceeds by replacement of the acyl ester group by the free acid (see Fig. 7) (Björkling et al. 1991).

Despite the advantages of using lipase as a biocatalyst, the process of isolation and purification is costly; when they are isolated from their natural environment, the structure is unstable. Moreover, being soluble in water and operating in homogeneous reaction systems, contaminate the product and cannot be recovered for reuse, which becomes an economic problem because the enzymatic activity is lost (Mateo et al. 2007). The enzyme action is increased when immobilized on a suitable support; in the industrial process in which is used, in order to improve properties such as operational stability, thermal stability, activity, specificity and selectivity, reduced inhibition of the reaction products is also possible. Therefore, the above

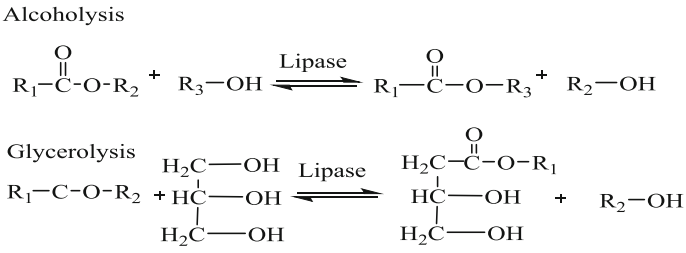


Fig. 5 Transesterification type with lipase

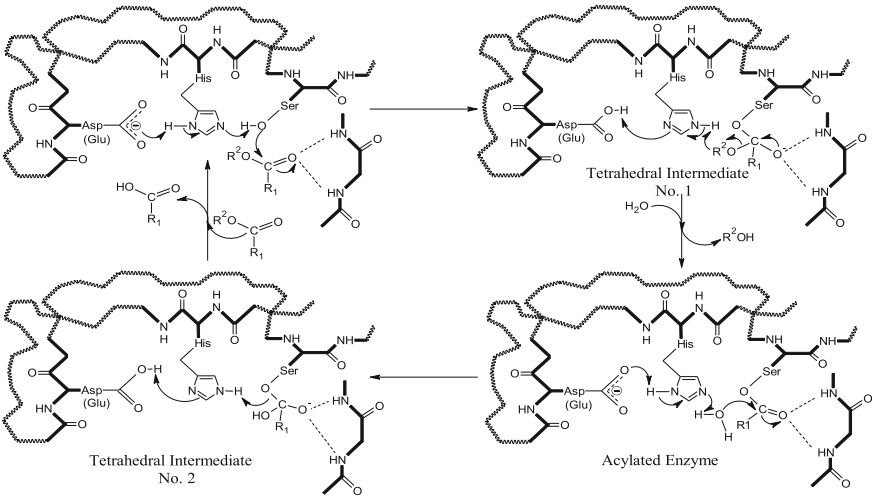


Fig. 6 Reaction mechanisms of lipases (taken from Naranjo et al. 2010)

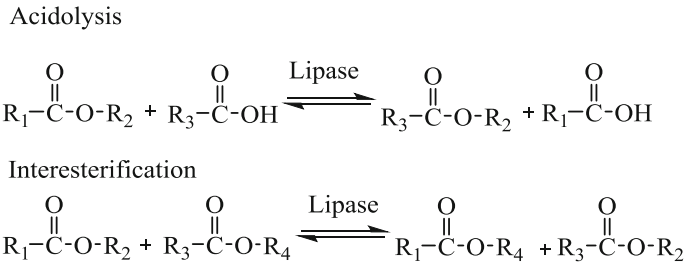


Fig. 7 Types of interesterification with lipases

depend heavily on the support and the locking protocol that is used (Verma et al. 2012; Mateo et al. 2007).

2.4.3 Immobilization of Enzyme

Immobilization is understood to be anchoring of the enzyme to an insoluble solid support, wherein the movement of the enzyme in the space is restricted or partially complete, resulting in a heterogeneous reaction system with the immobilized enzyme. This enables reactions, continuous enzymatic control, and product formation to be carried out, and facilitates the removal of the enzyme reaction mixture. Comparing the methodology when enzymes are free with when they are immobilized, the latter are more robust and more resistant to environmental changes (Saxena et al. 1999).

In the scientific literature, there is a method that is "standardized" for the immobilization of enzymes; this is due to its composition, differences in chemical characteristics, the different properties of the substrates, products and product applications, as well the advantages and disadvantages of each of the methods of restraint. Therefore, conditions for the investigation of an enzyme and its application are usually set based on the physical and structural properties of the support, the physical and chemical properties of the enzyme and the process specifications for the catalyst, to ensure the highest possible retention of enzyme activity and consider their performance, durability, cost and toxicity for immobilization reagent immobilization (Bengoagorostiza 2012; Saxena et al. 1999). The authors of this work have analyzed the most crucial variables in the process of immobilization of the *Lipase type II from porcine pancreas* and has found the most suitable conditions for immobilization to allow its use in the transesterification of fatty acids for the synthesis of biodiesel. Conditions, procedures, and results will be shown in this chapter. Immobilization was performed on granular and pelletised activated carbon made from coconut husks by thermal and chemical pretreatment to develop specific mesoporosity and proper surface chemistry that allows the high adsorption of lipase. The use of immobilized lipase in the transesterification reactions and subsequent synthesis of biodiesel has become a novel process due to its high specificity.

Methods of Immobilization by Chemical Bonding

- Covalent bonding

The covalent bonding methodology is based on the chemical activation of groups on the support by a specific reagent, which enables reactions with amino acid residues on the surface of the enzyme. The advantage of this method is the strength of unity and stability as a result of immobilization, whereas the disadvantages are the high costs and low yields, because the conformation of the enzyme and its activities are strongly influenced by the covalent union (Bommarius and Riebel 2004; Idris and Bukhari 2012; Bengoagorostiza 2012).

Methods of Immobilization by Physical Retention

- Adsorption

In adsorption, the enzyme is attached to an unfunctionalized support through reversible interactions, such as van der Waals forces and hydrogen bonds. It is the simplest, cheapest, and fastest method; chemical changes to the support or enzyme are not needed but this method has the disadvantage of loss of enzyme from the

support, steric hindrance occurring inside the support and the lack of specificity for this type of immobilization (Idris and Bukhari 2012; Bengoagorostiza 2012; Naranjo et al. 2010).

- Capture

In this method of immobilization, the enzyme is free in solution, but is limited in its movement by the lattice structure of the gel used for this procedure, which prevents the release of the protein without preventing penetration of the substrate. The immobilization process is carried out by suspending the enzyme in a monomer solution. Subsequently, the polymerisation is conducted by a temperature change or by adding a chemical reagent. The capture method has the disadvantage of the support acting as a barrier to mass transfer (Bommarius and Riebel 2004; Idris and Bukhari 2012).

- Encapsulation

The encapsulation method involves trapping the enzyme in various forms of spherical semi-permeable membranes with diameters ranging between 10 and 100 μm. Enzymes are physically contained within the membrane and the substrate and product molecules must diffuse through it freely if their sizes are small enough for this to be feasible. A disadvantage is the problem of acute dissemination, but an advantage may be the co-immobilization of different enzymes for special applications (Bommarius and Riebel 2004; Idris and Bukhari 2012; Naranjo et al. 2010).

Activated carbon includes a broad spectrum of materials consisting essentially of carbon, and is specially prepared to have high internal surface and high porosity, which allows different compounds to adsorb, both solids and liquids. Chemical analysis has shown that in addition to carbon, hydrogen and oxygen are present in its structure, allowing the formation of functional groups such as carbonyls, phenolics, esters, and carboxyl groups, among others, generating acidity or basicity in the coal, depending on their composition.

Activated carbon possesses a laminar structure consisting of microcrystalline parallel layers of carbon atoms arranged in regular hexagons; crystal regions are about 100 times lower than graphite and have a random orientation, allowing the existence of pores of different sizes to retain molecules of a gaseous medium or dissolution (Rodriguez 2003).

2.4.4 Supports for Immobilization

The physical and structural properties of the support and the physicochemical and chemical properties of the enzyme are critical for use as a guide in the selection of a suitable immobilization process, and in order to achieve a specific enzyme-support catalyst system with physicochemical properties and kinetics that are completely different from the free enzyme.

