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An Overview of Enzyme-Catalyzed Reactions and Alternative Feedstock for Biodiesel Production

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1. Introduction

There is an increasing interest in biodiesel production because of the environmental and economic advantages of this biofuel over traditional fuels derived from non-renewable resources (Akoh et al., 2007). Biodiesel is a renewable, non-toxic and biodegradable fuel (Van Gerpen et al., 2004) defined as the mono alkyl esters of long chain fatty acids derived from vegetable or animal fats, for use in compression-ignition (diesel) engines (American Society for Testing and Material [ASTM], 1989). This fuel can be used pure or in a blend with petroleum-based diesel (Van Gerpen et al., 2004). Currently, large-scale biodiesel production has been done by means of alcoholysis reactions of fats and oils (Reaney et al., 2005). The choice of the suitable alcohol for this process will depend on factors like its cost, availability, toxicity, easy of recovering and recycling, and the amount of alcohol needed for the reaction. Commercially, biodiesel has been produced using methanol as alkyl donor due to advantages like easy recovering and low cost (Van Gerpen et al., 2004). Anhydrous ethanol has also a great potential for biodiesel production in Brazil, one of the largest world ethanol producers (Schuchardt et al., 1998).

Renewable sources from both vegetable (Reaney et al., 2005) and animal (Lee et al., 2002) origin can be used as raw materials for biodiesel production. Microalgal oils have also received much attention as substrate for esters formation (Miao & Wu, 2006). Many agro-industrial processing operations generate a significant amount of lipids as by-products, such as chicken fat, beef tallow and fish oil. This feedstock, usually applied in livestock feeding, is a viable resource to produce biodiesel, and this use is a way for adding value to this material (Feltes et al. 2009). The conversion of used cooking oils and restaurant grease into alkyl esters is another proposal of particular interest in the field of alternative fuels (Lee et al., 2002). The use of biomass as feedstock is an attractive alternative for biodiesel production in tropical countries such as Brazil. The agricultural and farming industries are activities of great importance in many parts of this country. Also, government policies and legislative action may rapidly increase biodiesel production in such region (Ferrari et al., 2005; Meneghetti et al., 2006).

Chemical catalysis is a well established process for biodiesel production (Bournay et al., 2005). The homogeneous alkali-catalyzed transesterification process has been extensively

applied to the large-scale synthesis of alkyl esters, especially due to the low cost of base catalysts and their efficiency even at low concentrations. The chemical reaction, however, has some disadvantages as it is energy-intensive, requires several separation/purification steps and generates significant amounts of wastewater to be treated. Additionally, high-temperature alkali catalysis accelerates the oil oxidation, which is a serious drawback when dealing with a feedstock rich in polyunsaturated fatty acids (Damstrup et al., 2006). Also, ester phase processing and waste disposal are great problems when dealing with alkali-catalyzed reactions (Hájek & Skopal, 2010, Van Gerpen et al., 2004).

Many investigations have been conducted for the synthesis of biodiesel mediated by lipases. The enzymatic approach for the modification of oils and fats takes advantage of the specificity of some lipases, leading to high purity products, therefore reducing post processing operations and costs. Due to the mild conditions under which the enzymatic reaction occurs, oil oxidation becomes a negligible problem and less energy is required. Moreover, wastes are minimized and by-products are more easily purified (Gunstone, 1999). It is worth mentioning that the alcohol used in the reaction can be inhibitory to some enzymes (Van Gerpen et al., 2004). Moreover, compared to classical chemical catalysts, the relatively low stability of enzymes in their native state as well as the high cost of commercial lipases are important drawbacks to the use of these biocatalysts in industrial processes (Villeneuve et al., 2000). For these reasons, much effort has been directed for reducing lipases costs therefore allowing the development of competitive enzymatic processes with potential for industrial application. The production of lipases from new sources (Rigo et al., 2010; Wolski et al., 2009), the development of techniques for lipases immobilization (Villeneuve et al., 2000) as well as to perform enzyme-catalyzed reactions in compressed or supercritical fluids, such as propane, *n*-butane or carbon dioxide have appeared as attractive alternatives for reaching these goals (Dalla Rosa et al., 2009; Oliveira et al., 2006).

In light of the increasing interest in the development of alternative energy sources, the aim of this section is to make a review of an enzymatic approach for biodiesel production, focusing on a sustainable process. A section of this chapter will be dedicated to the raw materials that have been used as feedstock for biodiesel production, especially in Brazil, outlining the use of agro-industrial residues. We will survey recent researches for the production of lipases with great potential for biodiesel synthesis. The enzyme-catalyzed production of this biofuel in compressed or supercritical fluids will also be reviewed.

2. Biodiesel as fuel

Recently, there are several concerns about the dependence on foreign-controlled fuels sources of finite supply, reserves of petroleum shortages and rising prices of petroleum-based fuels. These problems have encouraged the development of alternative fuels, obtained especially from renewable sources (Reaney et al., 2005) like fats and oils. Oils can be used as liquid fuel for combustion in compression-ignition (diesel) engines directly or in a blend with other fuels. This use, however, has many problems (Rathore & Madras, 2007), that can be overcome by means of pyrolysis, micro-emulsification or transesterification in order to modify raw fats or oils. Transesterification is by far the most used process for oil modification, yielding fatty acid alkyl esters, the so-called biodiesel (Cerveró et al., 2008). This biofuel has a viscosity similar to that of the petrodiesel, as the oil viscosity decreases as transesterification reaction proceeds, as verified for alkyl esters of refined soybean oil (Costa Neto et al., 2004).

Biodiesel possesses several technical and environmental advantages over the conventional diesel. Besides being renewable and of domestic origin, advantages of biodiesel compared to petrodiesel include biodegradability, higher flash point, reduction of most regulated exhaust emissions, miscibility in all ratios with petrodiesel, compatibility with the existing fuel distribution infrastructure, and inherent lubricity (Knothe, 2008; Moser, 2009). Ferrari et al. (2005) produced ethyl esters from neutral soybean oil that were further mixed with petroleum-based diesel and tested as fuel in an energy generator. The diesel oil consumption ($0.6981L.h^{-1}$) was reduced when this fuel was mixed with up to 20% of biodiesel, when the fuel consumption was $0.6875L.h^{-1}$. Technical problems with biodiesel include oxidative stability, cold flow, and increased NOx exhaust emissions (Knothe, 2008). Solutions to some of the aforementioned problems have included the use of additives, the modification of the fatty acid composition of the feedstock, or mixtures of components with advantageous properties (Knothe, 2008).

In Brazil, the Law 11.097/2005 introduced biodiesel in the energy sources to be used in this country (Agência Nacional do Petróleo, Gás Natural e Biocombustíveis, 2005). Since January 2010, with the National Biodiesel Program, the blend of 5% of biodiesel has been mandatory, according to the Governmental regulation 6/2009 (Conselho Nacional de Política Energética, 2009). The biodiesel produced in Brazil in 2010 was about 2.4 million m^3 in 67 facilities whose total capability was $16,414.51m^3.day^{-1}$ (Agência Nacional do Petróleo, Gás Natural e Biocombustíveis, 2011).

