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Biodiesel: Production, Characterization, Metallic Corrosion and Analytical Methods for Contaminants

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1. Introduction

In face of recent changes in the edaphoclimatic conditions (climate and soil) occurring worldwide, it has been necessary reflections on the need to exploit natural resources in a sustainable manner. Sustainability is a systemic concept, relating to the continuity of economic, social, cultural and environmental aspects of human society. It proposes to be a means of configuring the civilization and human activity so that the society, its members and its economies can fulfill its needs and express its greatest potential at present, while preserving biodiversity and natural ecosystems, planning and acting to achieve pro-efficiency in maintaining undefined these ideals [1].

According to the International Energy Outlook -2011 (IEO 2011), the energy consumption in the world was 505 quadrillion of British thermal units (Btu) in 2008, while in 2006 this consumption was 472 quadrillion Btu. According to 2011 bulletin, the outlook for energy consumption in 2020 will be 619 quadrillion Btu, and 770 quadrillion Btu in 2035 for the countries of the Organization for Economic Cooperation and Development (OECD) [2]. This gradual increase in worldwide energy consumption increases the search for renewable energy, once the existing conventional sources are exhaustible ones, such as for example the oil. In this sense, biofuels have appeared, such as ethanol (bioethanol) and biodiesel, which emerged very strongly due to large government incentives.

Within the energy issues, Brazil, for its favorable natural conditions, presents a huge potential for the production of biofuels, especially ethanol and biodiesel, a fact which makes it a strategic country in relation to the sustainability of such market. There is an effort to consolidate energy

from renewable sources, as well as the use of byproducts from these industries, with the creation of programs and incentives by the federal government, such as Proálcool [3].

This text reports a brief historic background of biodiesel in Brazil, methods for biodiesel production indicating the main raw materials, and the regulated physical-chemical properties for the quality control of biodiesel discussing the consequences of cases of non-conformity and their regulated methods by European, American and Brazilian norms. A special topic is dedicated to metallic corrosion which is closely related to storage stability of biodiesel, and finally a comprehensive review of analytical methods developed for monitoring contaminants (glycerol and trace metal) in biodiesel is presented.

1.1. Historic background of biodiesel in Brazil

The first use of vegetable oil in diesel engine was tested at the request of the French government with the intention of stimulating energy self-sufficiency in its colonies in Africa, minimizing the costs relating to imports of coal and liquid fuels. The oil selected for the tests was from peanut, whose culture was abundant in tropical countries. The diesel engine produced by the French company Otto, powered by peanut oil, was presented at the Paris Exhibition in 1900. Other experiments conducted by Rudolph Diesel were held in St. Petersburg with locomotives powered by castor oil and animal oils. In both cases the results were very good and the engines showed good performance.[3] In chapter "Liquid Fuel" from Diesel's book "Die Entstehung des Dieselmotors" (The Emergence of Diesel Engines)[4], it mentions:

For completeness, it is important that, back in 1900, vegetable oils were already being used successfully in diesel engines. During the Paris Exhibition of 1900, the French company Otto demonstrated the operation of a small diesel engine with peanut oil. This experiment was so successful that only some of the people present realized the circumstances in which it was conducted. The engine, which had been built to consume oil, was operated with vegetable oil without any modification. It was also observed that the consumption of vegetable oil resulted in a use of heat literally identical to the oil.

Vegetable oils were also used as emergency fuel, among other applications, during the Second World War. For example, Brazil has banned the export of cottonseed oil because this product could be used to replace imports of diesel oil. Reductions in imports of liquid fuels were also reported in Argentina, which required greater commercial exploitation of vegetable oils. China produced "diesel", lubricating oils, "gasoline" and "kerosene", the last two by cracking processes, from tung oil and other raw material oilseeds. However, the requirements of war forced installation of cracking units of unusual technology base. Quickly, research activities with new oil sources were expanded, but with the subsequent decline in the price of crude oil barrel (post World War II), reaching more affordable price, these researches were abandoned, as happened in India [5].

The use of vegetable oil as an alternative renewable fuel to compete with diesel oil was proposed in the early 1980. The more advanced study with the sunflower oil happened in South Africa because of diesel oil embargo, and the first International Conference on Plants and Vegetable Oils was held in Fargo, North Dakota, in August 1982 [6].

In Brazil, since the 1930s, efforts have been made to incorporate renewable fuels in the energy matrix, which are mainly accomplished by government authorities, universities and research institutes. In addition to the PRO-ALCOHOL (created in 1975, this program was designed to ensure the supply of ethanol from sugarcane in the process of replacing gasoline, as government initiative to tackle the successive increases in oil prices), it was established the Production Plan of Vegetable Oils for Energy Purposes (PRO-OIL), which from the 1980s was named as the National Program of Vegetable Oils for Energy Purposes. This program was designed to generate a significant surplus of vegetable oils, able to make their production costs competitive with mineral oil. It was envisaged by legislation a mixture of 30% of vegetable oil in diesel oil, with prospects for full replacement in the long term. At this time, it was proposed as a technological alternative the transesterification or alcoholysis of vegetable oils, highlighting the studies conducted at Federal University of Ceará, using different sources of vegetable oils such as soybean, babassu, peanut, cottonseed and sunflower, among others. Unfortunately, this program was abandoned by the government in 1986, when the oil price fell again in the international market along with the high cost of production and the crushing of oilseeds, which were decisive factors for the slowdown of the program. However, even after the end of PRO-OIL, there was a considerable progress in researches on the production and use of biodiesel in Brazil, which were conducted in different universities and research centers, particularly the registration of the first Brazilian patent deposited by Chemical Engineer Expedito José de Sá Parente [7]. In accordance with Parente he did not developed a new method: *The transesterification process has been known for many years. What I have patented was the production of esters for use as fuel in diesel cycle engines, which is entirely different from what Rudolf Diesel did. Modern engines could not run for a long time using a vegetable oil under the conditions tested by Diesel.* Table 1 shows the evolution of fuels in Brazil since the 1970s.

In Brazil, especially from the year 2005, when there was the beginning of the National Biodiesel Program, these surveys were intensified due to growing importance in using this material, and three years later, in January became mandatory the addition of 2% (v/v) of biodiesel to commercial diesel, whose mixture was called B2 (B for blend). In March 2008, the National Energy Research Council (CNPE) determined the mandatory addition of 3% (v/v) from July. Since January 1, 2010, the diesel oil sold in Brazil contains 5% biodiesel. This rule was established by Resolution number 6/2009 of the National Energy Policy Council (CNPE), published in the Official Gazette (DOU) in October 26, 2009, which increased from 4% to 5% the mandatory blend percentage of biodiesel to diesel oil. Until mid-2011, two more increases were conducted over, and at that time the addition was 5% (v/v) of biodiesel to diesel, and such addition was put forward to year 2011, previously proposed for the year 2013, due mainly to the increase in diesel consumption and the consequent increase of the fleet of cars using this fuel.

Year	Event
1973	First Oil Shock
1974	Creation of the Pro-Alcohol
1977	Addition of 4.5% Ethanol to Gasoline
1979	Addition of 15% Ethanol to Gasoline
1980	Second Oil Shock
1983	Alcohol cars account for over 90% of total sales
1985	Percentage of Alcohol added to gasoline reaches 22%
1989	Oil prices fall and gasoline equates with alcohol
1992	Rio 92: Signing of the milestone about climate change
90's	Alcohol comes to represent 20-25% of Gasoline
2005	Founded PNPB
2007	Third Oil Shock
January 2008	Beginning of mandatory B2
March 2008	CNPE determines the mandatory use of B3 from July 2008
April 2008	Alcohol Consumption equates to Gasoline
July 2009	Validity of B4
January 2010	Validity of B5

Table 1. Evolution of fuels in Brazil since the 1970s.

This same law, published on January 13, 2005, introduced the biodiesel in the Brazilian energy matrix and expanded the administrative competence of the National Agency of Petroleum and Natural Gas (ANP), which became, since then, the National Agency of Petroleum, Natural Gas and Biofuels. Since the publication of the abovementioned law, ANP took over the assignment to regulate and supervise the activities related to production, quality control, distribution, sale and marketing of biodiesel and diesel-biodiesel blend [8]. The continued rise in the percentage of biodiesel added to diesel demonstrates the success of the National Program for Production and Use of Biodiesel and the experience accumulated by Brazil in production and large-scale use of biofuels, especially the biodiesel.

1.2. Aspects of the biodiesel market in Brazil

According to the Monthly Bulletin of Renewable Fuels of the Ministry of Mines and Energy (MME), based on deliveries of auctions promoted by ANP, it shows that the estimated production in May 2012 was 173,000 m³. The total of the year up to this month, the cumulative production was 958,000 m³. The installed production capacity, in May 2012, stood at 6.092 million m³/year (507,000 m³/month) of which 88% is related to companies holding the social seal [9]. The Social Fuel Seal is a component of identification created from the Decree No.