A wide variety of potentially viable supports are available for immobilizing an enzyme, using natural, synthetic, organic and inorganic compounds that differ in size, shape, density, and porosity. These take the form of sheets, tubes, fibers, and cylinders, but the most commonly used form is spheres; these should provide the system with permeability and have a suitable biotransformation surface area. Any material considered for the immobilization of enzymes must have certain characteristics, such as mechanical strength, high affinity for proteins, microbial resistance, the availability of reactive functional groups to direct reactions with the enzyme and/or chemical modifications, thermal stability, chemical durability, regenerability, biodegradability, and low cost (Mateo et al. 2007).

2.5 Carbon Activated as Catalytic Support

Activated carbon is a group of porous carbons obtained from the reaction of a carbonized material with oxidizing gases or by the carbonization of lignocellulosic materials impregnated with chemical dehydrating agents (Gómez et al. 2010). Its main characteristics are determined by its physical and chemical properties, such as: porous structure and its adsorption potential. Activated carbons are specially prepared to have a high internal surface and high porosity. Due to its textural properties, surface chemistry and mechanical resistance is used as a catalyst (Santos et al. 2006) and support for catalysts that allows to perform chemical processes under optimum conditions of environmental operation (Gómez et al. 2010).

The activated carbon as the carrier disperses the active phase (noble metals or transition metals) along its surface, generating a high active surface per gram of catalyst. This support must allow the diffusion of the reagents to the active phase, resistance to surface poisoning, good catalytic activity and migration of products; taking into account the type of process, gives the textural properties to the carbon (Gómez et al. 2010).

The activated carbon used as catalyst, has been used in processes of degradation of phenols (Omri and Benzina 2014), derivatives in aqueous effluents (Santos et al. 2006; Matsumura et al. 2002), hydrogenation (Merabti et al. 2010) and transesterification processes (Malins et al. 2015; Baroutian et al. 2010).

3 Methodology

The method used for the preparation of biodiesel from soybean and palm oils using activated carbon made from coconut shell and in the presence of lipases is presented in Fig. 8.

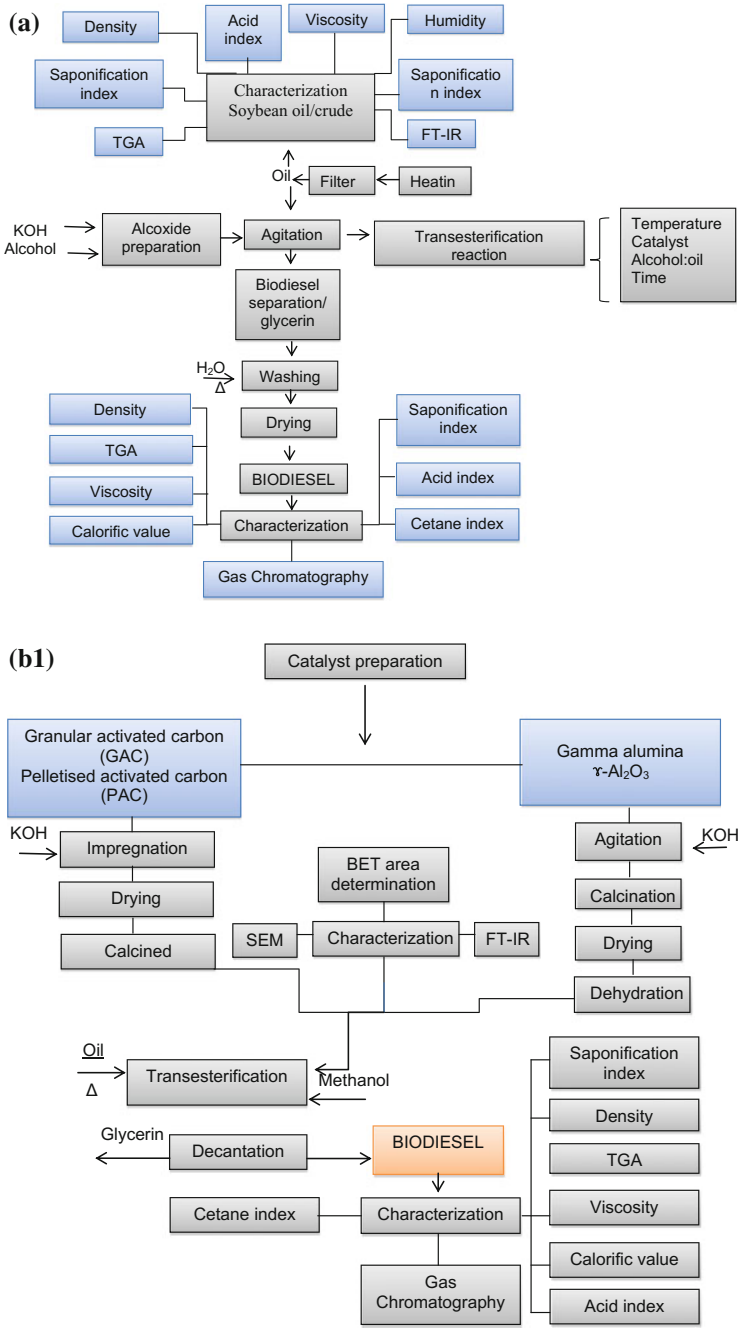


Fig. 8 Flow chart of procedures: **a** Homogeneous catalyst; **b1** and **b2** Heterogeneous catalysts

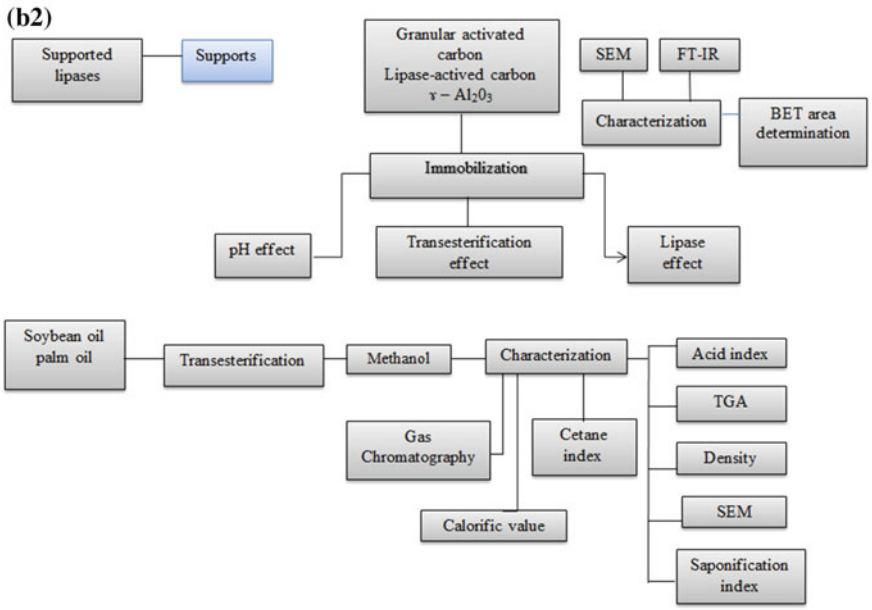


Fig. 8 (continued)

3.1 Homogeneous Catalysis

3.1.1 Characterization of Oil

Soybean and crude oil were characterized, measuring: density, humidity, acid index, viscosity, and saponification index. A thermogravimetric analysis and FT-IR were performed.

3.1.2 Preparation of Alkoxide

For the preparation of the alkoxide, the specific amount was weighed in each catalyst experiment (KOH) and the amount of alcohol was added, and then mixed in a reflux system, until complete dissolution, forming the alkoxide.

3.1.3 Preparation of Oil

The amount of oil for each test is measured, is heated to 110 °C for 30 min, and filtered. Subsequently, it is allowed to reach the desired study temperature, it is allowed to reach the desired study temperature.

3.1.4 Transesterification

To the alkoxide formed, the oil is added and left at reflux for 90 min by controlling the temperature and stirring at 100 rpm.

Effect of Temperature

The reaction was developed using a molar ratio of methanol to triglyceride of 6–1, of catalyst 0.7% (potassium hydroxide), the effect of the temperature in the range of 30–100 °C was studied.

Catalyst Effect

A molar ratio of alcohol:oil 6:1 at 60 °C was used, with stirring 100 rpm, for 90 min and the catalyst concentration varying 0.2–3.0%.

Molar Ratio of Alcohol:Oil

The molar ratio of alcohol:oil of 3–10, at 60 °C, was varied with 100 rpm of stirring, 0.7% of catalyst (KOH).