3. Interesterification reactions

Despite of some divergences between authors, acidolysis, alcoholysis (including glycerolysis), and interesterification, all fall under the heading transesterification. Interesterification or ester-ester interchange is the exchange of acyl groups between two esters, namely, two triacylglycerols. Acidolysis is the transfer of an acyl group between an acid and an ester. Alcoholysis is the process of exchanging acyl groups between an ester and an alcohol (Akoh et al., 2007). The most common method now in use for biodiesel production is reversible transesterification reactions in which the oil or fat is reacted with a monohydric alcohol in the presence of a catalyst (Figure 1), where the raw glycerol (glycerin) is obtained as co-product (Knothe & Dunn, 2005).
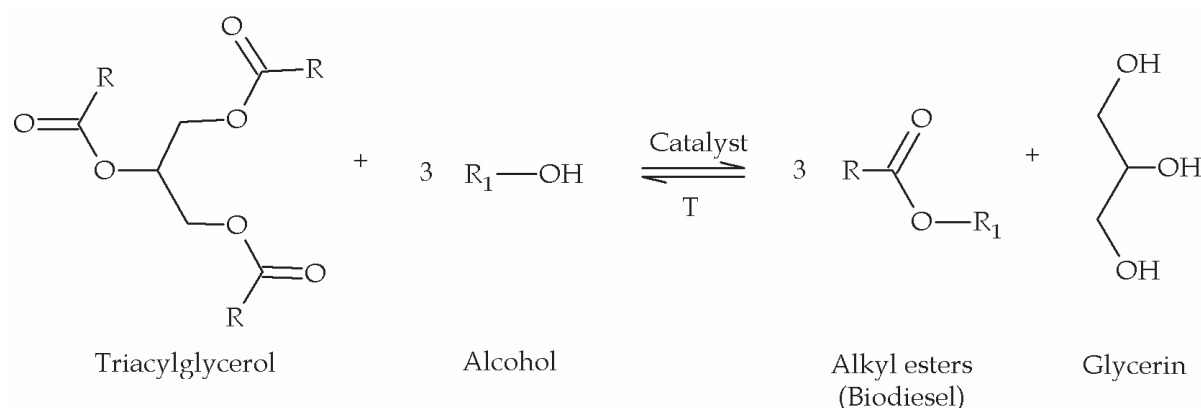
R, R_1: alkyl chain with different lenghts and/or saturation degrees

Fig. 1. Transesterification reaction of a triacylglycerol with an alcohol, for example, methanol or ethanol, as acyl acceptor (alcoholysis).

The glycerin obtained at large amounts and with a low cost in biodiesel production (Yazdani & Gonzalez, 2007) can be used as substrate for the synthesis of monoacylglycerols for application in the food industry, as suggested by Freitas et al. (2009). One has to consider, however, additional steps for products purification should be done when glycerin is obtained from the chemical-catalyzed biodiesel process, in order to assure a high-quality ingredient to human consumption (Hájek & Skopal, 2010).

Alternatively, as shown in Figure 2, methyl acetate can be used as acyl acceptor, where triacetyn, a valuable biodiesel additive, is produced as side product rather than glycerin, which has lower commercial value (Tan et al., 2010). Additionally, alkyl esters can be produced by means of direct esterification of fatty acids with alcohols (Figure 3). In this reaction, water must be removed to increase the yield, as the reverse reaction is the hydrolysis. Direct esterification is possible but not common for biodiesel preparation (Akoh et al., 2007).

Triacylglycerol   Methyl acetate   Methyl esters   Triacetylglycerin
                                                 (Biodiesel)

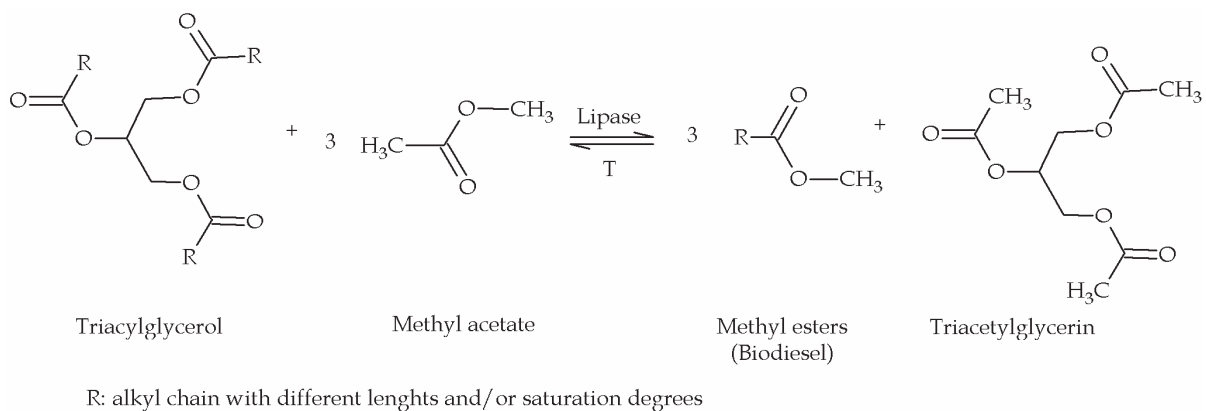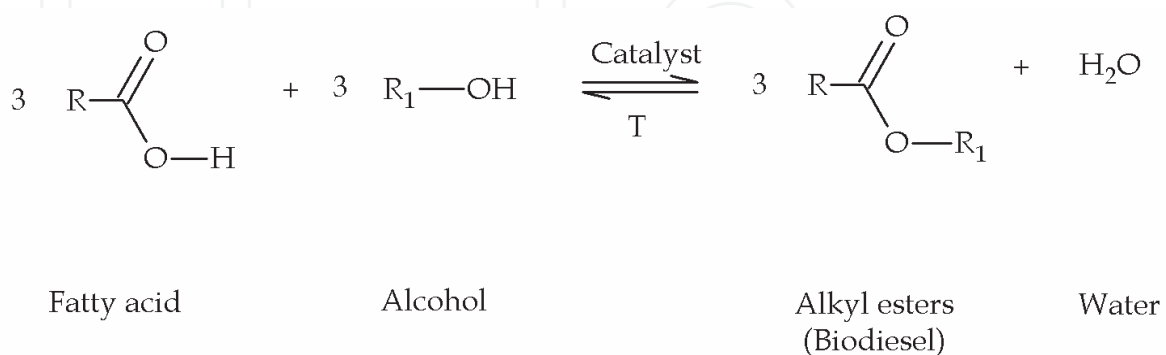R: alkyl chain with different lenghts and/or saturation degrees

Fig. 2. Transesterification reaction of a triacylglycerol with methyl acetate as acyl acceptor (interesterification or ester-ester interchange) (Adapted from Du et al., 2004).

Since reactions are reversible, it is advantageous to try to shift the equilibrium toward the synthesis, by using an excess of one of the substrates or continuously removing water eventually formed during the reaction (Villeneuve, 2007).

Fatty acid   Alcohol   Alkyl esters   Water
                                                (Biodiesel)

R, R1: alkyl chain with different lenghts and/or saturation degrees

Fig. 3. Transesterification reaction of a fatty acid with an alcohol as acyl acceptor (direct esterification). The reverse reaction is the hydrolysis.

3.1 Chemical versus lipase-catalyzed reactions

Currently, alcoholysis reaction by means of chemical catalysis is the most used method for the industrial production of biodiesel, especially because of the low cost of the catalysts, and high conversions reached in short time (Van Gerpen et al., 2004; Vicente et al., 1998). Many processes used in the oleochemical industry, however, are energy-intensive, requiring high temperatures (often above 200°C) and pressures. The resulting products generally need to be redistilled to remove impurities formed via thermal degradation. Moreover, highly unsaturated oils are thermally labile (Sheldon, 1996). Additionally, free fatty acids and water from the alcohol or the oil reduce ester yields, as they lead to the formation of soap and further free fatty acids, respectively (Fukuda et al., 2001). In the methanolysis of refined sunflower oil catalyzed by sodium hydroxide, for example, high temperatures (above 60°C) and catalyst concentrations (above 1.5%) led to the production of large amounts of soap (Vicente et al., 1998).