5297 of December 6, 2004, awarded by MDA to biodiesel producer who meets the criteria described in Instruction No. 01 of February 19, 2009. The Seal gives to the possessor the character of promoter for social inclusion of family farmers classified in the National Program of Family Agriculture (PRONAF).

2. Raw materials for biodiesel production

In general, biodiesel can be produced from any source having oil, either of animal or vegetable source; however, to ensure the quality of the final product, some factors must be observed, as, perennial crops, oil content and preferably with no potential for the food industry, as occurs primarily for soybean in Brazil. Furthermore, it should be noted the productivity per unit area, the agronomic balance and other aspects related to the life cycle of the plant (seasonal). These raw materials, along with the production processes, depend on the region concerned. The economic, environmental and social diversities have given distinct regional motivations for the production and consumption. Due to the favorable conditions of climate and soil, Brazil presents numerous possibilities for use as raw material for the biodiesel industry. It can be highlighted the use of soybean, castor, palm, cottonseed, sunflower, macauba, rapeseed, jatropha, animal fat (tallow) and residual oils, among which, the latter presents itself as an excellent alternative, because it is a material whose reuse at industrial level was insignificant until its application in the biodiesel industry. Oil contents of some of the oil seeds used in Brazil are shown in Figure 1.

Figure 1. Oil content of some of the seeds used in Brazil.

In Brazil the most relevant is the use of soybean for biodiesel production, followed by beef tallow and cottonseed oil as described by Monthly Bulletin of Biodiesel (published by ANP - reference month May 2012) as shown in Figure 2.

Figure 2. Oil sources used for biodiesel production in Brazil.

In Brazil, other species of oilseeds are cultivated and they are distributed by region as shown in Table 2.

Region	Raw materials that are available
Southeast	Soybean, castor, cottonseed, sunflower, jatropha and macauba
South	Soybean, corn, rapeseed, sunflower, jatropha and cottonseed
Centralwest	Soybean, castor, cottonseed, sunflower, jatropha, macauba, palm and animal fat
North	Palm, babassu and soybean
Northeast	Babassu, soybean, castor, palm, jatropha, cottonseed and coconut

Table 2. Distribution of oilseeds in Brazilian regions. Adapted from ref. [7].

Despite the many advantages in the use of biodiesel, there are two serious problems from the viewpoint of production of vegetable oils which can retard or impede the use of derivatives of vegetable oils as fuel. The first problem is the amount of oil produced. Some of the key raw materials used in Brazil and the corresponding harvest months and oil yields (t/ha) are listed in Table 3. The second problem is the quality of the extracted oil. Oils with high concentrations of polyunsaturated fatty acids are undesirable for biodiesel production, for decreasing their stability to oxidation [12]. Generally, the more unsaturated acids, such as linoleic and linolenic acids, respectively, with two and three unsaturations, are more susceptible to oxidation. In addition, these oils induce a higher carbon deposit than oils with high content of monounsaturated or saturated fatty acids as occurs to the palm oil.

Name	Scientific name	Harvest months/ years	Yield (ton/ha)
Peanut	<i>Arachis hypogea L.</i>	3	0.6-0.8
Cottonseed	<i>Gossipum Hirsutum L.</i>	3	0.1-0.2
Babassu	<i>Orbiguya phalenta Mart.</i>	12	0.1-0.3
Rapeseed	<i>Brassica napus L.</i>	3	0.5-0.9
Coconut	<i>Cocos mucifera L.</i>	12	1.3-1.9
Palm	<i>Elaeis guineenses L.</i>	12	3-6
Sunflower	<i>Helianthus annus L.</i>	3	0.5-1.9
Castor beans	<i>Ricinus communis L.</i>	3	0.5-0.9
Jatropha	<i>Jatropha Curcas</i>	12	0.5-2.5*
Soybean	<i>Glycine Max (L.) Merril</i>	3	0.2-0.4

Table 3. Name, Scientific name, harvests months and yields of raw materials for biodiesel production in Brazil. Adapted from refs. [10] and [11]*.

2.1. Cottonseed

The cotton is currently produced by more than 60 countries. China, India, U.S.A., Pakistan and Brazil emerge as major producers. Together, they account for 80% of world production of around 23.3 million tons. Only these five countries have more than 22 million hectares of planted areas, out of 35 million on average that are planted in different points of the planet [13]. The cottonseed oil shows 13-44% oleic acid (18:1) and 33-59% linoleic acid (18:2) [14], and gossypol, the main byproduct of the cotton industry, is a polyphenolic compound, yellow color, toxic, non-interfering in the biodiesel production process [15].

2.2. Palm

Over 70% of the world's oils come from 4 plant species: soybean, palm, sunflower and rapeseed, and currently, the palm oil (known in Brazil as "dendê") is the most traded oil in the world. It is used in the food industry in margarine, ice cream, biscuits, pies, and others. It is also used in the industry of hygiene and cleanliness in the composition of soaps, detergents and cosmetics. In the chemical industry, it is part of the lubricant composition and can also be used as biofuel [16].

In Brazil, the rise of the use of this oilseed is so great that the Federal Government launched in 2010, the Program for Sustainable Production of Oil Palm, which aims to regulate the expansion of oil production and offer tools to ensure a production in sustainable environmental and social bases [16].

This oilseed has the largest oil content among the raw materials for the biodiesel production reaching the yield of 6000 kg/hectare/year. This yield is about 15 times larger than the main raw material in Brazil, the soybean.

The palm oil presents approximately 35-47% palmitic acid (16:0) and 36-47% oleic acid (18:1) [14], and the main problem in using it as fuel is its high viscosity (≈ 20 Cst), about 10 times greater than oil diesel (≈ 2 Cts) (ASTM D445).

2.3. Rapeseed

The rapeseed (*Brassica napus*) is a winter oilseed of Brassica genus, from Cruciferae family and it has 39-45% oil with excellent quality for fatty acid composition. It is the main oil used for biodiesel production in Europe, and is usually known as “colza”. It started in Canada in 1974 and with plant breeding developed a seed without erucic acid, a substance which limited its use for human consumption [14]. It represents, in percentage, 15.32% of world production of vegetable oils, considering the 2011/2012 crop, behind only the production of palm oil (33.14%) and soybean (27.28%) [17]. The rapeseed oil contains mostly oleic acid (18:1), 62% followed by 32% linoleic acid (18:2) [18].

2.4. Jatropha

The jatropha (*Jatropha curcas*) is a drought-resistant plant, multipurpose, having a large amount of oil compared to other inedible plants [19]. The highest incidence and the center of diversity of this genus are the Central America and the Caribbean Islands. The genus has over 210 species growing in different regions of the globe [20-21]. This oleaginous has a different composition when it comes to saturated fatty acids [22-23], due to the presence of 16.4% palmitic acid (16:0) against 2.3% soybean and 7% corn [24]. The jatropha contains mostly 40.3% linoleic acid (18:1), 37% linolenic acid (18:2) which indicates a good quality of biodiesel to be produced from this source because the lower the amount of raw material unsaturation, the better is the stability to oxidation [25].

2.5. Soybean

In 2010/2011 harvest, Brazil produced about 75 million tons of soybeans, 65% of this production was exported, equivalent to 48.7 million tons (29.1 million tons of grain, 13.7 million tons of bran and 1.6 million tons of oil). China is the main destination of Brazilian exports of grain (66%) and oil (60%) and the European Union is responsible for the majority of imported bran (70%) [26]. In 2011/12 harvest, the soybean had a stake in percentage terms in the world production of vegetable oils of 27.28% and is the second after only to oil palm, with 33.14% [17].

In Brazil, in May 2012, the soybean had a participation of 80.09% in the production of biodiesel. Taking into account that the biodiesel production in Brazil in that same month was $188,367 \times 10^3 \text{ m}^3$ (ANP) then about $150,863 \times 10^3 \text{ m}^3$ of the biodiesel produced was derived from soybean oil. This fact is due to the growth of agricultural productivity of soybean and also the technical-industrial feasibility in this oilseed cultivation.

The soybean has about 19.0-30.0% of oleic acid (18:1) and 44.0-62.0% of linoleic acid (18:2) [14].

One aspect widely discussed in relation to this oilseed is the competition between the production for the biofuel market and food industry. In 2011/12 harvest, Brazil exported approximately 44.9% of the soybeans produced [10], due mainly to the increased buying power of emerging countries such as China and India and a small part (about 8%) was for national biodiesel production. China in this season was the largest buyer of Brazilian soybeans (66%) and processed refined oil (60%).