3.1.5 Separation of Biodiesel and Glycerine

After the reaction time, the resulting solution was transferred to a separatory funnel and allowed to stand for 24 h. Then, the two phases visualized in the lower part (glycerin) and in the upper (biodiesel) were separated.

3.1.6 Washing

Once the biodiesel is separated from the glycerin it must be washing because it may have contents of catalyst, alcohol, soaps, and glycerides without reacting, washes are performed until the residual water is clear and a pH near 7.0

3.1.7 Drying

The biodiesel previously washing is dried by adding 25% by weight of anhydrous sodium sulfate for 4 h, then filtered and was placed in the oven at 110 °C for three hours to completely ensure the presence of water.

3.2 Heterogeneous Catalysis

Three supports were used for the heterogeneous catalysis: granular activated carbon (GAC), pelletized activated carbon (PAC) and gamma alumina (ɤ-Al2O3), which were impregnated with potassium hydroxide (KOH); as alcohol methanol and the source of triglycerides: soybean oil and crude palm oil.

Preparation of the catalyst

3.2.1 KOH/Granular and Pelletized Carbon

All catalysts were prepared by impregnation with 15 mL of different concentrations of potassium hydroxide in aqueous solution (1–5% w/w), is left in constant agitation for 24 h, then the catalyst is dried at 110 °C for 24 h and calcined at 210 °C for 3 h. Finally the catalysts are characterized by FT-IR, BET and SEM. Its basic strength (H_) and basicity were determined by means of a Hammett titration.

3.2.2 KOH/ɤ-Al_2O_3

The alumina was impregnated with a solution of potassium hydroxide (1–5% w/w) for 24 h, with constant stirring. Then was calcined KOH/ɤ-Al_2O_3, a heating rate of 3 °C/min, were dried at 110 °C for 1 h, then dehydrated at a temperature of 250 °C for a period of 1 h, thereafter at a rate of 3 °C/min to 500 °C and left for 1 h. Finally, it was characterized by FT-IR, BET, SEM and its basic strength (H_).

3.3 Biocatalysis

3.3.1 Optimizing the Immobilization of Lipase on Granular Carbon, Alumina and Pelletized

In order to immobilize the lipase from porcine on different supports, immobilization conditions were optimized. To determine the effect of pH on immobilization of porcine lipase was used as 20 mM sodium phosphate buffer at pH 6.0; 7.0; 8.0, and 9.0. For the effect of temperature 125 mg support (granular carbon, pelletized, and aluminum oxide) were suspended in the lipase solution (100 mg porcine lipase dissolved in 0.500 mL of buffer 20 mM sodium phosphate pH 8.0) at different temperatures (4, 25, 37, 60, and 70 °C) for 6 h, then centrifuged at 10,000 rpm, the residue volume was 95% compared to the original solution of the lipase. The effect of incubation time on the immobilization of lipase was also studied, for that small aliquots were withdrawn during a time interval, the solutions were centrifuged and the residue (lipase) is stored at 5 °C.

3.3.2 Preparation of the Enzyme Tuned

100 mg of porcine pancreatic lipase was weighed and dissolved in 0.5 mL of 20 mM sodium phosphate buffer pH 8.00. Subsequently it is frozen at −20 °C and lyophilized for 48 h.

3.3.3 Preparation of Immobilized Enzyme

(a) 100 mg of porcine lipase was dissolved in 0.500 mL buffer 20 mM sodium phosphate pH 8.00 and mixed with 125 mg support (granular carbon, pelletized carbon, and aluminum oxide). After 24 h with occasional stirring, the support is removed; the residual liquid was stored for evaluating the amount of protein. Washes were performed with sodium phosphate pH 8.00 for one to two minutes each wash session. Then, the biocatalyst (lipase activated carbon) stored in refrigeration at 5 °C. Wash solutions were stored to determine your protein content.
(b) Enzyme activators carbon system was characterized by scanning electron microscopy (SEM).
(c) The amount of immobilized enzyme on the support was evaluated by performing the measurement of the concentration in the initial enzyme solution (initial mg protein) and concentration in the residue and wash solutions (final protein mg) using the method of Bradford (Naranjo et al. 2010).

3.3.4 Enzyme Activity

The lipase activity was determined using as substrate p-nitrophenyl palmitate (pNPP), a reaction mixture was realized, which contained 75 μL of pNPP (20 mM), 5 μL of the enzyme and 20 mM sodium phosphate buffer pH 8.00 to a volume of 3 mL, this was done for all the three supports. Subsequently, it incubated at 37 °C for 15 min and the reaction was terminated by adding 1 mL of 0.2 M sodium carbonate. The p-nitrophenol liberated was assessed by 410 nm spectrophotometry. One unit of enzyme activity is defined as the amount of enzyme capable of releasing 1 μmol of *p*-nitrophenol per minute (Soham et al. 2011).

3.3.5 Transesterification Catalyzed by Porcine Lipase

Soybean and palm oil (0.500 g) with methanol at molar ratio 1:6 (mol mol^{-1}) into vial. To this mixture is added 100 mg of enzyme preparation (tuned and immobilized) and incubated at 60 °C with an agitation of 300 rpm. The course of the reaction is performed by taking 200 μL aliquots and analyzing by gas chromatography (Naranjo et al. 2010).

3.4 Quantification of Biodiesel

Fuel characteristics were verified by EN and ASTM, based on this, determined properties such as: acidity, pH, density, calorific value, humidity, and percentage of esters.

Methyl esters (biodiesel) were analyzed using a gas chromatograph (GC) coupled to mass brand Shimadzu QP2010S Series with DB-225 MS column Agilent serial NºUS5268413H dimensions (20 m × 0.1 mm × 0.1 μm). The conditions set for the analysis were: injector temperature 220 °C, using helium as carrier gas with a flow of 1.0 mL/min. The sample injection 1.0 μL, automatic injection AOC 20s (Split mode, using a ratio 30:1. The temperature program in the oven was followed: initial temperature 60.0 °C for 1 min, then heating of 10 °C/min to 195 °C, after reaching this temperature heating of 3.0 °C/min to 205 °C and finally 8.0 °C/min to 220 °C, maintaining this temperature 30 min before giving for finishing the analysis.

4 Results and Analysis

The rate of chemical reactions is affected by many variables, so it is necessary for this kind of study prior to the synthesis of biodiesel. In this chapter, the authors present the results obtained in their laboratory to analyze the effect of five factors on the reaction yield following alkyl preparation for homogeneous, heterogeneous, and enzymatic transesterification from crude palm oil and soybeans.

4.1 Homogeneous and Heterogeneous Catalysis

4.1.1 Homogeneous Catalysis

In transesterification using basic solid catalysts, the first reaction step is the elimination of protons from the basic sites of the catalyst to form alkoxide; then an ester alkoxide attacks a carbonyl group of the triacylglycerides, forming a tetrahedral intermediate, which is subsequently divided into fatty acid alkyl esters and the corresponding anion of the diacylglyceride. Then, the monoacylglycerides and diacylglycerides are converted by the same mechanism as triacylglycerides, into a mixture of alkyl esters and glycerol (Jiang et al. 2010).

Before performing any type of reaction, the oil must be heated and filtered, in order to remove moisture and suspended solids. Then, the physical and chemical properties of oils are determined (see Table 3).

The data in Table 3 show that there is less than 1% acid in the case of vegetable oil (soybean), so pretreatments are not necessary, while palm oil has a high acid

Table 3 Physical and chemical properties of vegetable oil and palm oil used in the transesterification

Property	Vegetable oil (Soybean) (value)	Palm oil (value)
Density 15 °C (kg cm^{-3})	910	890
Viscosity 40 °C (cp)	38.0	37.5
Humidity (%)	0.08	0.04
Acid index (%)	1.00	3.10
Saponification index	185	190

content, i.e., has a higher percentage of free fatty acids, which is a problem when performing homogeneous alkaline catalysis, since the catalyst (KOH) neutralizes free fatty acids, promoting the saponification reaction, which in turn can also promote the generation of stable emulsions, preventing the separation of FAMES and glycerine (Jiang et al. 2010).

Tests with different concentrations of catalyst from 0.2 to 3.0% were performed, in which soap formation and the formation of FAMES was not evidenced. To avoid this, esterification was performed in two steps: in the first step, FFA methyl esters were formed with an acid (sulphuric acid) catalyst, and the percentage of acidity was redetermined, obtaining a value of 0.5%; in the second stage, an alkaline transesterification process was carried out. Once it was determined that the saponification reaction was not favored, the different parameters affecting catalysis were studied. Although the acid value obtained (3.10%) allows homogeneous acid catalysis, transesterification was not performed in this way because the acid catalysis has been reported to have a low reaction rate and high alcohol:oil molar ratios (Titipong and Ajay 2014), which prevent costs from being reduced in the production of biodiesel.