The development of green processes for biodiesel production has received much attention, involving the use of heterogenous catalysts, either chemical (Bournay et al., 2005; Boey et al., 2011) or enzymatic (Adamczak et al., 2009). In this sense, enzymatic processes can provide significant advantages over chemical process for biodiesel production. The mild conditions (pH, temperature and pressure), usually applied in the biocatalyzed reaction, allow energy saving and product quality improvement, because of the minimal thermal degradation of the substrates. Free fatty acids contained in waste oils and fats can be completely converted to alkyl esters, thus making their previous removal unnecessary (Van Gerpen et al., 2004).

In lipase-catalyzed reactions, glycerin can easily be recovered with simple separation processes (Cerveró et al., 2008). Moreover, the purification of fatty acid alkyl esters is simple to accomplish (Fukuda et al., 2001). These characteristics allow the reduction of residues treatment costs and of environmental concerns. Some disadvantages related to lipase-catalyzed reactions for biodiesel production include high cost (Rousseau & Marangoni, 2002), inactivation by acyl acceptors such as methanol depending on the enzyme, inactivation by minor components in the crude oil and waste oils, desorption from immobilization carrier, and fouling in packed bed bioreactors, as discussed by Akoh et al. (2007).

It should be emphasized that enzyme-catalyzed transesterification is a very good option to all chemical-catalyzed reactions; however, it must be developed for its industrial application for esters formation (Kulkarni & Dalai, 2006). In order to use lipases more economically and efficiently in aqueous and non-aqueous solvents, techniques for their modification, to increase their activity, selectivity or stability and to improve their solubility in organic solvents, have been the subject of increased interest (Villeneuve et al., 2007).

4. Lipases as catalysts

Lipases (triacylglycerol lipases, E.C. 3.1.1.3) are carboxylic ester hydrolases with catalytic activity not only in an aqueous solution, but also in nonaqueous solvents (Rousseau and Marangoni 2002). The amount of water present in the reaction mixture influences biocatalysis in several ways. Often optimal water content can be found; further water addition will cause decreased enzyme activity. Water probably activates the enzyme by increasing the internal flexibility of the catalyst molecule. However, water can also act as a substrate in the enzymatic reaction, especially in the reactions of hydrolytic enzymes. This results in side-reactions and lower product yields (Adlercreutz, 1996). The best way to characterize the degree of hydration of a biocatalytic system in organic media is to use the thermodynamic water activity (a_w) as the parameter (Adlercreutz, 1996; Halling, 2002).

The optimum water content to the highest enzyme activity depends on the catalyst. The best results for the majority of lipases preparations are with a_w values ranging between 0.25 and 0.45, which corresponds to an water content from 0.5% to 1%. The optimal water content, however, can vary up to values as high as 11% (w/v) (Villeneuve, 2007).

4.1 New sources of lipases

Enzymes are produced by microorganisms, plants and animals. Purified enzymes, though expensive, are easy to apply for biotransformation in organic media and their use is increasing. Barriers to the production of industrial enzymes include economic factor, the availability of optimal enzymes and safety issues. The production and purification processes differ for extracellular and intracellular enzymes, liquid and solid culture in the case of fermentation, and enzyme application. Liquid fermentation is useful for the production of both intracellular and extracellular enzymes, especially from bacteria and yeast. It is good for scale-up and reproduction. The use of *Escherichia coli* genetically modified is very popular for the production of the enzyme inside the cell. Solid fermentation is also used to produce many kinds of enzymes mainly from fungal species (Hirose, 2002).

Solid state fermentation could be a perfect technology for adding value for low-cost agro-industrial residues aiming the production of enzymes, and for helping reducing pollution problems (Soccol & Vandenberghe, 2003). Salum et al. (2010) produced a lipase from *Burkholderia cepacia* LTEB11 (234 units of hydrolyzing activity U against *p*-nitrophenyl palmitate per gram of dry solids) by solid-state fermentation of a low-cost agro-industrial substrate (sugarcane bagasse and sunflower seed meal). Ramani et al. (2010) used beef tallow, a slaughter house waste, as substrate for lipase production by *Pseudomonas gessardii*. This enzyme showed high hydrolytic activity towards oils from different origin. Nelofer et al. (2010) reported the optimization of the production of a thermostable and organic solvent tolerant lipase by submerged culture of a recombinant *Escherichia coli* BL21. The lipase activity was 50.2 $U.mL^{-1}$ against an olive oil emulsion.

Griebeler et al. (2009) carried out the solid state fermentation with soybean bran of different microorganisms isolated from agro-industrial sources. They reported the most promising fungi with hydrolytic activity against tributyrin belonged to *Penicillium* and *Aspergillus* genera. It is worth mentioning Zanotto et al. (2009) pointed out triacylglycerols such as triolein or tributyrin are suitable substrates for the determination of lipase and esterase activity, respectively. Wolski et al. (2009) reported the partial characterization of enzymatic extracts produced by a newly isolated *Penicillium* sp. in submerged and solid state fermentation with, respectively, 15.17$U.mL^{-1}$ and 11.28$U.mL^{-1}$, by using an olive oil emulsion. In the solid state fermentation, soybean bran was used as a low-cost substrate. Zanotto et al. (2009) were able to isolate three fungi from plants of the Amazon region with a great potential as providers of mycelium-bound lipases for use in biotransformations (good hydrolysis activity, and good esterification and transesterification capacity in organic media). Chen et al. (2009) attained 91.08% of fatty acid methyl esters in the alcoholysis of an waste cooking oil catalyzed by the lipase derived from *Candida* sp. 99-125, previously identified from sewage water in north China. The enzyme was immobilized on a carrier textile cloth by using co-fixing agents (polyethylene glycerol, tween and span, gelatine and lecithin).

Vargas et al. (2008) studied the lipase production by *Penicillium simplicissimum* isolated from the babassu oil industry by using soybean meal as substrate. The enzyme activity was

30U.g^{1} dry substrate in the reaction with an olive oil emulsion. Volpato et al. (2008) reported the use of the low-cost glycerin obtained as co-product in biodiesel production as an alternative feedstock for the production of an organic solvent tolerant lipase by submerged culture of *Staphylococcus caseolyticus* EX17 (activity of 127.3U.L^{-1} against *p*-nitrophenyl palmitate). Castilho et al. (2000) performed an economic analysis of the production of *Penicillium restrictum* lipase in submerged fermentation as well as by solid-state fermentation. The latter process turned out to be very attractive from an economic point of view, especially because of the extremely cheap raw material used as main substrate (babassu cake and olive oil).

The use of intracellular lipase as a whole cell biocatalyst immobilized within biomass carrier particles is significantly advantageous since immobilization can be achieved spontaneously during batch cultivation, and in addition, no purification is necessary (Fukuda et al., 2001). For these reasons, Robles-Medina et al. (2009) argued that the use of the whole cell biocatalyst can reduce both the cost of the biocatalyst and dependence on lipase manufacturers. For more details about microbial lipase production, the reader is referred to a recent review on this subject (Treichel et al., 2010).

According to Foglia & Villeneuve (1997), the high cost often associated with an enzymatic process can be overcome by the utilization of plant enzymes, which take the advantage of the lower cost, when compared to microbial lipases, and ready availability. These authors described high activity in synthesis reactions with *Carica papaya* latex lipase. Cambon et al. (2009) verified the lipase from the unripe fruit of the babaco plant (*Vasconcellea heilbornii*; ex. *Carica pentagona*, activity of 280IU.g^{-1} against olive oil), was able to catalyse the alcoholysis reaction of sunflower oil with alcohols varying in chains length.

5. Substrates and reaction media

The following sections are dedicated to an overview of sources of alcohol, fats and oils that have been used for biodiesel production, focusing on lipase-catalyzed reactions.