Regarding to soybean oil, Brazil in 2010/11 harvest produced about $7,434 \times 10^3$ tons, and $5,495 \times 10^3$ tons (74.85%) were used for domestic market and $1,758 \times 10^3$ tons (23.95%) for export [26]. In Brazil, this situation has fluctuated over the months, due to the offseason of soybeans and also because many other oilseeds arose that can be intended for biodiesel production, such as jatropha, cottonseed, moringa, among other.

2.6. Waste cooking oil

This raw material represents currently a potential use for biodiesel production worldwide since it is a residue from cooking process of industries, restaurants, bars, among other outlets. The high cost of biodiesel in the national and international market is mainly due to the soaring cost of industrialized vegetable oils and virgin oils [27,28].

The biodiesel made from these raw materials is most often cheaper than from sources such as soybean, rapeseed and sunflower, but the quality of the oil used must be monitored to obtain a high quality biodiesel. The free fatty acid (FFA) content and water content (moisture content) are the main parameters to be analyzed. For a reaction catalyzed by alkali, a FFA content of less than 3% is required, since the use of oil with a high FFA content produces the hydrolysis of triglycerides at high temperatures during the frying process [29]. The residual oil has as striking characteristic a high acidity index and it is previously necessary a transesterification reaction, an acid catalysis using a strong acid (generally H_2SO_4) to promote the esterification of fatty acids and thereby reduction of FFA to less than 1% [27,28]. The residual cooking oil has mostly in its composition, oleic acid (18:1) and linoleic acid (18:2) [30].

The use of recycled cooking oil for Biodiesel production has been relevant in recent times, and the flight KL705, originated from Amsterdam with destination to Rio de Janeiro, was carried out in part with this raw material, fueling a Boeing 777-200 [31].

2.7. *Moringa oleifera*

The *Moringa oleifera* belongs to the family Moringaceae, consisting of only one genus (*Moringa*) and fourteen known species, native to northern India. This species is found in natural conditions in India, Africa, Asia, Arabia, South America and the Pacific and Caribbean Islands [32]. *Moringa* is a multipurpose plant from the leaves to the seeds, showing different properties. The *Moringa* leaves are the source of a diet rich in protein for both humans and animals. Moreover, oil extraction from its seed enables the utilization of this raw material to produce biodiesel having a remarkable oxidation resistance, with shelf time between 4 and 5 years [33]. The seeds of *Moringa Oleifera* contain between 33 and 41% m/m oil [33]. This val-

ue is considered a good natural emollient for cosmetic, based on tactile properties, a lower natural occurrence of color and odor besides a high concentration of oleic acid (>73%) [34]. Recently studies showing the potential of Moringa oil extracted from seeds of India and Pakistan for the biodiesel production were published [35,36]. The Moringa has in its composition about 7% of palmitic acid (16:1) and 78% of oleic acid (18:1) [32]. Variations in oil content within countries and species are attributed to possible changes in environmental and geological conditions of the regions [37].

3. Methods for biodiesel production

Vegetable oils and animal fats contain, in addition to triacylglycerols, free fatty acids, phospholipids, sterols, water and other impurities. These compounds give special properties to these raw materials which prevent their use directly as a fuel, as, for example, the blockage point and the high viscosity [38]. These problems can be overcome with adjustments to the compression engines; however, such possibility makes the process very expensive and often impractical. The adaptation of the fuel becomes more interesting and it can be used directly to the existing fleet, without adjusting existing technologies by those that cause chemical modifications (cracking, esterification and transesterification). In the last decade some reviews on the different methods for biodiesel production were reported in the literature [39-47].

The transesterification (also called alcoholysis) is generally a term used to describe the important class of organic reactions in which a triacylglycerol reacts with alcohol in catalytic environment and becomes ester and glycerol [39]. The overall process is a sequence of three consecutive and reversible reactions, in which the mono- and diacylglycerols are formed as intermediates of reaction. In the transesterification reaction three moles of alcohol are needed for each mol of triacylglycerol. In practice, it is used an excess of alcohol in order to increase the ester productivity (shifting the reaction towards the product side) and to allow the separation of the obtained glycerol (the impure fraction containing glycerol is called glycerin).

Glycerin makes the oil more dense and viscous, therefore, during the transesterification process, the glycerin is removed resulting in a product of lower viscosity. In the transesterification, the reaction of biodiesel production creates alkyl esters and glycerol, and the glycerol layer is denser than the ester one and it deposits at the bottom of the reactor [39]. The process is based on the stoichiometric reaction of the alkyl glycerol with alcohol, which in most cases has short organic chain in the presence of a catalyst [39-42]. Alcohols such as methanol, ethanol, propanol or butanol can be used in the transesterification reaction and the (produced) monoesters are known as methyl, ethyl, propyl and butyl esters, respectively. The raw materials used as triacylglycerol source for biodiesel production were discussed previously in the text (Section 2).

The technology for the biodiesel production prevailing in the world is methyl transesterification, where, vegetable oils or animal fat are mixed with methanol that, along with a cata-

lyst, produce the biodiesel. This option occurs mainly because of high ethanol cost and operational facilities. The advantages of biodiesel production using methanol include: ester phase separation (biodiesel) of glycerol that occurs instantaneously; the alcohol recovery that is completed and can be returned to the process; the obtaining of glycerol which is feed-stock to the chemical industry; the synthesis is more attractive under the industrial viewpoint, because it is faster and cheaper than the others.

In Brazil, the enterprises that are in operation adopt the methylic route; however, due to ethanol production in Brazil is established beyond favorable environmental factors, there are enterprises that adopt the ethylic route (at industrial level). The ethyl route is recognized as more ecologically friendly, because ethanol is a renewable source. Production of ethyl ester is slightly more complex and requires more steps and the use of specific centrifugal pump and optimized for a good separation of glycerol from esters. The more alcohol is added to the oil, the faster is the conversion to ester, however, an excess can stabilize the emulsion and complicate the separation of glycerol.

The catalysis used for biodiesel production can be chemical or biological, homogeneous or heterogeneous, acidic or basic. The process for producing biodiesel by basic catalysis is faster than the process of acid catalysis, the biodiesel produced presents less corrosivity, [43,44] and the most used catalysis are the potassium hydroxides, sodium hydroxide, potassium methoxide and methoxide sodium. In Brazil, KOH is more expensive than NaOH, however, there is less soap formation using KOH [45]. The base catalysis is the preferred procedure when there are oils with low water content and low acidity index. On the other hand, if oils and fats have a high content of free fatty acids, it is recommended a pre-treatment or the acid catalysis, followed by an alkaline transesterification [46].

In general, the transesterification of oils or fats can be affected by several factors such as [48]: presence of free fatty acids, moisture [50], type of alcohol used, the molar ratio of alcohol/oil, concentration and type of catalyst, time, temperature of reaction [49] and the intensity of shaking [41].

The content of free fatty acids and moisture are important parameters to determine the feasibility of the basic process of transesterification of vegetable oils and animal fats, requiring low levels of free fatty acids and moisture in the raw material for higher conversion efficiency. Studies have shown [43] that the transesterification of beef tallow catalyzed by NaOH in the presence of free fatty acids (FFA) and water has its yield compromised. Therefore, the raw material that has a high content of free fatty acids can be purified by saponification or using acid catalysis for previous esterification of these acids [51].

The production of ethyl esters via basic catalysis becomes more difficult compared with the production of methyl esters, due to the undesired formation of stable emulsion during the reaction. Methanol and ethanol are immiscible in triglyceride at room temperature, and the reaction media are commonly kept under mechanical stirring to increase mass transfer. In methanolysis, these emulsions easily form two layers: a bottom one rich in glycerol and a top one rich in esters; in ethanolysis the phases are more stable complicating the separation and purification of esters [39,50].

The molar ratio of alcohol/oil is one of the most important variables affecting the yield of esters in the transesterification reaction. The transesterification is a reaction in equilibrium, which requires an excess of alcohol to drive the reaction in the formation of esters. For a maximum conversion of esters, the molar ratio should be greater than or equal to 6:1 [39], but a very high molar ratio of alcohol/oil interferes with the separation of glycerol since its solubility increase takes place. When glycerin remains in solution, it favors the reaction equilibrium for the reactants and then decreasing the yield of esters [52]. Some researchers [42,53] have studied the molar ratios between 3:1 and 15:1 with ethanol, and they have observed arise in the yield of esters with molar ratio increase up to a value 12:1. The best results were between 9:1 and 12:1 [48].

The catalyst concentration is another factor extremely important for the alkaline transesterification [53] since the excessive addition of catalyst can promote an acidity reduction, but, on the other hand, it leads to the formation of soap, hampering the separation of glycerol from esters and consequently decreasing the yield of the reaction.