Effect of Time

This type of test was used to find the minimum time for the total conversion of fatty acids to methyl esters.

Figure 9 shows the evolution of conversion along with transesterification, expressed as a percent of triacylglycerides (TG) to fatty acid methyl esters. The minimum time to ensure 100% conversion was 90 min, so tests were conducted allowing this reaction time.

Effect of Catalysts

Of the possible catalysts used, due to cost and by the catalytic activity was more efficient than acidic catalysts, NaOH and KOH were assessed; was decided to use KOH due to the glycerine generated being less toxic than when prepared with sodium, and this product is used in the preparation of fertilizers.

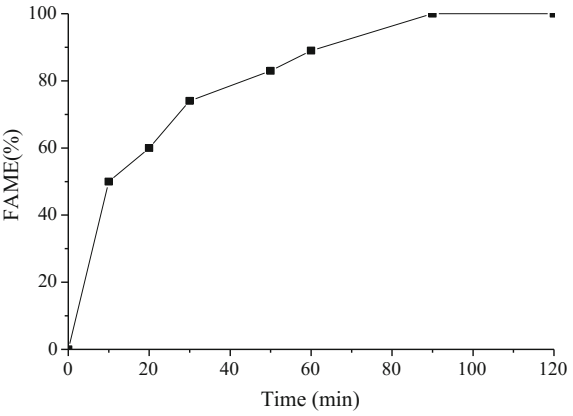


Fig. 9 Conversion of methyl esters from fatty acids as a function of time, with a molar alcohol:oil ratio of 6:1, with 0.7% KOH to vegetable oil (soybean)

Table 3 summarizes the results showing that the higher percent yield were obtained when 0.7% catalyst was used for both palm oil and soybean oil. For values lower than 0.7%, a worse performance was noted because there is a decrease in the reaction rate in the intermediate stages. For larger values, a significant decrease in reaction yields was evidenced as a result of the secondary saponification reaction for triacylglycerides oil, producing soaps which are dissolved in the glycerol phase because this is the more polar phase. These reaction yields were quantified by measuring the mass of methyl esters resulting from the transesterification process (Table 4).

Effect of Molar Ratio of Alcohol:Oil

The results of these studies are shown in Fig. 10; it can be observed that even a molar ratio methanol:oil of 6, presented an increase in performance for both oils, displacing of the reaction towards product formation. Furthermore, at higher values of 6 the yield of methyl esters decrease especially for the oil palm, because the high alcohol content affects the separation of glycerine due to increased solubility; here

Table 4 Percentages of catalyst used in the basic homogeneous transesterification at 60 °C for 90 min, molar ratio methanol:oil 6:1

Molar relation oil:alcohol	Vegetable oil (Soybean)		Palm oil	
	% Catalyst KOH	% Yield	% Catalyst KOH	% Yield
6:1	0.2	80	0.4	82
	0.4	85	0.7	94
	0.7	96	1.1	91
	1.1	92	1.6	82
	2.1	90	2.1	76
	3.0	59	3.0	49

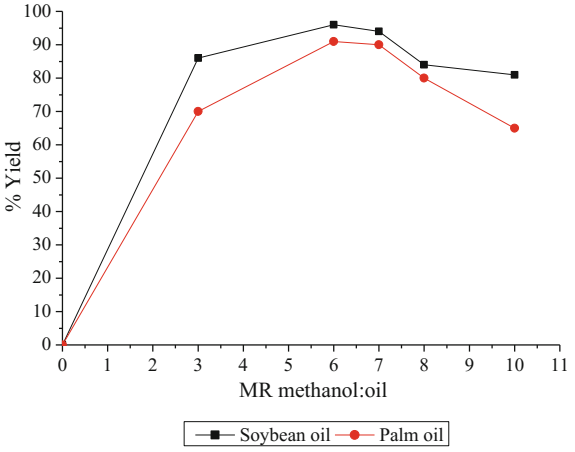


Fig. 10 Effect of methanol molar ratio (MR):oil in a homogeneous basic transesterification process, at 60 °C for 90 min, with 0.7% KOH and 100 rpm agitation

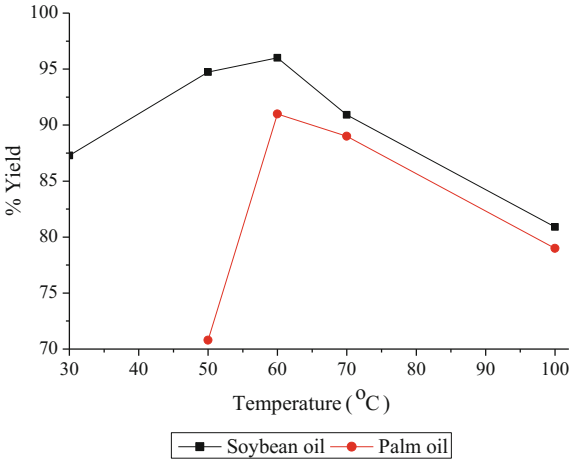


Fig. 11 Effect of temperature on the basic homogeneous transesterification process, for 90 min, with a molar methanol:oil ratio of 6:1, with 0.7% KOH and 100 rpm agitation

the glycerine solution has a shift in equilibrium towards the formation of reactive species (left), finally creating a mixture of triacylglycerides, glycerine and FAMES.

Effect of Temperature

This study shows that palm oil at a temperature of 30 °C did not react, since oil at temperatures below 40 °C is still solid, while a significant increase in performance occurs from 50 to 60 °C, which is higher compared to vegetable oil (soybean). It was found that the highest yield for both vegetable oil (Soybean Oil) and palm oil was seen at 60 °C, the boiling point of methanol. At higher values, performance decreases in a linear manner (see Fig. 11).

It is interesting to note that although the process of homogeneous alkaline transesterification is relatively fast and has a high conversion rate, the catalyst (KOH) cannot be reused because it requires neutralization and purification steps; also, it generates important polluting effluents at the end of the reaction, a process that ultimately has technical and ecological disadvantages, generating overruns in the separation and purification of the end-product (Knothe et al. 2005).

Figure 12a, b show the results obtained using the technique of differential thermogravimetric analysis (TGA-DTA) for the soybean and palm oil samples used

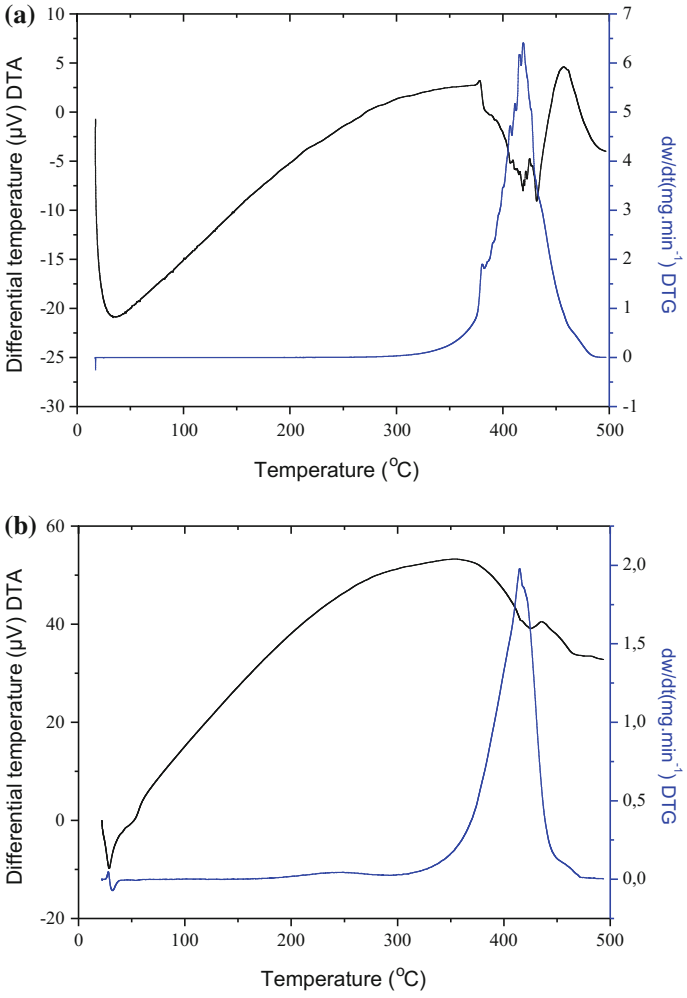


Fig. 12 **a** Thermogravimetric analysis of palm oil, up to 500 °C with a nitrogen flow of 100 mL min^{-1} and a heating rate of 10 °C min^{-1} and **b** Thermogravimetric analysis of soybean oil to 500 °C with a nitrogen flow of 100 mL min^{-1} and a heating rate of 10 °C min^{-1}

in a typical transesterification reaction in our laboratory. The mass starts to decrease after 290 °C for palm oil and 180 °C for soybean, continuing down until all oil present in the sample was completely decomposed at temperatures of 420 and 410 °C for palm oil and soybean oil, respectively. The two oils present endothermic processes, which is more noticeable for palm oil than soybean oil.