5.1 Sources of alcohol

For economic reasons, methanol is most commonly used in transesterification reactions to the commercial production of biodiesel, although other alcohols can also be applied as alkyl donors (Moser, 2009), such as ethanol, propanol, and butanol (Knothe & Dunn, 2005). In general, improved conversion is achieved by adding extra alcohol (Reaney et al., 2005). It is known that largest conversions to methyl esters, for example, are obtained for a methanol to triacylglycerols molar ratio of 6:1 (Vicente et al., 1998). Methanol in excess, however, may lead to lipase inactivation. According to Soumanou & Bornscheuer (2003), particularly with water-miscible alcohol substrates such as methanol, water required to maintain lipase structure can be stripped leading to lower activity and eventual biocatalyst inactivation. This problem can be overcome, for example, by using methyl acetate as acyl acceptor, as already verified in the transformation of soybean oil for biodiesel production catalyzed by the commercial lipase Novozym 435 from *Candida antarctica* (Du et al., 2004). Alternatively, a method of adding methanol stepwise was successful for avoiding the enzyme inactivation in the methanolysis of degummed soybean oil catalyzed by the immobilized *Candida antarctica* lipase (Watanabe et al., 2002). Another possibility to prevent methanol inactivation during lipase-catalyzed reactions is the addition of *tert*-butanol (Akoh et al., 2007).

Rodrigues et al. (2008b) presented the alcoholysis of different vegetable oils (soybean, sunflower and rice bran) catalyzed by three immobilized commercial lipases. Novozym 435 from *Candida antarctica*, Lipozyme TL-IM from *Thermomyces lanuginosus* and Lipozyme RM-IM from *Rhizomucor miehei* presented higher activity in methanolysis (with an alcohol:oil molar ratio of 5:1), ethanolysis (7:1) and butanolysis (9:1), respectively, in the range of 30–35°C. The assessment of enzyme stability over repeated batches was carried out by washing the immobilized enzymes with different solvents. When washing with *n*-hexane, approximately 90% of the enzyme activity remained after seven synthesis cycles.

Higher molecular weight alcohols have lower polarity and therefore their separation from alkyl esters becomes more difficult after reaction completion, which can be overcome by mixing higher alcohols with methanol, glycerol or water (Reaney et al., 2005). Ethanol has a great potential for use in biodiesel production in countries like Brazil, where it is a cheap and abundant commodity produced from the fermentation of sucrose from sugarcane (Rodrigues et al., 2008b). In 2009, Brazil produced 26.10 million m³ of ethanol, whose major producer was by far São Paulo State (58% of the total production) (Agência Nacional do Petróleo, Gás Natural e Biocombustíveis, 2009). In spite of its renewable origin, the ethanolysis reaction has some drawbacks to the industrial production of biodiesel. The yield of fatty acid ethyl esters is less compared to methyl esters and separation of glycerol is the main constrain in the process of ethanolysis (Meher et al., 2006).

Propanol or butanol can also be used in alcoholysis reactions, especially because these two alcohols promote a better miscibility between the alcohol and the oil phases (Iso et al., 2001). Soumanou & Bornscheuer (2003) were able to produce fatty acid alkyl esters from various vegetable oils (cottonseed, peanut, sunflower, palm olein, coconut and palm kernel) by transesterification with different alcohols (methanol, ethanol, 1-propanol, 1-butanol and isobutanol) using commercial immobilized lipases (AK lipase from *Pseudomonas fluorescens*, RM lipase from *Rhizomucor miehei*, and lipase from *Thermomyces lanuginosa*). Using *n*-hexane as organic solvent, all immobilized lipases tested were found to be active during methanolysis. Highest conversion (97%) was observed with *Thermomyces lanuginosa* lipase after 24h.

5.2 Sources of fats and oils

Along with vegetable oils, animal fats and oils also have a great potential to be used as substrate for biodiesel production. The choice of the source depends largely on the country and its climate (Knothe & Dunn, 2005), and also on economics (Rodrigues et al., 2008b). A wide variety of feedstock is expected to be used in Brazil for biodiesel production. Currently, the main resource for biodiesel production in this country is soybean oil (*Glycine max*), comprising about 80% of total feedstock, followed by tallow, cottonseed oil and other sources such as chicken fat, pork fat, waste cooking oil, oilseed radish (*Raphanus sativus* L.) (Agência Nacional do Petróleo, Gás Natural e Biocombustíveis, 2011).

The use of biodiesel has not expanded in developing countries mostly due to the high production cost which is associated with the expensive high-quality virgin oil feedstocks (Hoque et al., 2010). The cost of the raw materials currently represents about 70% of the total production costs (Robles-Medina et al., 2009).

Fats and oils of differing origin have different fatty acid compositions (Table 1). The properties of fats and oils, and hence their commercial value, depend on the fatty acid composition and of the triacylglycerol structure (Sheldon, 1996). For example, a high content of saturated fatty acids in the raw feedstock (i.e. myristic, palmitic, stearic acids) rises

turbidity and the cetane number, decreases NOx emissions, and shows better stability. Furthermore, a high ratio of polyunsaturated fatty acids (i.e. linoleic or linolenic acids) has the opposite effect (Cerveró et al., 2008). Knothe & Dunn (2005) discussed in detail the influence of fats and oils compositions on biodiesel properties.

One of the main criteria for the quality of biodiesel is the storage stability which, in turn, depends on the fatty acid composition of the feedstock. Vegetable oil derivatives especially tend to deteriorate owing to hydrolytic and oxidative reactions. Their degree of unsaturation makes them susceptible to thermal and/or oxidative polymerization, which may lead to the formation of insoluble products that cause problems within the fuel system, especially in the injection pump (Meher et al., 2006). Santos et al. (2010) outlined the high amount of unsaturated fatty acids found in fish oil may result in higher oxidation problems. It is also a critical parameter when dealing with phytoplankters and seaweeds rich in long-chain polyunsaturated fatty acids. Autooxidation of unsaturated oils can be limitated by addition of suitable antioxidants (Lands, 2005).

Fatty acid	Soybean oil[a]	Cotton seed oil[b]	Beef tallow[b]	Chicken fat[b]
C12:0, lauric			1	
C14:0, myristic		1	4	1
C16:0, palmitic	11	23	26	25
C16:1, palmitoleic		1	4	8
C18:0, stearic	4	2	20	6
C18:1, oleic	25	17	28	41
C18:2, linoleic	53	56	3	18
C18:3, linolenic	7			1
Other			14	

[a] From Liu (2004); [b] From Gunstone & Harwood (2007).

Table 1. Typical fatty acid composition (wt%) found in vegetable and animal oils and fats that may be used for biodiesel production.

Knothe & Dunn (2005) outlined biodiesel is susceptible to start-up and operability problems when subjected to cold temperatures. Biodiesel made from feedstock containing larger amounts of high-melting point long-chain saturated fatty acids tends to have relatively poor cold flow properties. This drawback can be overcome by the transesterification of triacylglycerols with medium chain-length (C3-C8) or branched-chain alkyl alcohols.

Despite of the economic, environmental and social advantages of biodiesel as an energy source, there is concern about the utilization of edible oils as substrates for its production. Taking it into consideration, non-edible and waste oils have been investigated as starting material (Robles-Medina et al., 2009).

5.2.1 Vegetable fats and oils

Traditional oilseed feedstocks for biodiesel production predominantly include soybean, rapeseed/canola, palm, corn, sunflower, cottonseed, peanut, and coconut oils (Knothe & Dunn, 2005; Moser, 2009).

According to Liu (2004), the range oil content in soybean is 12-30% (dry weight; typical oil content 20%), whose major components are triacylglycerols. There is a large genetic

variation in fatty acid composition of soybean oil, mainly resulting from plant breeding. The range of fatty composition (% relative to total oil) among soybean germplasm is palmitic acid, 4-23%; stearic acid, 3-30%; oleic acid, 25-86%; linoleic acid, 25-60%; linolenic acid, 1-15%. This oil contains minor components (phospholipids, unsaponifiable material, free fatty acids, and trace metals) that are reduced after oil refinement. These substances affect lipase activity, as verified in the methanolysis of unrefined soybean oil, containing phospholipids, catalyzed by the immobilized *Candida antarctica* lipase (Watanabe et al., 2002).