The catalysts most used during transesterification are the alkoxides [54,55], hydroxides [56] and carbonates of sodium or potassium. The alkoxides of alkali cations such as potassium methoxide (CH_3ONa) are the most reactive catalysts, since they exhibit high yields ($> 98\%$) in a short reaction time (30 min), even at low molar concentrations (0.5 mol L^{-1}). The greater efficiency of the catalyst CH_3ONa relative to NaOH is described by Freedman and colleagues [48,54] which had a similar conversion of oil at concentrations of 1% NaOH and 0.5% CH_3ONa , while in the work developed by Ma and colleagues [45], NaOH showed better performance in ester than CH_3ONa in the transesterification of beef tallow. Vincent and colleagues [57] reported good yields obtained with methoxide catalyst, but higher conversion rate was obtained with NaOH and lower with CH_3OK at 65°C , in methanol/oil ratio of 6:1 and catalyst concentration of 1%. Hydroxides of alkaline cations (KOH and NaOH) are more accessible in price than the respective alkoxides, but are less reactive.

Stirring of the reaction medium is an important factor in the transesterification process. Stirring should be intense to transfer amounts of mass of triglycerides from oil phase to the interface with methanol, as the reaction mixture is heterogeneous, consisting of two phases. In this case, the greater the stirring the higher is the mass transesterification [58]. Ma and colleagues [45] added NaOH and MeOH to the tallow beef melted in a reactor and found that after a certain reaction time without stirring anything occurred, suggesting the need of stirring for the reaction be initiated.

Some oils and fats, which may be used as raw materials for the production of biodiesel, have high levels of free fatty acids. The presence of free fatty acids impairs the synthesis of biodiesel via homogeneous basic catalysis [59]. In this sense, heterogeneous acid catalysts, which simultaneously promote reactions of alcoholysis of triglycerides and esterification of free fatty acids, present themselves as promising substitutes of homogeneous basic catalysts [60]. Moreover, such catalysts have the advantages inherent to heterogeneous catalysis, such as significantly reducing the number of purification steps of products as well as the possibility of reuse and enable the production of biofuel by a continuous process with fixed bed reactors [60].

For the production of biodiesel there are other technological routes besides the transesterification, as for example, the esterification catalyzed by an acid, preferably the sulphonic or sulfuric acids. The obtained yield is very high (99%), but the reaction is slow, requiring high temperatures (above 100°C) over 3 hours to reach the mentioned output [45,46,61,62]. Furthermore, it is necessary to use a large excess of alcohol to ensure a high yield reaction. The acid catalysis is suitable for oils with high content of free fatty acids and moisture. In this case the process is the esterification of free acids and not the transesterification of triacylglycerol.

The enzymatic catalysis (biological) allows the simple recovery of glycerol, the transesterification of triglycerols with high content of fatty acids, the total esterification of free fatty acids, and the use of mild conditions in the process, with yields of at least 90%, making it a commercially viable alternative. In this type of catalyst there are no side reactions that result in by products, which reduce the expenditure of further purification. Some enzymes require cofactors, metal ions or organic compounds (coenzymes). These co-factors will influence the activity of biological catalyst [47]. The advantages of the process are: lack of aqueous alkaline waste, lower production of other contaminants, greater selectivity and good yields. The main drawbacks of this methodology are the high cost of pure enzymes, the high cost of extraction and purification process of macromolecules and their instability in solution, which represent an obstacle to the recovery of the biocatalyst after its use [47]. On the other hand, the immobilization of enzymes allows their reuse, reducing the process cost. In the case of biocatalysis in nonaqueous media, immobilization also results in improvement in enzyme activity. Thus, many processes of transesterification using immobilized lipases have been developed [51,63,64].

Another possible and effective reaction is the supercritical transesterification using methanol, which allows a conversion of 60-90% in only 1 minute and more than 95% in 4 minutes. The better reaction conditions are: temperature of 350°C, pressure of 30 MPa and the volumetric ratio between methanol and oil of 42:1 for 240 seconds. The supercritical treatment of lipids with suitable solvent such as methanol depends on the relationship between temperature, pressure and thermo physical properties such as dielectric constant, viscosity, specific mass (density) and polarity [65,66]. The process is attractive since it overcomes problems such as oil/fat waste that is rich in free fatty acids and also the problem of the presence of water that often favors the formation of soap. However, side reactions involving the unsaturated esters occur when the reaction temperature exceeds 300°C, resulting in loss of material. There is also a critical residence time value in high temperature above which the efficiency decreases [51,67].

The H-BIO process was developed to introduce the processing of renewable raw materials in the scheme of petroleum refining and allow the use of existing facilities. The vegetable or animal oil is blended with fractions of petroleum diesel to be converted to units of Hydrotreating (HDT), which are employed in refineries, especially for reducing the sulfur content and improving the diesel oil quality, adjusting the fuel characteristics to the legal specifications. It was already conducted tests with up to 30% vegetable oil in the HDT load, mixed with diesel fractions, generating a product that has the same characteristics as petroleum

diesel, but the use of such a high proportion of vegetable oil, in existing industrial HDT units meet operational constraints and limitations of some equipment that were not rated for such task in its original design. A patent of this method was registered (INPI PI0900789-0 A2) and a summary of this method is available at the webpage of Petrobras (Brazilian Oil Company) [68].

4. Physical-chemical properties of biodiesel

Because of the importance of biodiesel and regulations for its use in the country, the concern of the ANP (National Agency of Petroleum and Natural Gas and Biofuels) is to ensure a quality fuel in any situation, through the establishment of quality standards for biodiesel. The federal law 11.097/2005 establishes in the country the introduction of minimum percentages of mixture of biodiesel to diesel, and also the monitoring of such insertion. The Brazilian specification of biodiesel is similar to European and American ones, with some flexibility to meet the characteristics of domestic raw materials. This specification is issued by ANP decree. This agency has developed standards in recent years with respect to biodiesel, among them there are the resolutions 15, 41 and 42. Resolution No. 42 of ANP [69] provides a specification for biodiesel (B100) according to the provisions contained in the Technical Regulation No. 4/2004, part that composes such Resolution. B100 can be added to diesel oil in proportions defined by volume, sold by various economic agents authorized throughout the national territory. Any vegetable oil can be used as fuel for diesel engines, but some oils have better performance in terms of their thermodynamic properties [70].

Visual observation of biodiesel formed which should be presented clear and free of impurities, either in suspension, precipitated material or any other. This parameter is adopted only by ANP and it is a simple but important test which is performed in a tube without graduation because this parameter is a qualitative indication of quality.

Tables 4 and 5 present the specifications for biodiesel in accordance with Brazilian, European and American standards and the corresponding analytical methods, respectively.

The specific mass (density) is connected with the molecular structure, i.e. the longer the carbon chain of the ester alkyl, the greater is the density which is, however, reduced by the presence of unsaturations. It is an important parameter for the vehicle injection system. Biodiesel has a specific mass greater than the diesel. This parameter is variable according to the raw material, alcohol excess, among others; very high values may indicate contamination with soap and/or vegetable oil, and alcohol excess causes a decrease in density. The European standard presents as specification limit the values of 860-900 kg m⁻³, according to the manual method EN ISO 3675 using glass hydrometers and the automatic method EN ISO 12 185 using digital densimeters, and the later with better repeatability. ASTM D6751 norm does not consider the specific mass as a measure of quality of biodiesels. ANP provides the specification range with values between 850 to 900 kg m⁻³,

which adopts the European standard methods, in addition to ASTM D1298 (manual) and ASTM D4052 (automatic), corresponding to NBR 7148 and NBR 14065, respectively. The Brazilian specification differs from European only for this parameter. However, as the reference temperature in Brazil is 20°C and assuming that the lower the temperature of the test, the higher is the density, it can be concluded that the European specification is more restrictive.

The kinematic viscosity (measurement of internal resistance of liquid flow) is an important parameter for the vehicle injection system and fuel pumping system. It depends on the efficiency of the process (reduction of viscosity of raw material). The viscosity reaches high levels with polymerization processes and/or thermal or oxidative degradation. The EN 14214 standard provides an acceptable range from 3.5 to 5.0 mm²/s (EN ISO 3104 method), while the ASTM D6751 standard allows a broader range from 1.9 to 6.0 mm²/s (ASTM D445 method). The Brazilian standard adopts, besides the methods already mentioned, also the ABNT NBR 10441 method, with the allowed viscosity range from 3.0 to 6.0 mm²/s. The bottleneck for this parameter is the upper limit of the specification which is the value of 6.0 mm²/s for ANP and ASTM, while the standard EN 14214 has a limit 5.0 mm²/s, which may restrict the use of some raw materials as for example the biodiesel from castor beans.

The flash point corresponds to the lowest temperature at which the product generates steam enough to ignite when a flame is applied under controlled conditions. This analysis measures the power of self-igniting the fuel and is essential for safety in stocking, handling, transport and storage of fuel. Biodiesel has a flash point much higher than diesel, and low flash point is commonly linked to the presence of alcohol residue in the process. ANP states that the flash point presents at least 100°C and adopts the EN ISO 3679 method, the same of European standard, ASTM D93, the same used by ASTM D6751, and also recommends ABNT NBR 14598.