Differential thermal analysis (DTA) shows maximum peak decomposition in the temperature range between 210 and 500 °C for palm oil, which is attributed to the decomposition of palmitic acid. For soybean oil, this occurs in a range of 220–460 °C, which is attributed to the decomposition of stearic acid. Greater decomposition occurs in soybean oil compared to palm oil, because palm oil is mainly saturated fatty acids (C16) while soybean has a higher level of unsaturated fatty acids (C18), therefore requiring less energy to break the carbon–carbon single bonds (Muhammad and Tat 2013).

Figure 13a shows the infrared spectrum corresponding to soybean oil. This spectrum shows representative bands of low intensity at 3470 cm^{-1}, corresponding to O–H stretching due to oil moisture; medium intensity 3007 cm^{-1}, indicating HC=CH vibration; intense bands at 2924 and 2853 cm^{-1}, associated with C–H aliphatic stretch; an intense band at 1746.51 cm^{-1}, associated with C=O ester stretching; a medium intensity band at 1464 cm^{-1}, denoting bending in the H–C–H plane; a high intensity band at 1163 cm^{-1}, associated with C–O stretching and a low intensity band at 722 cm^{-1}, linked to C=C–R in alkenes.

On the other hand, Fig. 13b shows the corresponding infrared spectrum for palm oil which displayed the following characteristic bands: medium intensity bands at 2917 and 2850 cm^{-1}, associated with aliphatic C–H stretching; a medium intensity band at 1737 cm^{-1}, associated with C=O unsaturated aliphatic ester stretching; a medium intensity band at 1470 cm^{-1}, corresponding to flexion in the H–C–H plane;

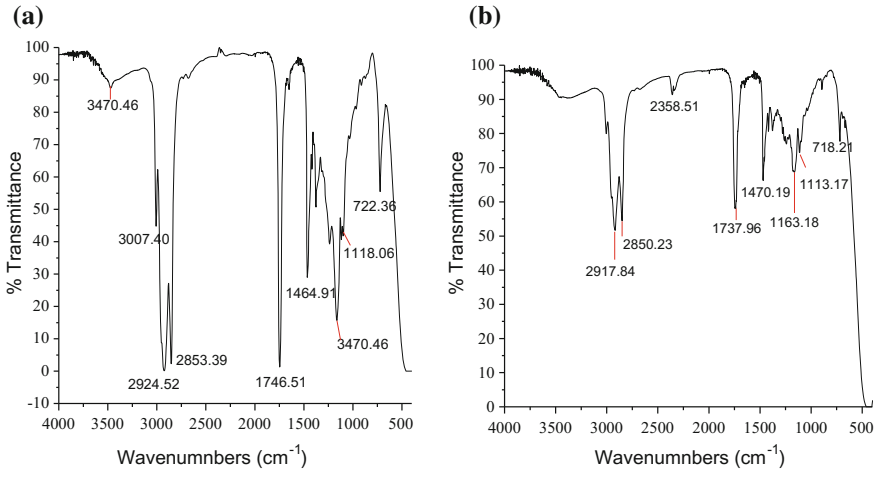


Fig. 13 The corresponding oils used in our investigation. **a** Soybean oil and **b** oil palm

a low intensity band at 1163 cm^{-1}, corresponding to C–O stretching; and a low intensity band at 718 cm^{-1}, corresponding to C–C–R in alkanes.

4.1.2 Heterogeneous Catalysis

Catalysts Characterisation

Table 5 shows the textural properties of the catalysts prepared in our laboratory. The supported catalyst pelletised activated carbon (PAC) has the largest surface area and pore volume, followed by granular activated carbon (GAC) and finally ɤ-lumina, where there is a decline in both the area and total pore volume due to the addition of potassium hydroxide in all cases. This reduction is mainly caused by the preparation process of each solid, which alter the pore volume, apparent surface area and pore distribution according to the starting material and both the chemical and heat treatments used. Decreasing the area is additionally associated with locked pores and the interaction of metal cations with the catalyst support (Ebiura 2005). With a major area, the probability of having active sites is increased and the catalytic reaction type can be adjusted.

Figure 14 shows SEM micrographs taken at our laboratory of heterogeneous catalysts; it can be seen that there is higher porosity in coals in ɤ-Al_2O_3. Activated carbon (called GAC and PAC) has a non-uniform porosity. In the catalysts prepared (called KOH/GAC and KOH/PAC), there is reduced porosity with respect to GAC and PAC, due to thermal and chemical actions to which they are subjected during preparation. Alumina (ɤ-Al_2O_3) has a non-uniform appearance and granular KOH/ɤ-Al_2O_3 has a particle size less than when ɤ-Al_2O_3 is used as precursor.

Table 6 shows the results for the elemental analysis by SEM; some catalysts in which the ɤ-Al_2O_3 decreases the percentage of aluminum and carbon content develop calcination products. Regarding GAC and PAC, the carbon percentage decreases due to a merger of salt, thus increasing the surface coverage of the support. Detections match the nature of the sample, but a complete comparison cannot be performed regarding the composition of all catalysts used, as this analysis was performed on a given area of the sample, which cannot allow the composition of the entire sample to be generalized.

Table 5 Textural parameters of the catalysts prepared from the calculated isotherms of N_2 at −196 °C

Catalyst	A_{BET} ($m^2\ g^{-1}$)	V_p ($cm^3\ g^{-1}$)	D_p (nm)
ɤ-Al_2O_3	144	0.49	1.2
KOH/ɤ-Al_2O_3	121	0.42	–
Granular activated carbon (GAC)	842	0.34	1.7
KOH/GAC	830	0.31	
Pelletised activated carbon (PAC)	1131	0.59	1.9
KOH/PAC	1120	0.45	–

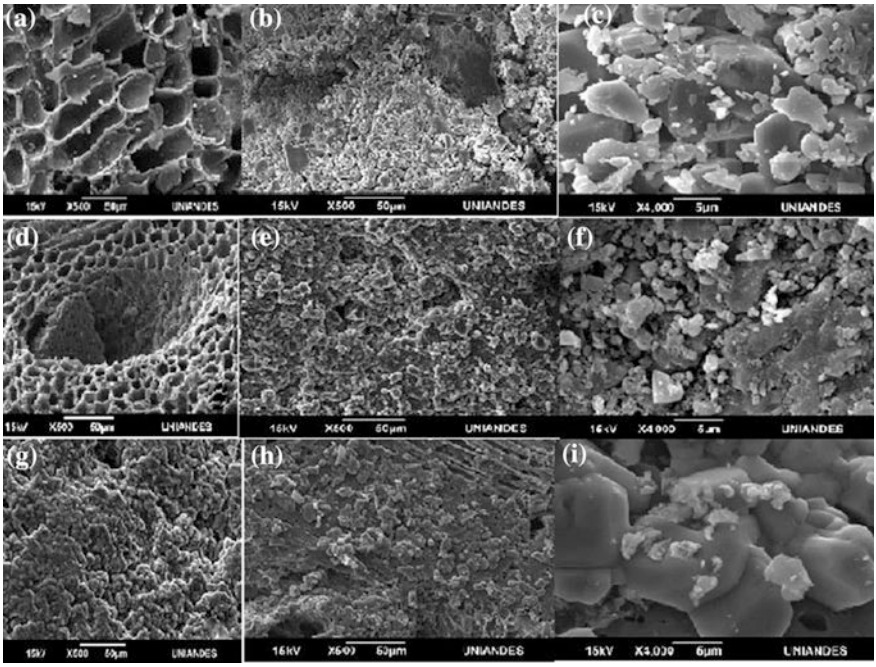


Fig. 14 SEM catalysts prepared, **a** GAC, **b** and **c** KOH/GAC, **d** PAC, **e** and **f** KOH/PAC, **g** ɤ-Al_2O_3, **h** and **i** KOH/ɤ-Al_2O_3