Palm-base alkyl esters are potential substitutes for diesel fuel, as already investigated by Dors (2011). Palm trees are an attractive alternative feedstock, especially because of their great productivity (610 gallons of oil per acre) (Kurki et al., 2010). These plants produce two types of oils, palm oil from the fibrous mesocarp and lauric oil from the palm kernel. The former contains mainly palmitic (44.0%) and oleic acids (39.2%), while the latter is rich in lauric (48.3%), myristic (15.6%) and oleic acids (15.1%) (Teoh, 2002). The world´s two largest producing nations are Indonesia and Malaysia (United States Department of Agriculture, [USDA], 2011). Batistella et al. (2002) proposed an approach to recover carotenoids, valuable pigments for the food industry, and biodiesel from palm oil by means of suitable techniques. *Jatropha curcas* oil, a non-edible vegetable oil, has been considered as a potential alternative feedstock for biodiesel production. *Jatropha curcas* is a large shrub or tree native to the American tropics but commonly found and utilized throughout most of the tropical and subtropical regions of the world. It is a hardness plant with rapid growth, easy propagation and wide ranging usefulness. The oil content of jatropha seed ranges from 30 to 50% by weight and the kernel itself ranges from 45 to 60%. The major fatty acids found in jatropha oil are the oleic and linoleic acids. The seeds and oil are toxic due to the presence of cursive and curcasive (Pramanik, 2003). The properties of this oil make it suitable for alkyl esters production, as they have lower viscosity than the starting material. Berchmans & Hirata (2008) produced biodiesel from crude *Jatropha curcas* L. seed with a high content of free fatty acids (15%) by means of a two-step chemical reaction.

Castor oil is a unique commercial resource containing ricinoleic acid (12-hydroxy-9-octadecene acid; C18:1, OH) with a major share of up to about 90%. Castor oil is obtained from the seeds of *Ricinus communis*, with a yield of about 50%. It is non-edible, virtually insoluble in water, chemically stable and fully biodegradable. It is widely used in oleochemical applications, mainly in special productions. The countries of production are primarily India and Brazil, where the plant grows native with high hectare yields. Cvengros et al. (2006) suggested a dilution of castor oil-based methyl and ethyl esters with esters based on oils/fats without -OH group, or a blending with conventional diesel fuel and addition of a cetane enhancer, as alternatives for reaching EN 14214 standards. Oliveira et al. (2004a, 2004b) investigated the lipase-catalyzed transesterification of castor oil to produce biodiesel.

An alternative feedstock for biodiesel production is the non-edible oil extracted from babassu (*Orbinya martiana*), a palm tree available in great amount in tropical forests. Their coconuts contain in average 7 wt% of almonds with 62 wt% of oil rich in lauric acid. The possibility of the whole usage of the coconut for other purposes makes babassu a potential source for biodiesel production (Freitas et al., 2009). Rubber tree (*Hevea brasiliensis*) is another potential feedstock for biodiesel production (Ramadhas et al., 2005). Rubber seed kernels (50–60% of seed) contain 40–50% of brown color oil, consisting of 18.9% saturated (palmitic and stearic acids) and 80.5% unsaturated fatty acids (oleic, linoleic and linolenic acids). It may contain up to 17% free fatty acids.

5.2.2 Animal fats and oils

Although animal fat such as beef tallow is considered an industrial residue, and not a renewable source of energy, if compared to oleaginous seeds, there is an increasing interest in its use as feedstock for biodiesel production. Additionally, the use of animal fat from residues as starting material for alkyl esters production plays an important role in the pollution reduction (Krause, 2008). According to Lee et al. (2002), lard is an inexpensive co-product of the meat-packing industry that could be further converted to value-added products such as biodiesel. However, the relatively high contents of saturated fatty acids in lard result in the poor cold temperature properties of its alkyl esters. To improve cold temperature properties, the content of saturated fatty acid in lard could be reduced through a fractionation process before esterification. As a consequence of this procedure, biodiesel from fractionated lard resists forming crystals at low temperatures, thus improving cold-temperature properties.

Chicken fat obtained from processing by-products is a promising alternative feedstock for biodiesel production because of its great availability and low cost (Feltes et al., 2009). Brazil is the third world´s largest producer of chicken meat and the first largest exporter of chicken meat, whose total production was 10.98 million tonnes in 2010 (Associação Brasileira dos Exportadores de Frango, 2011). Boye et al. (2011) studied the methanolysis of low free fatty acids chicken fat by using shells as catalysts. Krause (2008) described the economic feasibility of biodiesel production from animal fats (beef tallow and chicken fat), by using the alkaline methanolic route. The same research group reported the alkali-catalyzed methanolysis of biodiesel from beef tallow in a pilot plant (Cunha et al., 2009). Hoque et al. (2010) demonstrated a high potential of producing economically viable biodiesel from low cost feedstock (beef fat, chicken fat and used cooking oil) with proper optimization of the process parameters.

According to Food and Agriculture Organization of the United Nations [FAO] (2010), capture fisheries and aquaculture supplied the world with about 142 million tonnes of fish in 2008, excluding aquatic plants. Of this, 27.2 million tonnes were used for non-food purposes. The dominant species in marine fishery catches were anchoveta (*Engraulis ringens*), Alaska Pollock (*Theragra chalcogramma*) and Atlantic herring (*Clupea harengus*). China remained by far the largest fish-producing country, with production of 47.5 million tonnes in 2008. Brazil was one of the major producer countries of inland capture fisheries, accounting for an estimated amount of 243,000 tonnes in 2008.

Total fish oil production by the five main exporting countries (Peru, Chile, Iceland, Norway and Denmark) in 2009 was 530,000 tonnes, a decline of 100,000 tonnes compared with 2008. Fish-oil prices reached US$950/tonne in March 2010, which was 50 percent higher than an year earlier. For fish oil, almost 85 percent of production was used as an ingredient in fish and shrimp feeds (FAO, 2010). This oil could be applied as substrate for the production of biodiesel (Feltes et al., 2010).

In 2009, the total fisheries production in Brazil was more than 1.24 million tonnes. The Northeast region was the major national producer (34%), but the state of Santa Catarina, at the South, was the largest producer (more than 199,000 tonnes). The sardine (*Sardinella brasiliensis*) was the main fish resource caught in this country (more than 83,000 tonnes) in the aforementioned year (Ministério da Pesca e Agricultura, 2010). According to Feltes (2006), the oil extracted from sardine (*Sardinella brasiliensis*) viscera and heads by the Bligh & Dyer method, contains 5.43% of lipids (wet weight), with 12.1% of EPA and 16.5% of DHA. These residues are therefore an alternative substrate for the production of biodiesel in this region.

Santos et al. (2010) investigated the chemical production of biodiesel from oil extracted from the viscera of *Oreochromis niloticus*, containing 15.9%, 12.1%, 18.1% and 15.8% of palmitic, oleic, eicosapentaenoic and docosahexaenoic acids, respectively. These authors converted the Nile tilapia oil into free fatty acids, further esterified with methanol by using sulphuric acid as catalyst in ultrasonic system. Chiou et al. (2008) showed that despite the differences in the fatty acid profile, waste Alaska pink salmon (*Oncorhynchus gorbuscha*) oil and corn oil methyl esters had comparable physical properties (viscosity, volatility, low temperature properties, oxidative stability, heating value, acid value, and specific gravity), and could be used as automotive fuel.