Water and sediment in biodiesel are usually higher than in diesel, as biodiesel is hygroscopic. The water may generate an unwanted reaction, producing free fatty acids, growth of microorganisms, corrosion and malfunctions of the engine [71]. ASTM D6751 standard adopted this parameter as a quality control for biodiesel using ASTM D2709 method, while ANP and European standard recommends coulometric method (Karl Fischer) EN ISO 12937, and the Brazilian standard also recommends ASTM D6304 method. Comparing the methods, it seems that the coulometric method has greater sensitivity, higher repeatability and lower response time compared to the volumetric method (ASTM D2709).

The total contamination is mainly originated from the raw material, from soaps formed during the process and from unsaponifiables such as wax, hydrocarbons, carotenoids, vitamins and cholesterol (animal origin and used oils). The unsaponifiables present higher boiling point and create waste and soaps in engines and which result in sulphated ashes and finally in abrasion. ASTM standard has not adopted this parameter that is indicated by the European and Brazilian standards, which recommend the same method of analysis described by EN 12662, with maximum specification limit of mg kg⁻¹.

Property	Unit	Limits		
		ANP 07/2008	EN 14214	ASTM D6751
Aspect	---	Limpid and without impurities		
Density	kg/m ³	850-900 (20°C)	860-900 (15°C)	---
Kinematic viscosity (40°C)	mm ² /s	3.0-6.0	3.5-5.0	1.9-6.0
Water and sediment, max.	%vol.	---	---	0.050
Flash point, min.	°C	100	101	130
Distillation, 90% recovered vol., max.	°C	---	---	360
Carbon residue, max.	% mass	0.050 (100% of the sample)	0.3 (10% Distillation residual)	0.05 (100% of the sample)
Sulfated ash, max.	% mass	0.020	0.02	0.020
Sulfur content, max.	mg/kg	10	10	15
Copper strip corrosion, 3h at 50°C, max.	Rating	1	1	3
Cetane number	---	Note	51 (min.)	47 (min.)
Cold soak filterability	°C	By region	By region	---
Pour point	°C	---	By region	---
Cloud point	°C	---	---	Note
Sodium and potassium, max.	mg/kg	5	5	5
Calcium and magnesium, max.	mg/kg	5	5	---
Phosphorus content, max.	mg/kg	10	4.0	10
Total contamination, max.	mg/kg	24	24	---
Ester content, min.	% mass	96.5%	96.5%	---
Acid value, max.	mg KOH/g	0.50	0.5	0.5
Free glycerol, max.	% mass	0.02	0.02	0.020
Total glycerol, max.	% mass	0.25	0.25	0.240
Monoglycerides	% mass	0.80 (max.)	0.80 (max.)	---
Diglycerides	% mass	0.20 (max.)	0.20 (max.)	---
Triglycerides	% mass	0.20 (max.)	0.20 (max.)	---
Methanol or Ethanol, max.	% mass	0.20	0.20	0.20
Iodine value	g I ₂ /100 g	Note	120 (max.)	---
Oxidation stability at 110 °C, min.	H	6	6	3
Water content, max.	mg/kg	380	500	---
Linolenic acid	% mass	---	12 (max.)	---
Polyunsaturated methyl esters (with more than four double bonds)	% mass	---	1 (max.)	---

Table 4. Comparison of limits for the quality control of biodiesel in accordance with Brazilian, European and American standards.

The ester content is the main property as it indicates the degree of purity of the biodiesel produced and the efficiency of the production process used. It depends on the amount of unsaponifiables in the raw material and process variables (time, temperature, agitation speed, molar ratio, catalyst concentration, type of catalyst, water, free fatty acids, and alcohol). The low content of esters indicates a low yield of the transesterification reaction, i.e., most of the triacylglycerols has not reacted, which can cause difficulties in combustion and carbonization of engine cylinders. This parameter is required by the ANP and EN 14214 standards, whose minimum percentage is 96.5 % wt. using the EN ISO 14103 method. Brazilian standard also recommends ABNT NBR 15342 method for biodiesel from animal origin or for blends in which there is the presence of biodiesel from castor beans.

The carbon residue indicates the tendency of a fuel to form carbon deposits in engines. These residues are deposited in the nozzles and other parts of the engine, reducing their useful life. They correspond to the amount of triacylglycerols, soaps, leftover of catalyst and unsaponifiables, which are present in the final biodiesel. American and Brazilian standards adopt the same ASTM D4530 method, also having the same specification limit, no more than 0.050 % wt. Now the European standard indicates EN ISO 10370 method in which the maximum allowed is 0.3 % wt.

Sulphated ashes cause saturation of filters and wear on various parts of the engine and may be present in the form of abrasive solids, soluble metal soaps and catalyst residues. The maximum content of sulphated ashes in biodiesel is 0.020 % wt. set by the EN 14214 standard (EN ISO 3987 method), ANP (EN ISO 3987, ABNT NBR 6294 and ASTM D874 methods) and by ASTM D6751 (ASTM D874 method).

The sulfur content derived from the raw material generates toxic emissions affecting the performance of vehicle emission control system. Brazilian standard adopts methods of analysis described by ASTM D5453 (molecular fluorescence), EN ISO 20 846 (also by molecular fluorescence) and EN ISO 20 884 (dispersive X-ray fluorescence), with the maximum acceptable limit of 50 mg kg⁻¹. As to ASTM D6751, the maximum limit is 15 mg kg⁻¹ (ASTM D5453 method). The most restrictive is the European standard which recommends the methods given by EN ISO 20846 and EN ISO 20884, with the maximum value of 10 mg kg⁻¹.

Group I (Na + K) and group II (Ca + Mg) metal ions cause the formation of deposits of insoluble soaps, as well as catalyze polymerization reactions. They derive from catalysts employed in the production of biodiesel, as KOH, NaOH and / or CH₃ONa or CH₃OK. Calcium and magnesium may also be present as impurities in the NaOH or KOH used.

Phosphorus can damage catalytic converters used in emission control systems of the engine. They comes mainly from the raw material and, eventually, from residues of the phosphoric acid used in the neutralization. Phosphorus is determined in biodiesel via optical emission spectroscopy with inductively coupled plasma. Both the European standard (maximum 4.0 mg kg⁻¹) as the Brazilian (maximum 10.0 mg kg⁻¹) recommends the analytical method EN ISO 14107. The Brazilian standard also recommends the method ABNT NBR 15553 and ASTM D4951. The latter is also indicated in the American standard (maximum 10.0 mg kg⁻¹).

Property	Methods				
	ANP 07/2008			EN 14214	ASTM D6751
	ABNT NBR	ASTM D	EN/ISO		
Aspect	---	---	---	---	---
Density	7148	1298 4052	EN ISO 3675 EN ISO	EN ISO 3675 EN	---
	14065		12185	ISO 12185	
Kinematic viscosity (40°C)	10441	445	EN ISO 3104	EN ISO 3104	D445
Water and sediment	---	---	---	---	D2709
Flash point	14598	93	EN ISO 3679	EN ISO 2719 EN ISO 3679	D93
Distillation, 90% recovered vol.	---	---	---	---	D1160
Carbon residue	---	4530	---	EN ISO 10370	D4530
Sulfated ash	6294	874	EN ISO 3987	EN ISO 3987	D874
Sulfur content	15867	5453	EN ISO 20846 EN	EN ISO 20846 EN	D5453
			ISO 20884	ISO 20884	
Copper strip corrosion, 3h at 50°C	14359	130	EN ISO 2160	EN ISO 2160	D130
Cetane number	---	613 6890	EN ISO 5165	EN ISO 5165	D613
Cold soak filterability	14747	6371	EN 116	---	D7501
Pour point	---	---	---	---	---
Cloud point	---	---	---	---	D2500
Sodium and potassium	15553 15554 15555	---	EN 14108	EN 14108	EN 14538
	15556		EN 14109 EN 14538	EN 14109 EN 14538	
Calcium and magnesium	15553 15556	---	EN 14538	EN 14538	---
Phosphorus content.	15553	4951	EN 14107	EN 14107	D4951
Total contamination	---	---	EN 12662	EN 12662	---
Ester content	15342	---	EN 14103	EN 14103	---
Acid value	14448	664	EN 14104	EN 14104	D664
Free glycerol	15341	6584	EN 14105	EN 14105 EN	D6584
			EN 14106	14106	
Total glycerol	15344	6584	EN 14105	EN 14105	D6584
Monoglycerides	15342	6584	EN 14105	EN 14105	---
	15344				
Diglycerides	15908	6584	EN 14105	EN 14105	---
	15342				
	15344				
Triglycerides	15908	6584	EN 14105	EN 14105	---
	15342				
Methanol or Ethanol	15343	---	EN 14110	EN 14110	EN 14110
Iodine value	---	---	EN 14111	EN 14111	---
Oxidation stability at 110 °C	---	---	EN 14112	EN 15751 EN	EN 15751
				14112	
Water content	---	6304	EN ISO 12937	EN ISO 12937	---
Linolenic acid	---	---	---	EN 14103	---

Table 5. Established methods for the quality control of biodiesel in accordance with Brazilian, European and American standards.