Table 6 Chemical analysis by dispersive energy of some heterogeneously prepared catalysts

Sample	% Weight			
	C	O	Al	K
GAC	100	–	–	–
PAC	100	–	–	–
ɤ-Al_2O_3	–	58.10	41.99	–
KOH/GAC	71.33	27.66	–	1.01
KOH/PAC	71.30	28.13	–	0.57
KOH/ɤ-Al_2O_3	15.20	56.21	27.55	1.04

The basic strength and the basicity of the prepared catalysts were determined using the Hammett titration method. In general, all sites have a basic strength between $7.2 < H^+ < 9.3$. In heterogeneous catalysis, for the reaction between the liquid reactant (methanol) and the solid catalyst (KOH/ɤ-Al_2O_3, KOH/GAC, KOH/PAC), thermodynamically viability is essential in certain stages, such as (Ebiura 2005):

(1) Diffusion of reactive molecules to the solid surface
(2) Chemisorption of some reactive species on the surface

(3) Chemical reaction on the surface
(4) Desorption of surface products
(5) Diffusion of products to dilute phase

The slowest step determines the reaction rate. Steps (1)–(5) depend on the pressure, temperature, and gas viscosity and are usually fast, while steps (2), (3), and (4) are slow and thus determine the reaction rate.

Step (2) is usually rapid, since it is a process with zero energy or close to zero activation. In contrast, (4) needs some activation energy $E_{a,des} = E_{a,adsP} - \Delta H_{adsP} = E_{a,adsP} + \Delta H_{desP}$ (where ΔH_{desP} is enthalpy of desorption of the product P, a normally positive, and ΔH_{adsP} is enthalpy of adsorption of the product P); this step is not determinant of the process but may present inhibition if the catalyst is blocked by non-desorption. Finally, step (3) is the determining step, which results in the conversion of reactants to products (Ebiura 2005).

As shown in Fig. 15, the greatest percent yield in the production of biodiesel was reached at 90 min for the two catalytic systems, both homogeneous and heterogeneous. The homogeneous system has higher yields using soybean oil (SO) and crude palm oil (OP) compared to the heterogeneous system. In the heterogeneous system prepared using different catalysts (KOH/ɤ-Al_2O_3, KOH/GAC and KOH/PAC), higher yields were presented for KOH/PAC and lower yields for KOH/ɤ-Al_2O_3; these results are associated with the surface area catalysts, where PAC is the best support for catalyst preparation and is suitable for transesterification, since it has more active sites available. The ɤ-Al_2O_3 has less A_{BET}, thus generating low yields in the production of biodiesel. After 90 min, the conversion does not increase, but favors the backward reaction (hydrolysis of esters) reducing of product yield (Leung and Guo 2006).

It is also established that, under these experimental conditions, crude palm oil has lower yields compared to soybean, because of the amount of free fatty acids which are reacting with the catalyst, generating side reactions which influence the

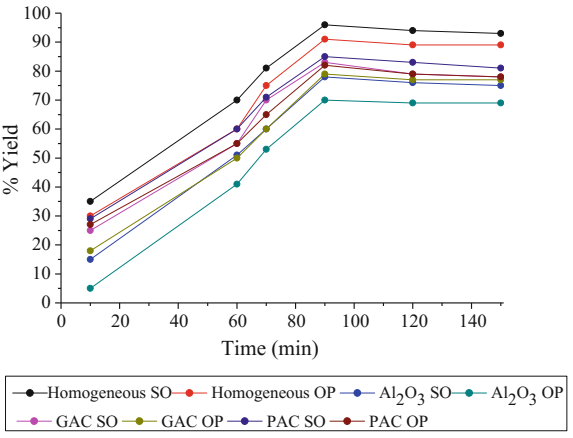


Fig. 15 Effect of reaction time on the yield of biodiesel produced in homogeneous and heterogeneous catalysis using as ɤ-Al_2O_3 support, pelletised activated carbon (PAC) and granular activated carbon (GAC) with KOH, at 60 °C and 100 rpm. The molar ratio of alcohol:oil was 6:1 and 0.7% catalyst was used for palm oil (OP) and soybeans oil (SO)

separation of the two phases (FAMES and glycerol) and thus the overall performance.

Each study for the synthesis of biodiesel is very particular, so it is necessary to investigate each parameter. In this study, the reaction conditions employed were found to be optimal in homogeneous catalysis, meaning that it was necessary to optimize the heterogeneous system to maximize the yield of biodiesel.

It is necessary to point out that in the case of the homogeneous catalysis compared to the heterogeneous, presents higher yields to the same reaction conditions, because the presence in solid basic catalysts in transesterification, the reaction mixture is three-phase catalyst-methanol-oil, where the methoxide which is thought to be the active species in the transesterification are formed upon adsorption of methanol on the catalyst surface, and the transesterification reaction becomes mass transfer-controlled (Dossin et al. 2006; Xie and Peng 2006).

Effect Molar Ratio of Alcohol:Oil

By stoichiometry (Fig. 1), it can be seen that the transesterification requires a molar ratio of methanol:oil of 3:1; as it is a reversible reaction, the effect of the molar ratio of methyl ester on performance is very important. The authors of this work conducted research into such parameters to assess the molar ratio of oil:methanol in the range from 3:1 to 12:1.

It should be noted that the transesterification by heterogeneous catalysis had limited mass transfer, resulting in a low yield of biodiesel, as shown in Fig. 16. The results show that for each catalyst (KOH/ɤ-Al_2O_3, KOH/GAC, KOH/PAC), when the molar ratio of methanol:oil was increased to 9:1, the yield of biodiesel increased, obtaining a yield between 86 and 84% for soybean oil (SO) and oil palm oil (OP) on ɤ-Al_2O_3; 91 and 89% for soybean oil and crude palm oil on GAC; and finally 92 and 91% for soybean oil (SO) and crude palm oil (OP) on PAC. Beyond the molar ratio of 9:1, the excess amount of methanol has a negative effect on

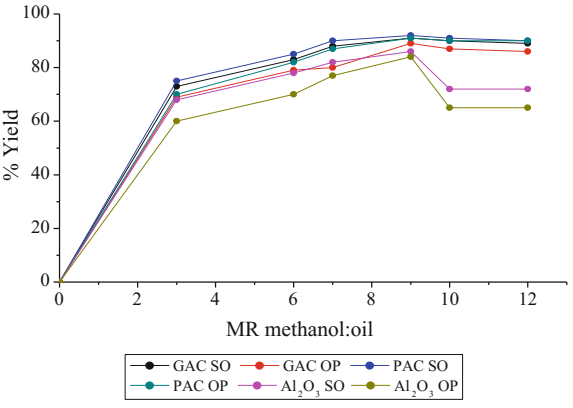


Fig. 16 Effect of molar ratio (MR) of methanol:oil in the basic heterogeneous transesterification process, at 60 °C for 90 min, with 0.7% KOH and 100 rpm agitation. Results for crude palm oil (OP) and soybean oil (SO) using different catalysts

performance, which makes the amount of biodiesel decrease; this is because the high dissolution of oil in alcohol and the high percentage of water content in the alcohol that reduce the percentage FAMES (Kim et al. 2004).

As the transesterification reaction is a balance where an excess of alcohol is required or by separation of any of the products from the reaction mixture, to handle the reaction to the right. However, a high proportion of alcohol:oil interferes in the separation of glycerine, due to an increase in solubility. When glycerin remains in solution, it helps to drive equilibrium back to the left, lowering the yield of esters (Meher et al. 2006).

The literature reports in other studies, with residual sunflower oil, methanol to oil ratio of 6:1 as an optimum proportion corresponding to a conversion of 89.8%, similarly using waste rapeseed oil presents higher yields at an optimum oil ratio alcohol:oil of 7:1 (Yuan et al. 2008). Another study with frying oil reveals that the optimal conditions are given in molar ratio of alcohol:oil between 5:1 and 8:1, after which there is already a constant behavior (Leung and Guo 2006).

Effect of Catalysts

To determine the influence of the amount of catalyst on the yield of biodiesel, it was varied in the range from 1 to 5% by weight. The results obtained are shown in Fig. 17.