5.2.3 Waste fats and oils

Low-cost sources such as restaurant greases (Lee et al., 2002) and soapstock can be used for biodiesel production. These sources, however, are of lower quality than refined vegetable oils, especially because of the high content of free fatty acids, what is a major problem when dealing with transesterification reactions catalyzed by alkali (Knothe & Dunn, 2005). Along with free fatty acids, restaurant grease contains other substances formed during cooking, especially deep-frying (Akoh & Min, 2008), that must be removed prior to alcoholysis in order to a high yield in alkaline-catalyzed reactions (Lee et al., 2002).

The use of vegetable oils as frying oils produces significant amounts of used oils which may present a disposal problem. Their use for biodiesel production has the advantage of their low price. Free fatty acids present in this feedstock can be a drawback when dealing with alkaline catalyzed-processes, but acid or enzymatic reactions can be a practical solution to the production of this biofuel (Knothe & Dunn, 2005). Besides free fatty acids, this starting material contains other degradation products as polymers, as revised by Kulkarni & Dalai (2006). These compounds can affect the catalyst activity, but they can be removed by means of simple filtration. Waste oils have higher amounts of saturated fatty acids, leading to less favourable cold flow properties (Knothe & Dunn, 2005).

Charoenchaitrakool & Thienmethangkoon (2011) investigated the optimum conditions in biodiesel production from waste frying oil using two-step catalyzed process (acid catalysis followed by alkali-catalysis). Although liquid mineral acids can be used to catalyze the modification of an oil with a high free fatty acids content, Silva et al. (2011) pointed out serious drawbacks, such as high corrosiveness, large effluent generation, the impossibility of catalyst reuse, compromise their application. Contrarily, solid acid catalysts appear to be an attractive option; however, the water present or generated during free fatty acids esterification causes the leaching and deactivation of these catalysts.

Coconut and palm kernel distillates containing a high content of free fatty acids (71.3 and 61.2%, w/w as lauric acid), rich in saturated fatty acids, especially lauric acid, a low content of unsaponifiable matter and neutral acylglycerols (Nandi et al., 2005) could be used as starting material for biodiesel production.

5.2.4 Aquatic plants

According to FAO (2010), procedures for the industrial preparation of biofuel not only from fish waste but also from seaweeds are being developed. Aquaculture produced 15.8 million tonnes (live weight equivalent) of aquatic plants in 2008.

Chile was the most important seaweed culturing country outside Asia, producing 21,700 tonnes in 2008, followed by Africa (14,700 tonnes). In 2008, the highest production of cultured seaweed was of Japanese kelp (*Laminaria japonica*, 4.8 million tonnes), followed by

Eucheuma seaweeds (*Kappaphycus alvarezii* and *Eucheuma* spp., 3.8 million tonnes), Wakame (*Undaria pinnatifida*, 1.8 million tonnes), *Gracilaria* spp. (1.4 million tonnes) and Nori (*Porphyra* spp., 1.4 million tonnes) (FAO, 2010).

The production of algae culture in freshwater was 68,400 tonnes in 2008, especially of *Spirulina* from China (62,300 tonnes) and Chile (6,000 tonnes) (FAO, 2010). Some microalgae produce substantial amounts of lipids (up to 70% w/w of biomass) with widely varying fatty acid composition in their biomass. The microalgae *Phaeodactylum triconutum*, for example, can produce 20% of oil (w/w) in biomass (5g lipids.m^{-2}.day^{-1}) (Robles-Medina et al., 2009). *Chlorella vulgaris* can produce 39% (w/w) of biomass rich in oleic acid (58% w/w of total) (Weber & Mukherjee, 2008). The latest development in freshwater algae culture is the development of lipid-rich species for biofuel production (FAO, 2010). The production cost of high grade algae oils, however, is still high, despite of the recent advances in photobioreactors, hindering the use of this feedstock in the short term, as outlined by Robles-Medina et al. (2009).

5.3 Reaction media

Several approaches have been made in order to optimize transesterification reactions for biodiesel production in batch systems (Table 2). Many studies are carried out in order to reduce enzymatic processes costs. In an attempt to produce biodiesel from used palm oil and ethanol using immobilized lipases in a solvent-free system, Tongboriboon et al. (2010) verified higher conversions could be achieved using the combination of Lipase AK (from *Pseudomonas fluorescens*) and Lipase AY (from *Candida rugosa*).

As can be seen in Table 2, several enzymatic reactions are performed in organic media. An organic solvent is used in order to dissolve the reactants, to enable interesterification rather than simple hydrolysis to occur, because excess water would cause the enzyme to desorb from its support (Cheetham, 1995), and also to improve the mass transfer during the reaction, increasing its rates. In all the solvent-containing biocatalytic systems, however, the nature of the solvent influences the activity and the stability of the enzyme to a large extent. The tendency of solvents to inactivate enzymes does not depend only on their hydrophobicity, expressed as their log *P* value (partition coefficient of the solvent in an octanol/water biphasic system), but also on other physicochemical characteristics of solvents, such as the solvating ability and molecular geometry (Adlercreutz, 1996).

Hydrophilic solvents with log *P* values < 2 such as *tert*-butanol often lead to enzyme deactivation if present in high concentrations; in contrast, apolar solvents with log *P* ≥ 4 (*n*-heptane) are compatible with enzymes, leaving the essential layer of water molecules on the polar surface regions unperturbed. The results using solvents with intermediate values of log *P* (2-4) such as *n*-hexane are unpredictable and depend very much on the individual case (Kula, 2002). Laane et al. (1987) indicated lipases show a good stability and activity in hydrophobic solvents with 2 < log *P* < 4. Enzymes generally remain active and will not be rapidly deactivated when using a solvent with log *P* > 3 (Villeneuve, 2007). The choice of the suitable solvent for an enzymatic reaction must to be made taking into consideration both enzyme stability and substrate solubility (Adlercreutz, 1996).

When using Novozym 435, a commercial immobilized lipase, as catalyst, *tert*-butanol proved to be a suitable solvent for assure substrates homogeneity in the continuous biodiesel production from palm oil. In the ethanolysis of palm oil in the same system, however, the lipase AK immobilized on polysiloxane-polyvinyl alcohol hybrid support was able to produce ethyl esters without the use of this tertiary alcohol. These finding is extremely interesting for process costs (Dors, 2011). For industrial-scale production, despite

of the benefits, the presence of organic solvents introduces problems like reduction of capacity (as the solvent takes up volume), environmental issues (toxicity, emissions) and costs (recovery and losses), as discussed by Nielsen et al. (2008).

Lipase	Oil or fat source	Acyl acceptor	Result	Conditions and additional information	Ref.
Lipozyme IM (Novo Nordisk) from *Rhizomucor miehei*, IM	Castor oil	Ethanol	99.5% yield	*n*-hexane; 6.5:1 ethanol to oil molar ratio; 6h; 200rpm; 50°C; 12.5% lipase (w/w based on oil and alcohol)	Oliveira et al. (2004a)
Lipozyme IM-77 (Novo Nordisk) from *Rhizomucor miehei*, IM	Soybean oil	Methanol	92.2% yield	*n*-hexane; 3.4:1(methanol to oil molar ratio); 6.3h; 200rpm; 36.5°C; 0.9BAUN* of lipase; added water 5.8wt% of oil	Shieh et al. (2003)
Lipozyme TL IM (Novozymes) from *Thermomyces lanuginosus*, IM	Soybean oil	Ethanol	96% yield	7.5:1 ethanol to oil molar ratio; 31.5°C; 5h; enzyme content, 15wt% (based on oil); added water, 4wt% (based on oil)	Rodrigues et al. (2008a)
Novozym 435 (Novozymes) from *Candida antarctica*, IM	Refined soybean oil	Methanol	95.9% yield	3:1 alcohol to oil molar ratio; methanol added in three steps; 48h; 130rpm; 30°C; 4wt.% lipase	Watanabe et al. (2002)
Novozym 435	Degummed soybean oil	Methanol	93.8% yield	3:1 alcohol to oil molar ratio; methanol added in three steps; 48h; 130rpm; 30°C; 4wt.% lipase; 25 cycles of reuse with no loss of activity	Watanabe et al. (2002)
Novozym 435	Crude and refined soybean oil	Methanol	-	Lipase inactivation with a molar ratio of methanol to oil > 1:1	Du et al. (2004)

Table 2. (Continued). Lipase-catalyzed reactions for biodiesel production in batch systems.