Corrosivity to copper (copper strip corrosion) due to sulfur compounds, as well as due to free fatty acids, can lead to problems of corrosion in storage tanks and some engine parts. As acids are included in this parameter, it keeps a relationship with the acid index. The maximum acceptable values are degree 1, for standard EN 14214 and ANP, and degree 3 for ASTM D6751 (the degree number corresponds to a visual comparison with the copper strip established by ASTM D4951).

The acid number can increase or accelerate corrosion of the engine. It also measures the presence of free fatty acids and other acids and is related to the quality of the process. In injection systems that work at higher temperatures, faster biodiesel degradation may occur increasing the level of acidity and causing problems in filters. All standards have adopted as the specification limit for that parameter the value of 0.5 mg de KOH/g. The ANP standard recommended the methods ABNT NBR 14448, ASTM D664 and EN ISO 14104, and these last two methods are also adopted by European standard and American standard, respectively.

The cetane number measures the ignition quality of fuel. A low cetane number indicates a poorer ignition, which can form deposits and wear on pistons as well as provide greater fuel consumption. It depends upon the feedstock besides the oxygenate content in biodiesel. The number of cetane is measured with the aid of a special motor and the cetane index is calculated. The cetane index is a useful tool to estimate the number of cetanes according to ASTM standard [51].

The content of total glycerol and free glycerol as well as monoglyceride, diglyceride and triglyceride, reflects the quality of biodiesel. A high content of these can cause problems ranging from the formation of crystals, crusts inside the fuel storage tank, contributing to the formation or deposit of waste on pistons, injectors, valves, rings (of segments), filters, up to clogging the nozzles, decreasing the engine life. These are intermediate products of the process that ended up not reacting. The free glycerin (a byproduct) depends on the efficiency of separation of esters/glycerin.

The presence of residual alcohol in the biodiesel may cause corrosion on items of aluminum and zinc, and also can influence the flash point, reduce the cetane number and decrease the lubricity of the engine [51]. The alcohol content is determined by the chromatographic method EN ISO 14110 indicated by the standards EN 14214, ASTM D6751 and ANP. The Brazilian standard also recommends the method ABNT NBR 15343.

The iodine value is related to viscosity and cetane number, and indicates the quantitative degree of unsaturation of esters that form the biodiesel. The method suggested by the Brazilian and European standards is the same EN ISO 14111, though the latter is the only one to provide a limit to the parameter of 120 g I₂/100 g.

The oxidation stability determines the degradation of biodiesel, and mixtures thereof. It is related to the time required to degrade the biodiesel under controlled heating and in the presence of oxygen. The method adopted by the ANP standard and also EN 14214 is given in EN 14112, where the specification limit for both is 6 h at least (induction time by Rancimat method). For ASTM D6751 standard this limit is 3 hours and the recommended method is EN 15751.

In addition to the mentioned properties, other parameters may also be important and relate directly to the raw material used in the production of biodiesel: the soap content, the boiling range of esters, the peroxide index (that express the oil oxidation degree), the filterability (that express the difficulty to filter the oil before injecting into the engine) and the gum content, which expresses the amount of gums formed by polymerization of unsaturated oil components during the combustion [51].

In Brazil, the National Program for Production and Use of Biodiesel in one of its aspects provides the Program for Engine Test and Experiment, which is coordinated by the Ministry of Science and Technology (MCT). This program establishes test performances on vehicles and stationary engines and the gradual use increase of the blend biodiesel/diesel and evaluating its consequent technical feasibility. Currently in Brazil, B5 blend is used and it is extremely important to carry out tests to ensure commercial guarantees to vehicles moved with this blend and the quality of the blend commercialized. Currently in Brazil, B5 blend is used and test performing is extremely important to ensure commercial guarantees to vehicles moved with this blend and the quality of the blend commercialized.

At low temperatures, biodiesel tends to partially solidify or increase its viscosity (fluidity loss) inhibiting or even stopping the flow of fuel with the consequent clogging of filters, damaging the starting system of the engine. In tropical countries like Brazil, where most states have high temperatures throughout the year, the effects of these engines operating at low temperatures are minimized, and such problems are mainly present in countries in North America and Europe. Some properties analyzed in such conditions are: cloud point (ASTM D2500), which is the temperature at which, in a process of fuel cooling, it is observed the formation of first crystals, cold filter plugging point (EN 116), which is the temperature where the fuel ceases to be filtered when a cooling occurs and pour point (EN 3016) which is the temperature that the fuel loses its fluidity at established test conditions.

Thus, a major problem is the biodiesels from raw materials that have a high content of saturated compounds in its chain, as occurs for the tallow and the palm oil [72]. Biodiesels from raw materials of animal source when compared to those of vegetable origin have higher pour point, cold filter plugging point and cloud point.

Despite having standards for performing tests, there are no fixed and established values for these parameters. In Europe, they are measured for each country in terms of its climate. In U.S.A., they are dependent on the seasons and climate and in Brazil they depend on the states and months of the year, as shown in Table 6.

Despite the problems that can arise with the use of this fuel in cold seasons and countries with low average temperature, the biodiesel has many advantages over petroleum diesel as, it is a renewable fuel with lower emissions of particulate matters, polycyclic aromatic hydrocarbons, and sulfur compounds; storage and handling of this material is safer, since it has a flash point much higher than petroleum diesel, and also a higher flammability point which ensures a greater safety in loading/unloading and handling of this material by the drivers and operators [72].

Brazilian Federation Units	Maximum limit, °C											
	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
SP - MG - MS	14	14	14	12	8	8	8	8	8	12	14	14
GO - DF - MT - ES - RJ	14	14	14	14	10	10	10	10	10	14	14	14
PR - SC - RS	14	14	14	10	5	5	5	5	5	10	14	14

Table 6. Maximum limits of cold filter plugging point for the Brazilian Federation states according to the months of the year in accordance with the ANP resolution number 14 (May 2012). For the other federation states (not included in the table), the value remains in 19°C.

5. Metallic corrosion in biodiesel

Although biodiesel has many properties that assist in good yield as a fuel as a relatively high flash point and good lubricating properties compared to diesel, some of these properties facilitate its self-oxidation, and oxidation of the metallic materials which they are in contact. The metallic corrosion becomes extremely important since many of the engine parts are composed of variety metals such as aluminum, copper, stainless steel and alloys. The percent of aluminum in engine parts includes piston (100%), cylinder heads (70%), and engine blocks (19%). Pumps and injectors are composed of copper and its alloys. Parts composed of stainless steel include fuel filter, valve bodies, nozzle and pump ring [73-75]. The metallic corrosion may occur due to the following factors:

- biodiesel is an ester so makes hydrogen bonds with water; then it becomes much more hygroscopic compared to diesel which is composed by hydrocarbons. Water acts on the corrosion of metallic materials, or it causes the hydrolysis of biodiesel, resulting in fatty acids and glycerol which increase metallic corrosion, or it promotes microbial growth and thereby microbial corrosion [73,75-77].
- the presence of impurities like water, methanol, free glycerol, free fatty acid, catalyst residues (Na and K) due to incomplete conversion or inadequate purification can also result in metallic corrosion [73,75].
- due to its good lubricity, biodiesel dissolves more metallic parts than diesel, and these trace metals in solution enhance biodiesel degradation and promote metallic corrosion [73,75].
- metals into biodiesel like brass, copper and aluminum act as catalysts for biodiesel oxidation. Therefore, the acid number of biodiesel increases proportionally with the corrosion rate for different metals [78-81].

Generally, the corrosion tests are performed by immersion tests with metallic coupons in biodiesel [79,80,82,83]. After immersion, the weight of the coupons was measured and the corrosion was analyzed by measurement of corrosion rate, according Equation 1:

$$\text{Corrosion rate} = \frac{W \times 534}{D \times T \times A} \quad (1)$$

Where:

Corrosion rate = mpy (stands for mils (0.001 inch) per year);

W = weight loss (mg);

D = density (g cm⁻³);

A = exposed surface area (square inch);

T = exposure time (h).