It is evident that when there is an insufficient amount of catalyst, biodiesel yields are low; for example, when the amount of catalyst in the reaction was 1%, the following yields were obtained: 78 and 70% for crude soybean oil and palm oil on ɤ-Al_2O_3; 83 and 79% when soybean oil and crude palm oil, respectively, on GAC, and 85 and 82% for SO and OP, respectively, when PAC is used. By increasing the

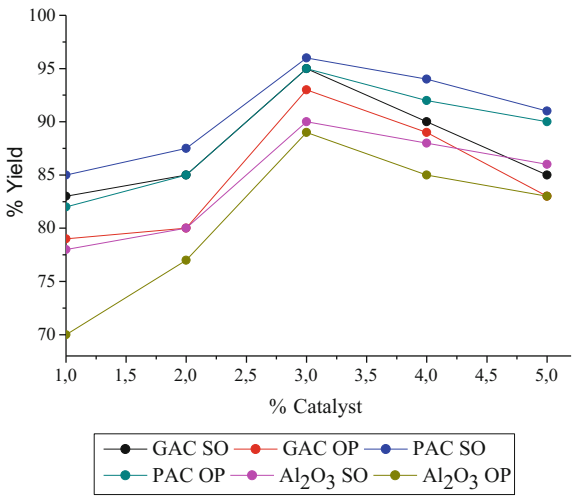


Fig. 17 Effect of catalyst on Transesterification, at 60 °C for 90 min, 100 rpm agitation and MR 9:1

catalyst amount up to 3% by weight, the biodiesel performance for all systems increased. Above 3%, the reagent mixture became too viscous, resulting in emulsification, promoting salt formation, and creating problems of mass transfer, thus decreasing biodiesel performance. By increasing the amount of catalyst in the transesterification, increases the amount of basic sites and the possibilities of contact with the reactants, increasing the yield of biodiesel. Comparing the % catalyst optimal with the literature, studies have shown that the maximum conversion (98%) using vegetable oil is 1% by weight of the alkaline catalyst concentration and then tends to constant values (Leung and Guo 2006).

Effect of Temperature

The rate of conversion to product in the transesterification reaction is greatly influenced by temperature. Despite this, the reaction can be carried out within a wide temperature range as long as the reaction time is sufficient to achieve good performance (Banerjee and Chakraborty 2009).

Transesterification is present at different temperatures depending on the type of oil and alcohol; the results are shown in Fig. 18. As the temperature increases, the yield and reaction time increase until maximum conversion is reached, due to the higher energy state of the molecules, generating greater collisions and higher solubility of the reactants, increasing the rate of reaction (Xie and Peng 2006).

When exceeding the boiling point of methanol (65 °C), performance decreases, as it is vaporized to produce bubbles that limit the reaction between the catalyst–methanol–oil–biodiesel interface. The catalytic process has lower relative yields of 89 and 90% for OP and SO respectively, generated using the catalyst KOH/ɤ-Al_2O_3, which is associated with fewer active sites than the highest yields

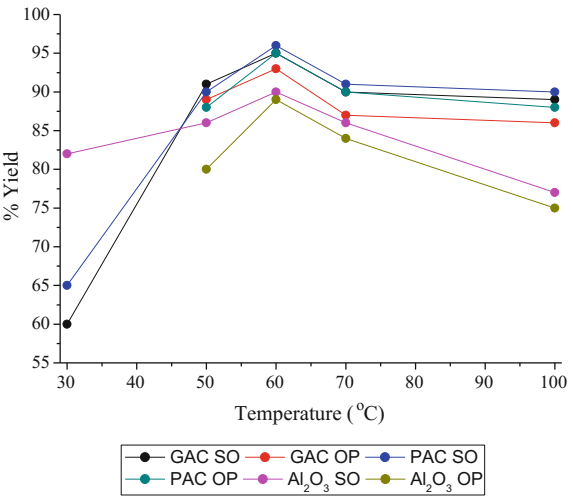


Fig. 18 Effect of temperature heterogeneous basic transesterification process, for 90 min, with a molar ratio methanol:oil of 9:1, with 3.0% KOH and 100 rpm agitation

for the catalyst KOH/PAC, generating yields of 95 and 96% using palm oil and soybean oil, respectively. The results are consistent if one considers that this catalyst (Table 5) has a greater surface area (Marchetti et al. 2007; Jagadale and Jugulkar 2012). Additionally, the result (65 °C) coincides with that reported in the literature, where the maximum yield of esters occurs in the temperature range 60–80 °C, at a molar ratio of alcohol:oil 6:1 with 1% catalyst in a time of 60 min (Cvengros and Cvengrosova 2004), but according to previous studies of optimum operating temperature, it can be concluded that the transesterification can be carried out at various ranges of temperature from room temperature to the boiling point of each alcohol used. For example, in transesterification studies of waste sunflower oil the reaction occurred efficiently at 55 °C, whereas waste rapeseed oil is used without changing the other conditions, the maximum yield occurs at 48.5 °C (Yuan et al. 2008).

No yields were recorded at 30 °C for cases where palm oil was used as it is still in the solid phase at temperatures below 40 °C.

When comparing the basic transesterification processes homogeneous catalysis and basic heterogeneous catalysis, and when using the same catalyst for both types of oil, homogeneous catalysis generates higher yields of biodiesel, but removal of the catalyst is technically difficult and is not necessary for heterogeneous catalysis. Here, the separation of catalysts (KOH/GAC, KOH/PAC, and KOH/ɤ-Al_2O_3) from the reaction products is easily achieved by using a filtering process. The catalyst has the advantage that it can be reused, although an additional cost is incurred by recovering their activity; additionally, when using heterogeneous catalysis in the transesterification, cleaner products (FAMES and glycerol) are obtained by simplifying the separation and purification steps, but heterogeneous catalysis for the synthesis of biodiesel requires more reagents and energy, increasing production costs. Additionally, presents diffusion processes as the reagents and the catalyst are in different state (Dossin et al. 2006).

4.2 Lipase

Lipase type II from porcine pancreas (Sigma-Aldrich; L3126) was used and the transesterification process was carried out with the free enzyme and the supported

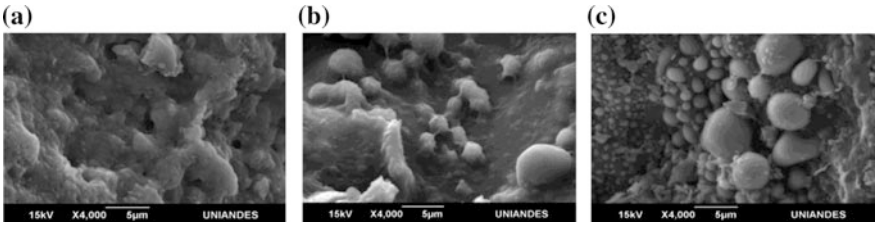


Fig. 19 Study of scanning electron microscopy. **a** ɤ-Al_2O_3-immobilized porcine lipase, **b** GAC-immobilized pig lipase and **c** PAC-immobilized porcine lipase

enzyme for this study: activated carbon, coal and pellet ɤ-Al_2O_3; as a solvent for the process, ethanol, methanol, isopropanol, and isobutanol were used, and palm oil and soybean oil were the raw materials.

In Fig. 19a–c, scanning electron photomicrographs are shown in which *Lipase type II from porcine pancreas* was adsorbed on the respective support (ɤ-Al_2O_3, GAC, and PAC), showing a greater amount of lipase supported on PAC. This is in good agreement with the results obtained in the textural characterisation of each type (Table 5), since PAC has a larger pore size, which allows the lipase to more easily reach the pores, and also enables greater interaction and thus a larger amount of supported lipase.

The optimal conditions for the free lipase were: 37 °C and pH 8.0. It is determined that the amount of protein absorbed (Pg) to GAC is 20 mg g^{-1}, to PAC is 18 mg g^{-1} and to ɤ-Al_2O_3 is 9 mg g^{-1}; i.e., GAC is supported more appropriately compared with PAC and ɤ-Al_2O_3, a result which was unexpected since PAC has a pore diameter that is greater than that of GAC and ɤ-Al_2O_3, which should facilitate its adsorption. This is due to the number of points on the surface hydrophobic interaction and the morphology of PAC (Diaz and Balkus 1996).