Novozym 435	Crude and refined soybean oil	Methyl acetate	92% yield	12:1 methyl acetate to oil molar ratio; 14h; 150rpm; 40°C; 30wt% enzyme (based on oil); 100 cycles of reuse with no loss of activity	Du et al. (2004)
Novozym 435	Soybean oil	Ethanol	100% conversion	6:1 ethanol to oil molar ratio; 6h; 1000rpm; 65°C; 50bar; 5wt% lipase (based on oil and ethanol); compressed propane (solvent to substrates weight ratio of 2:1)	Dalla Rosa et al. (2008)

* Abbreviations: IM: immobilized. BAUN: Batch Acidolysis Units Novozymes

Table 2. Lipase-catalyzed reactions for biodiesel production in batch systems.

The key issue in enzymatic methods is to reduce the cost of the catalyst or to improve its operational life (Du et al., 2004). The reuse of the catalyst is crucial to make the process economically feasible to a large scale application (Villeneuve et al., 2000). Furthermore, different reaction media or technologies have been applied for the development of a cost-effective enzymatic system for biodiesel production, as for example the use of low-frequency (10–60 kHz) ultrasound (Rokhina et al., 2009; Kumar et al., 2011), ionic liquids (Gamba et al., 2008). Supercritical fluids have also received much attention, as it will be discussed in the next section.

6. Enzymatic reactions in compressed and supercritical fluids

When a compound is compressed and heat above its critical point, it enters a phase that is referred to as its supercritical phase. The compound in the supercritical region is called as supercritical fluid (Dunford, 2004). An experimental setup used for alkyl esters production with immobilized lipase in pressurized fluids is schematically shown in Figure 4. Potential advantages with supercritical media are the high diffusivity and low surface tension which lead to low mass-transfer resistance (Adlercreutz, 1996).

High temperatures and pressures are needed during the non-catalytic transesterification process (Kulkarni & Dalai, 2006; Silva et al., 2007; Tan et al., 2010). For energy saving, transesterification reactions in supercritical fluids can be enzyme-catalyzed (Adlercreutz, 1996). Moisture content, particle size, and solvent flow rate are important parameters that must be optimized when using supercritical fluid technology for oil and oilseed processing (Dunford, 2004). Supercritical and near-supercritical fluids have been used for enzymatic reactions in a large number of studies. The most widely used medium in this group is supercritical carbon dioxide, whose main advantage is the easy removal after the reaction by just decreasing the pressure. Furthermore, supercritical carbon dioxide has low toxicity compared to organic solvents. As solvent, it resembles hexane and accordingly,

hydrophobic compounds can be dissolved, but the solubility of more polar compounds is low, but this can be overcome by addition of organic co-solvents, when necessary. Enzymes are normally not soluble in the supercritical fluids, and therefore heterogeneous systems with solid enzyme preparations are normally used (Adlercreutz, 1996).
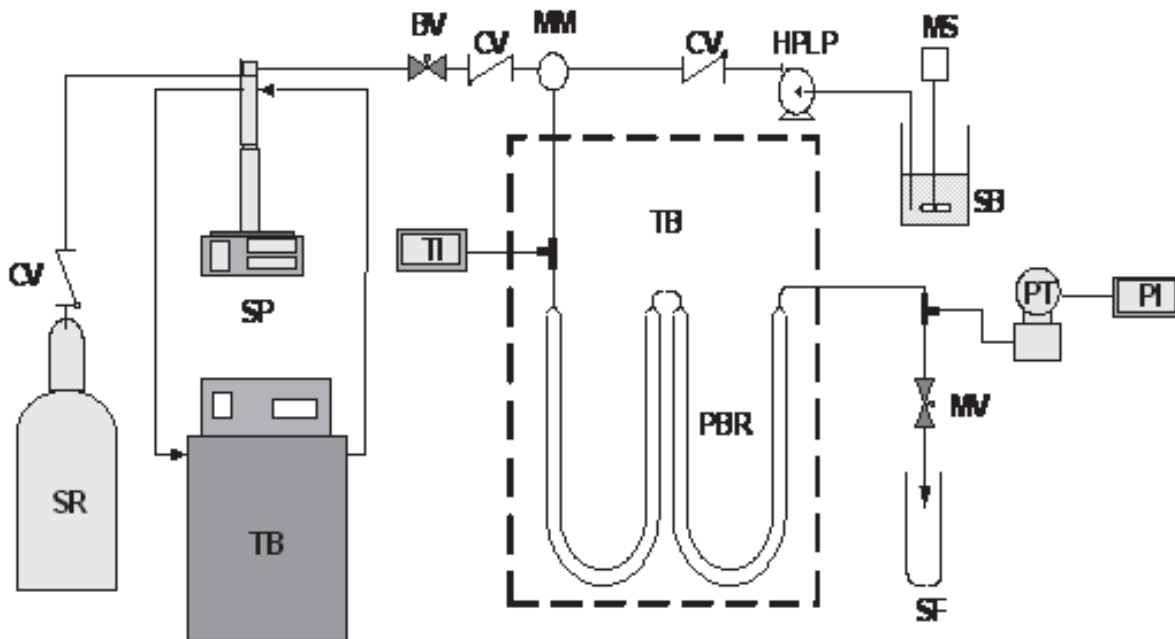

Fig. 4. Schematic diagram of the packed-bed experimental apparatus: SR- solvent reservoir; TB- thermostatic bath; SP- syringe pump; CV- check valve; MM- micro mixing; TI- temperature indicator; PI- pressure indicator; PBR- packed-bed reactor; HPLP- high-pressure liquid pump; SB- substrates; MS- mechanical stirrer; MV- micrometering valve; SF- sample flask collector. From Dalla Rosa et al. (2009).

Dalla Rosa et al. (2009) indicated the continuous lipase-catalyzed ethanolysis of soybean oil in a packed-bed reactor using compressed propane as solvent as a potential alternative to conventional process for biodiesel production. Good reaction conversions were obtained at mild temperature (70°C) and pressure (60bar), opening possibilities to manipulate process variables as well as reactor configurations towards reaching acceptable yields. Rathore & Madras (2007) investigated the synthesis of biodiesel from edible (palm oil, groundnut oil) and non-edible oils (*Pongamia pinnata* and *Jatropha curcas*) in supercritical methanol and ethanol without using any catalyst from 200 to 400°C at 200bar. Very high conversions (>80%) were obtained within 10min and nearly complete conversions were obtained after 40min. Biodiesel was also produced by the lipase Novozym 435 in presence of supercritical carbon dioxide. Conversions of only 60-70% were obtained in the enzymatic process even after 8h. One should consider, however, temperature reaction for the lipase-catalyzed method (45°C) was strongly lower than that used for the non-catalytic reaction. Moreover, the authors discussed stepwise methanol feeding and glycerol removal by dialysis could be adopted to avoid strong substrate and product inhibition, respectively, therefore increasing reaction conversion.