Kaul et al. [80] studied the corrosivity of biodiesel from different oil sources by static immersion tests using metallic (aluminum alloy) piston for 300 days at 15 to 40 °C. In this study the authors observed that the corrosion rate varied with the chemical composition of each oleaginous. The corrosion tests were performed with *Jatropha curcas*, *Karanja*, *Madhuca indica* and *Salvadora* biodiesels. Corrosion rates values were 0.0117, 0.0058, 0.0058, and 0.1236 mpy, respectively. The *Salvadora oleoides* biodiesel presented the highest rate due to its higher content of total sulfur (1200 ppm), while other biodiesels presented lower concentrations of total sulfur (around 1 ppm), except *Madhuca indica* biodiesel (164.8 ppm). *Jatropha curcas* biodiesel was slightly more corrosive than *Karanja* and *Madhuca indica* biodiesels because of its elevated concentration of C18:2 (19–41%) fatty acid, which is more prone to oxidation due to presence of two double bonds.

The metal corrosivity also depends on the nature of the metal exposed to the biofuel. Haseeb et al. [82] performed static immersion tests with coupons of copper and leaded bronze (87% Cu, 6% Sn, 6% Pb) in palm biodiesel at room temperature (25–30 °C) for 840 h; the corrosion rates for copper and bronze were 0.042 and 0.018 mpy, respectively. Additionally, at 60 °C in 2640 h, the corrosion rates of both metals were relatively higher, 0.053 mpy for copper and 0.023 mpy for bronze. The corrosion resistance of bronze was believed to be related to the presence of alloying elements such as tin (Sn) in the alloy. These results clearly show that copper is more prone to corrosion by biodiesel.

Similar behavior was observed by Fazal et al. [83], which performed static immersion tests and evaluated the corrosion rates of copper, brass (Cu: 58.5 %; Zn:41.5), aluminum, cast iron (C:3 %; Si:1.84 %; Mn: 0.82 %; P:0.098%; S:0.089; Fe: balance) at room temperature (25–27 °C) and for 120 days in palm biodiesel. The authors verified that copper presented higher corrosion rates (0.39278 mpy) followed by brass (0.209898 mpy), which contained zinc its composition that probably reduced its corrosion. Aluminum presented higher corrosion rates (0.173055 mpy) than cast iron (0.112232 mpy). This is accordance with Geller et al. [84], which reported that copper alloys are more prone to corrosion in biodiesel than ferrous alloys.

Recently, Hu et al. [85] compared the corrosion rates of several metals in rapeseed biodiesel and proposed corrosion mechanisms through static immersion tests at 43 °C for 60 days. The obtained corrosion rates of copper, carbon steel, aluminum, and stainless steel were 0.02334,

0.01819, 0.00324, and 0.00087 mmy, respectively (which correspond to 0.9336, 0.7276, 0.1296, and 0.0348 mpy, respectively). This study indicates that copper and carbon steel presented higher corrosion rates than aluminum and stainless steel.

Using scanning electron microscope with energy dispersive X-ray analysis and X-ray photoelectron spectroscopy to analyze the effects of biodiesel on the corrosion of different metallic materials, Hu et al. [85] reported that the corrosion process of metal surfaces in biodiesel was mainly attributed to the chemical corrosion and the products after corrosion were primarily fatty acid salts or metal oxides, depending on the studied metal. Elements of copper and iron are catalysts for the decomposition of biodiesel because they enabled various chemical reactions to easily take place. According to Hu et al. [85], metals were oxidized by oxygen and active oxygen atom dissolved in biodiesel, resulting in the formation of metal oxides (CuO , Cu_2O , Fe_2O_3 , etc.). Copper and carbon steel were easily oxidized but aluminum and stainless steel were protected by films of metal oxide and then their corrosion rates were lower. The protective metal oxide layer prevented the metal surface from contact with the oxygen and atom oxygen as well as from contact with the oil sample.

Fazal et al. [86] has also found that the oxygen concentration increases with increasing temperatures, which may explain the high corrosion rates at higher temperatures. However, water and fatty acids are also responsible for metallic corrosion and need to be considered [80].

Similarly to Hu et al. [85] which carried out static immersion tests at 80 °C for 600 h in rapeseed biodiesel, Norouzi et al. [78] verified corrosion rates of 0.9 mpy for copper and 0.35 mpy for aluminum and confirmed that increase in temperature enhanced corrosion rates.

Another limiting factor that should be considered is the stirring. Fazal et al. [79] obtained corrosion rates of 0.586, 0.202, and 0.015 mpy for copper, aluminum, stainless steel, respectively, in immersion tests at 80 °C for 50 days under 250 rpm stirring in palm biodiesel. The corrosion rates presented superior values than those obtained in static immersion tests. Table 7 presents corrosion rates of different metallic materials in various biodiesels.

Analyzing the data in Table 7, palm biodiesel is less corrosive than rapeseed biodiesel. This different behavior can be correlated by the chemical composition of each biodiesel; rapeseed biodiesel presents 68.821 % oleic acid (C18:1) and 19.5927 % linoleic acid (C18:2) [78], whilst palm biodiesel presents 41.8 % oleic acid (C18:1) and 9.10 % linoleic acid (C18:2) [87].

The content of metal released to biodiesel during corrosion can be quantified. In the static immersion tests in rapeseed biodiesel performed by Hu et al. [85], 41.088 mg L⁻¹ Cu (copper coupon), 3.544 mg L⁻¹ Fe (carbon steel coupon) and 2.756 mg L⁻¹ Fe and 9.02 mg L⁻¹ Cr (stainless steel coupon) were obtained. On the other hand, Haseeb et al. [80] quantified lower amounts of metals in palm biodiesel (copper: 5 ppm Cu, bronze: 5 ppm Cu, 4 ppm Pb and 10 ppm Zn) because this biodiesel is less corrosive. According to McCormick et al. [88] the high concentration of metals in biodiesel generates higher oxidation of the biofuel. Biodiesel containing more than 6 ppm of metal exhibits very short OSI (oil stability index) induction time [88]. The negative effect of the presence of metal contaminants on the biodiesel oxidation

stability was also reported by other authors who performed experiments adding organometallic standards or powdered metals into different biodiesels [25,89-94].

Operation	Biodiesel	METALS (mpy)							Ref.
		Aluminum	Copper	Bronze	Carbon steel	Stainless steel	Brass	Cast iron	
300 days 15-40 °C static	<i>Jatropha curcas</i>	0.0117	-	-	-	-	-	-	[80]
	<i>Karanja,</i>	0.0058	-	-	-	-	-	-	[80]
	<i>Madhuca</i>	0.0058	-	-	-	-	-	-	[80]
	<i>Salvadora</i>	0.1236	-	-	-	-	-	-	[80]
60 days 43 °C static	Rapeesed	0.1296	0.9336	-	0.7276	0.0348	-	-	[85]
25 days 80 °C static	Rapeesed	~ 0.35	~ 0.9	-	-	-	-	-	[78]
50 days 80 °C 250 rpm	Palm	0.202	0.586	-	-	0.015	-	-	[78]
120 days 25-27 °C static	Palm	0.173055	0.39278	-	-	-	0.209898	0.112232	[83]
35 days 23-30 °C static	Palm	-	0.042	0.018	-	-	-	-	[82]
		-	0.053	0.023	-	-	-	-	[82]

Table 7. Corrosion rate of some metals in different biodiesels.

Fazal et al. [79] and Hu et al. [85] have reported that biodiesel is more corrosive than diesel oil (based on the corrosion rates in different metallic materials). Figure 3 displays data from both reports [79,85] on the corrosion rate of copper, aluminum, stainless steel, and carbon steel in biodiesel and diesel. See that the first work only evaluated the corrosion of copper, aluminum, and stainless steel [79], while the second work also evaluated all the previous materials and in addition carbon steel [85]. The higher corrosion rates were also confirmed by the metal release verified in diesel and biodiesel, especially copper and iron [85]. Figure 3 also indicates that biodiesel presents more corrosive behavior than diesel oil and copper is not compatible with biodiesel.

Figure 3. Corrosion rate of metals in biodiesel and diesel oil: a) adapted from Ref. [79]; b) adapted from ref. [85].

An alternative way to reduce the corrosive behavior of biodiesel is to use it as blends with diesel oil. According to Norouzi et al. [78], the greater the amount of diesel in blends, the lower corrosion rate as well as the lower total acid number (TAN). Figure 4 shows the variation of (a) corrosion rate of aluminum and copper coupons and of (b) TAN number of biodiesels exposed to these metallic materials through static immersion tests.

Figure 4. a) Corrosion rate of the Al and Cu and b) TAN of the rapeseed biodiesel-diesel blends in contact with both metals (adapted from ref. [78]).

There is a clear relationship between corrosion and TAN of biodiesel; the higher the corrosion rates the higher the TAN numbers. These results show that copper was more corrosive than aluminum and that as long the biodiesel-diesel blend was richer in biodiesel, higher corrosion rates were verified and consequently higher TAN numbers.