4.2.1 Effect of Time

Figure 20 shows that the yield of methyl esters increases as a function of time up to 360 min for all cases. At higher times, yields are constant; for that reason, 360 min was selected as the optimal time for transesterification. In transesterification with no catalyst and a set temperature, the time is important, since the process may be stopped at an intermediate step if the time is not optimal, preventing the complete conversion of triacylglycerides to methyl esters.

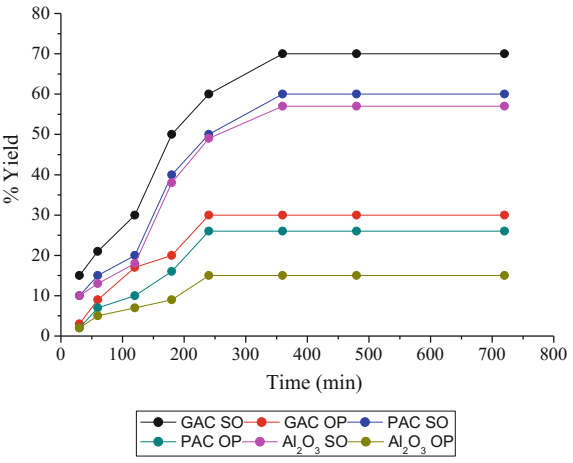


Fig. 20 Effect of time in the process of biocatalysis, with a molar ratio

4.2.2 Catalysts

Increasing the amount of lipase to 6% by weight of oil increases the amount of methyl esters; the yield for the corresponding systems is shown in Fig. 21. Further increases show no significant effect, because after the saturation point of the substrate is reached, the addition of more enzymes will not enable the substrate to serve as a reactant (Torres and Otero 2001). A relatively low enzyme concentration of 1–2% causes inhibition, and can result in low conversions (see Fig. 21).

4.2.3 Effect of Temperature

In this case, unlike homogeneous and heterogeneous catalysis, at temperatures below 60 °C, where the miscibility between oil and alcohol occurs, is quite low. As evidenced, an increase in the yield of methyl esters to a temperature of 37 °C occurs, which is then diminished appreciably because the optimum temperature of the enzyme is 37 °C; after this, the lipase begins to denature. It should be noted that at high temperatures, the system attempts to find a balance, as at low temperatures, again causing a decrease in the yield of methyl esters. Furthermore, the vibration and movement of the enzyme caused by high temperatures affects hydrogen bonds and other lipase bonds, altering the primary and tertiary structure. As shown in Fig. 22, in the case where palm oil (OP) is used, no results were obtained at temperatures below 40 °C as palm oil is still solid at this temperature.

It should be noted that although the optimal temperature is 37 °C, the temperature used in this study was 40 °C as the palm oil was liquid at this temperature and it was therefore possible to compare the two processes. When palm oil is used, lower yields were obtained than with soybean oil, which may be due to problems with mass transfer between the interfaces.

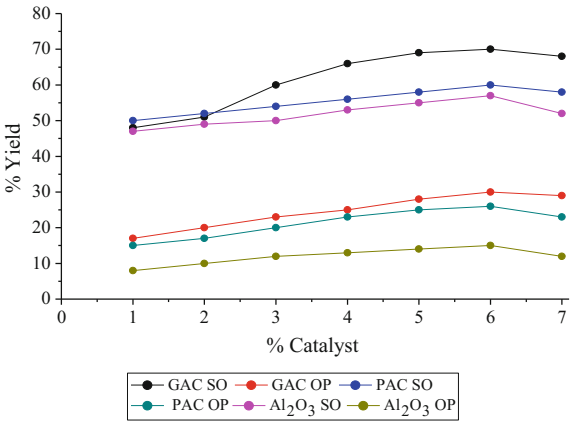


Fig. 21 Effect of the amount of enzyme in the process of biocatalysis, with a molar ratio of methanol:oil of 6:1, with 6.0% by weight of oil lipase, 300 rpm agitation at 40 °C and a 6 h reaction time

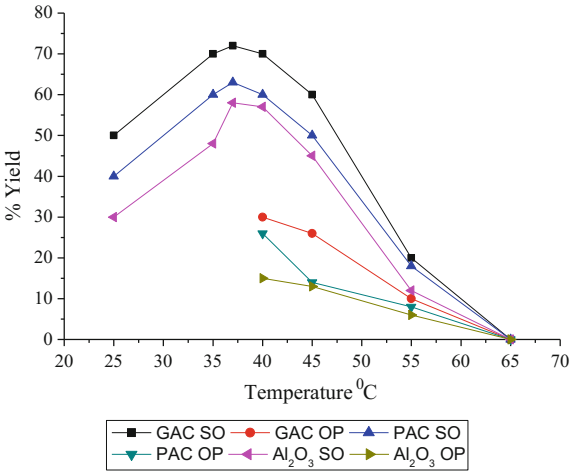


Fig. 22 Effect of temperature on the process of biocatalysis for 6 h, with a molar ratio of methanol:oil of 6:1, with 6.0% lipase by weight of oil and 300 rpm agitation

4.2.4 Oil:Alcohol Molar Ratio

The results of a study of this type are shown in Fig. 23. The highest yield was obtained at a molar ratio of 6:1, which then decreased due to the fact that excessive alcohol destabilizes the enzyme (Shimada et al. 2002), which sometimes hinders the interaction of the enzyme with the substrate (Kanasawud et al. 1992). Increased performance for GAC with soybean oil of 70% was obtained, while yields were lower when using palm oil due to the temperature of this study (40 °C).

Lipases as biocatalysts have high-potential transesterification, but have the disadvantage that the amount of lipase adsorbed on the support is less than 50% the initial amount that was dissolved, meaning that a greater amount of spent lipase does not yet occur, leading to high costs when compared with other catalysts. The

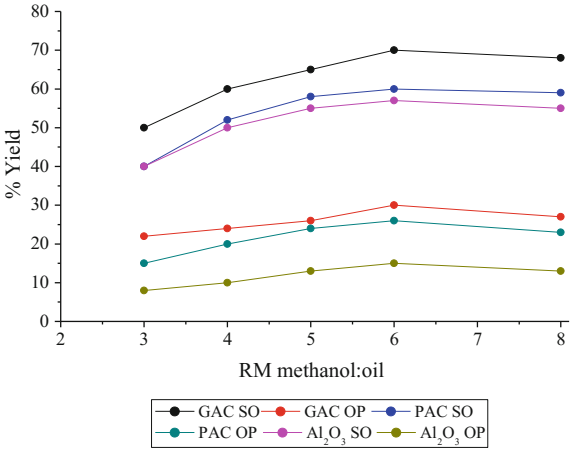


Fig. 23 Effect of methanol: oil molar ratio, the transesterification process for biocatalysis, at 40 °C, for 6 h, with 6.0% lipase and 300 rpm agitation

ability to select catalysts for the transesterification allows the products obtained to be pure and saves costs in purification and washing. Comparing the homogeneous and heterogeneous basic catalysis, using KOH provides a method wherein pre-treatment of the oil is not required, since the catalyst is selective (lipase), it consumes less energy and causes less waste, making it environmentally more attractive.

To remedy the problems of the adsorbed amount of lipase, further studies are needed, where the conditions of the supports will be varied, providing the surface with greater affinity to the lipase; as a result, it will be fully immobilized and recovered and reused, reducing the costs associated with biodiesel production.

5 Conclusions

Higher yields in the homogeneous transesterification were obtained for 0.7% KOH, at 60 °C, 100 rpm and 90 min. The heterogeneous catalysts KOH/ɤ-Al_2O_3, KOH/GAC, and KOH/PAC can be used in the transesterification of crude palm oil and soybean oil, presenting high yields (greater than 85%) under optimum conditions of 3.0 wt% catalyst, a molar ratio of alcohol:oil of 9: 1, the temperature of 60 °C, at 100 rpm for 90 min.

The heterogeneous catalytic process generates lower yields of 89 and 90% for palm oil (OP) and soybean oil (SO), respectively. Therefore, KOH/ɤ-alumina should be used as a catalyst, with KOH/PAC having a higher performance of 95 and 96% for palm oil and soybean oil, respectively. Biodiesel yields are directly related to the surface area of the catalyst, with PAC having a greater area and thus also the highest yield of biodiesel of 95% for palm oil and 96% for soybean oil.

Biocatalysis is a method that generates significant returns, and as a selective method is a huge draw for the production of biodiesel. Optimal conditions for biocatalysis are: molar ratio of methanol:oil of 6:1, 6.0% lipase by weight of oil, a temperature of 40 °C and 300 rpm.

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