A preliminary study comparing conventional and supercritical media for the ethanolysis of palm kernel oil was carried out using two commercial immobilized lipases (Lipozyme IM from *Mucor miehei* and Novozym 435) (Oliveira & Oliveira, 2001). Higher conversions (up to

77.5%) were obtained in the presence of *n*-hexane for the reaction catalyzed by Lipozyme IM. Despite of enzyme activity losses and lower conversions (up to 63.2% with Novozym 435), supercritical carbon dioxide showed to be an appropriate solvent for enzymatic alcoholysis. Other studies involving lipase-catalyzed in pressurized fluids indicated this process as a potential technology for biodiesel production (Hildebrand et al., 2009).

According to Oliveira et al. (2006), the enzyme behaviour in compressed fluids is of primary importance as the loss of enzyme activity may lead to undesirable poor reaction rates and low yields of target products. Enzyme stability and activity may depend on the enzyme species, characteristics of compressed fluid, water content of the enzyme/carrier/reaction mixture and process variables manipulated. These authors investigated the behaviour of two commercial immobilized lipases submitted to compressed carbon dioxide, propane and *n*-butane under different reaction conditions (temperature from 35 to 75°C, pressure range of 10-280bar, exposure times from 1 to 6h, with distinct decompression rates). The lipase Lipozyme IM from *Mucor miehei* showed activity losses in all compressed solvents, markedly in carbon dioxide. For Novozym 435, treatment in carbon dioxide also led to activity losses, while the use of propane and *n*-butane promoted enhancements of the enzyme activity. Thermogravimetric analyses showed that the thermal profiles of Novozym 435 treated in *n*-butane and in carbon dioxide were similar to that of the untreated enzyme. Scanning electron micrographs (SEM) of Novozym 435 indicated that the material submitted to carbon dioxide presented morphological alterations when compared to the untreated enzyme (Figure 5).
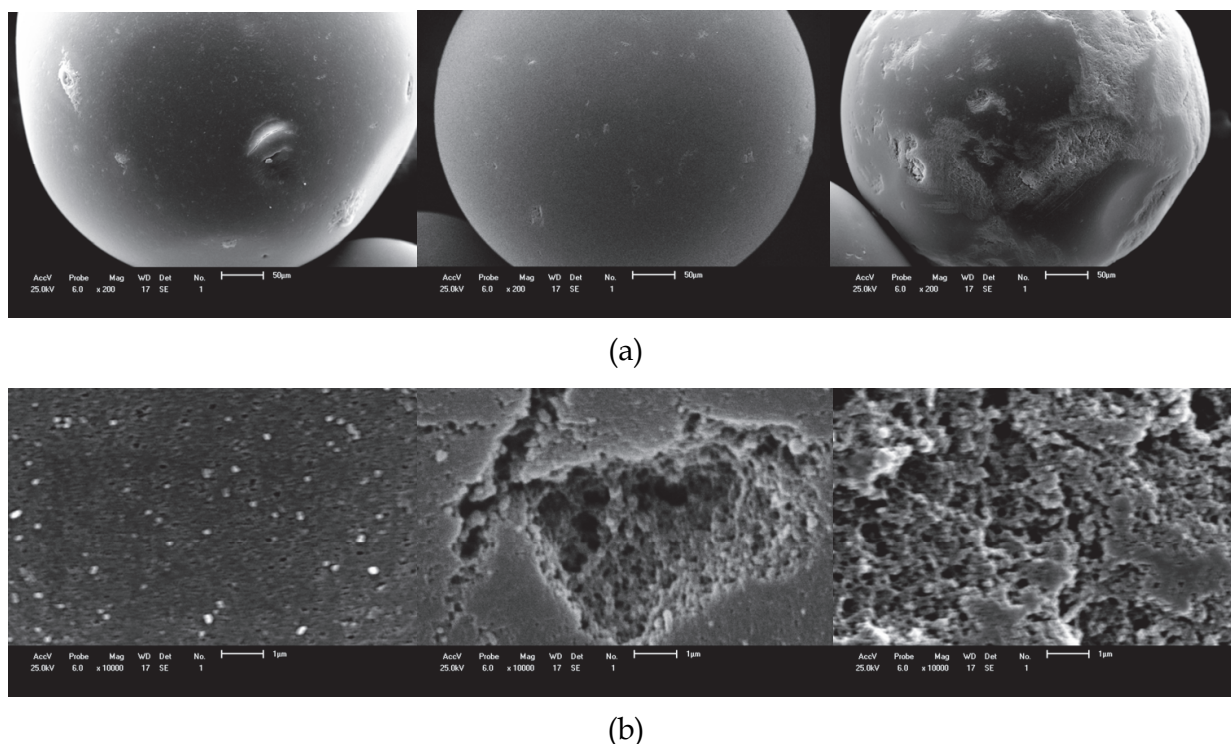

(a)

(b)

Fig. 5. SEM micrographs of Novozym 435 from *Candida antarctica* samples: (a) amplification of 200 times, (b) amplification 10,000 times. From left to right side, SEM micrographs refer to untreated enzyme, treated in *n*-butane (75°C, 250bar, 6h, decompression rate 5050bar.min^{-1}), and in carbon dioxide (75°C, 6h, decompression rate 200kg.m^{-3}.min^{-1}, reduced density 1.6, and related pressure 276.4bar), respectively. From Oliveira et al. (2006).

Lanza et al. (2004) observed the commercial immobilized lipase from *Candida antarctica* (Novozym 435) was stable in supercritical carbon dioxide under the investigated conditions (30 to 70°C, pressure range 70-250bar, 60-360min, decompression rates 10-200kg.m^{-3}.min^{-1}). These results therefore indicate the tested lipase can be used with low activity loss at typical conditions of temperature and pressure employed in many biotransformation reactions of raw materials in this supercritical fluid.

The commercial exploitation of supercritical fluids technology has been limited to a large-scale biodiesel production. One factor that may contribute to the development of this technology is the study of the phase behaviour of the high-pressure multicomponent systems found in transesterification reactions, as it is essential for the design of any supercritical fluid reaction system. Furthermore, these experimental data are of primary importance for selecting appropriate operational conditions to accomplish enzyme-catalyzed transesterification reactions in supercritical media. The phase behaviour of castor and soybean oils and their fatty acid ethyl esters in carbon dioxide at high pressures was already investigated, for example, by Ndiaye et al. (2006).

7. Conclusion

Several approaches have been made for a greener biodiesel production by means of biocatalyzed reactions. Lipases production from alternative sources, enzymes immobilization, as well as the use of alternative reaction media, such as supercritical fluids, have appeared as potential alternatives to reach this goal. Besides, the use of alternative feedstock, focusing on agro-industrial residues, seems to be a practical solution to reduce biodiesel production costs while ensuring a minimal environmental impact.

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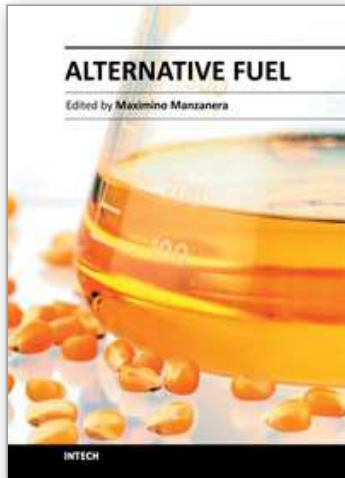
Alternative Fuel

Edited by Dr. Maximino Manzanera

Renewable energy sources such as biodiesel, bioethanol, biomethane, biomass from wastes or hydrogen are subject of great interest in the current energy scene. These fuels contribute to the reduction of prices and dependence on fossil fuels. In addition, energy sources such as these could partially replace the use of what is considered as the major factor responsible for global warming and the main source of local environmental pollution. For these reasons they are known as alternative fuels. There is an urgent need to find and optimise the use of alternative fuels to provide a net energy gain, to be economically competitive and to be producible in large quantities without compromising food resources.

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