Some studies reported that the corrosiveness of biodiesel can be reduced by using corrosion inhibitors or antioxidants. Corrosion inhibitors act by the formation of adsorbed monolayer

films at the metal-solution interface. The common corrosion inhibitors in oil are imidazoles, primary amines, diamines, amino-amines, oxyalkylated amines, naphthanoic acid, phosphate esters, dodecyl benzene sulfonic acids, etc [95]. Amine inhibitors can reduce the dissolution of metal oxide layers into biodiesel by forming stable oxide layers on the metal surface [95]. Fazal et al. [95] reported the inhibition effect on the corrosion of grey cast iron (C: 3%, Si: 1.84%, Mn: 0.82%, P: 0.098%, S: 0.089%, Fe: balance) immersed (static) in biodiesel containing 100 ppm of ethylenediamine (EDA), n-butylamine (nBA), tert-butylamine (TBA) at room temperature for 1200 h. The corrosion rates in each case are shown in Figure 5. The results indicate that EDA is the most powerful corrosion inhibitor under the experimental conditions. However, the analysis of fuel properties revealed that the biodiesel containing EDA was more degraded.

Figure 5. Corrosion rate of cast iron (duplicate and respective standard deviation) in palm biodiesel with and without corrosion inhibitors (EDA, nBA and TBA) and their respective chemical structures (adapted from ref. [95]).

Antioxidants can be classified into two groups: chain breakers and hydroperoxide decomposers. The chain breakers have two most common types of antioxidants: phenolic and amine-types. These antioxidants present a highly labile hydrogen that is able to abstract a peroxy radical and thus stops oxidation reactions in the ester chain [96]. The chain breakers antioxidants can be re-classified into natural antioxidants (tocopherols present in vegetables oils) and synthetic antioxidants such as butyl-hydroxytoluene (BHT), tert-butylhydroquinone (TBHQ), butyl-hydroxyanisole (BHA), pyrogallol (PY), and propyl gallate (PG) [96].

Liang et al. [97] have reported that synthetic antioxidants presented superior antioxidant activity in palm biodiesel than natural antioxidants. Other authors investigated BHT, BHA, TBHQ, and natural antioxidants as potential antioxidants in soybean oil biodiesel and TBHQ presented a superior antioxidant activity [98,99]. Jain and Sharma compared the efficiency of eight synthetic antioxidants in different biodiesels and they concluded that only three antioxidants significantly increased the stability of biodiesel in the order of TBHQ > PY

> PG [96]. The addition of antioxidants can also overcome the low oxidation stability of biodiesels promoted by metal contamination (experiments performed by adding organometallic standards) in order to re-establish the required 6-hour induction time [25,90-94]. This statement is especially essential considering the presence of metals in biodiesel due to corrosion of containers and engine components.

Almeida et al. [100] evaluated the effect of the synthetic antioxidant TBHQ on the corrosive character of biodiesel against copper coupon through static immersion tests. Due to the strong catalytic effect of copper towards biodiesel oxidation, the oxidation stability of biodiesel decreased tremendously after 24 h of exposure even in the presence of TBHQ. The copper content in biodiesel continuously increased with the exposure time and the metal concentration was much higher in the non-stabilized biodiesel. Then, the presence of TBHQ decreased the corrosion rate of the copper coupon and the authors claimed that the antioxidant may have acted as a corrosion inhibitor through the formation of a protective layer on the metallic surface. Performing mass-spectrometry (MS) and MS-MS analysis of deteriorated biodiesel containing TBHQ (after the metal corrosion for 24 h and 168 h), the authors have also identified the formation of new molecules of high molecular weight formed by the association between oxidized antioxidants molecules and free radicals of long-chain molecules (fatty acid derivatives). These results give light to possible side-reactions between phenolic antioxidants and esters molecules of biodiesel under oxidative conditions.

6. Analytical methods for contaminants in biodiesel

6.1. Free and total glycerol

The determination of the content of total glycerol and free glycerol in biodiesel is required by the different regulatory agencies and upper limits are established as listed in Table 4. Glycerol is the major by-product in the biodiesel production and its removal is necessary. A high content of total glycerol (sum glycerol, mono-, di- and triacylglycerols) and free glycerol can cause problems ranging from the formation of deposits in injectors, sediments inside the fuel storage tank, reducing the engine life. The recommended method for the determination of total and free glycerol in biodiesel is gas chromatography (GC) (Table 5). Both ASTM and EN methods require a derivatization step (silylation reaction) using N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA) with a few differences in the standard solution concentrations and column temperature conditions. Table 8 and 9 present a comprehensive list of analytical methods developed for the determination of free glycerol and total glycerol, respectively. Generally, all analytical methods listed in Tables 8 and 9 required a sample preparation step. GC methods often required a derivatization step (modification of a functional group of the analyte such as the silylation reaction) which improves the chromatographic performance. Derivatization reagents (when required) are cited and in a separate column other sample preparation steps (analyte extraction procedures) are detailed in accordance with information contained in the literature.

Method	Derivatization reagents	Analyte extraction procedures	Detection limit	Ref.
Enzymatic assay	No	2 g sample + 6.0 mL of 0.1 mol L ⁻¹ HCl	Not cited	[101]
GC-FID/ MS	BSTFA	No	10 ⁻⁴ -10 ⁻⁵ % wt	[102]
HPSEC	No	No	Not cited	[103]
GC-FID	MSTFA/ pyridine	No	No cited	[104]
GC-FID/ MS	BSTFA	No	10 ⁻⁴ % wt	[105]
HPLC-PAD	No	4 g sample + 45 g H ₂ O + 50 mL hexane stirred for 30 min at 40°C, 2 h standing	1 µg g ⁻¹	[106]
GPC-RI	No	PTFE membrane filtration	Not cited	[107]
Greenhill enzymatic assay	No	Not cited	5 ppm	[108]
UV-Vis	NaIO ₄ , acetylacetone	1 g sample + 4 mL hexane + 2 mL distilled water + 2 mL ethanol; 5 min vortex-stirring, 15 min centrifugation at 2000 rpm	Not cited	[109]
HPSEC	No	No	Not cited	[110]
HPLC-RI	No	4-20 g sample + 4.5 mL distilled water; 30 min stirring, 2 h standing	4x10 ⁻⁴ % wt	[111]
GC-FID	MSTFA/ pyridine	No	Not cited	[112-114]
CE-UV-Vis-DAD	NaIO ₄	200 mg sample + 800 mg water + 200 µL chloroform; 10 min vortex-stirring, 15 min centrifugation at 2000 rpm	4.3 mg L ⁻¹	[115]
SFC-MS-UV-ELSD	No	Methanol dissolution	Not cited	[116]
IC-PAD	No	5 g sample + 45 g distilled water; 5 min shaking, 5 min standing	7x10 ⁻⁵ % wt	[117]
UV-Vis-DAD	NaIO ₄ , acetylacetone	Aqueous extraction, 30 min heating	0.011 % wt	[118]
Cyclic voltammetry	No	2 g sample + 6.0 mL water; 5 min vortex, 10 min centrifugation; C18 filtration	2.3 mg L ⁻¹	[119]
FIA-UV-Vis	KIO ₄ , acetylacetone	1 g sample + 4 mL distilled water; 30 min shaking, 5 min centrifugation at 3000 rpm	4x10 ⁻⁴ % wt	[120]

Method	Derivatization reagents	Analyte extraction procedures	Detection limit	Ref.
Enzymatic assay with amperometry	No	400 µL sample + 800 µL distilled water + 800 µL ethanol + 1600 µL heptane; 2 min vortex-stirring and centrifugation	1x10 ⁻⁵ % wt	[121]
Enzymatic assay with amperometry	No	400 µL sample + 800 µL distilled water + 800 µL ethanol + 1600 µL heptane; 2 min vortex-stirring and centrifugation	0.013 % wt	[122]
Titration	NaIO ₄ , C ₂ H ₄ (OH) ₂	Aqueous Extraction	9x10 ⁻⁴ % wt	[123]
GC-MS	BSTFA, TMCS, MSTFA	No	0.04 µg mL ⁻¹	[124]
HPAE-PAD	No	Aqueous extraction	0.5 µg kg ⁻¹	[125]
UV-Vis	NaIO ₄ , acetylacetone	Aqueous extraction	0.5 mg L ⁻¹	[126]
UV-Vis	No	Solid phase extraction	0.004 % wt	[127]
FIA-Amperometry	No	250 mg sample + 5.0 mL water; 5 min vortex, 10 min centrifugation	5 mg kg ⁻¹	[128]
Enzymatic assay with colorimetric detection	No	400 µL sample + 800 µL ethanol + 800 µL distilled water + 1600 µL heptane; 1 min vortex-mixing, 2 min centrifugation	7.1x10 ⁻⁶ % wt	[129]
FIA-PAD	No	1.0 g sample + 4.0 mL water; 5 min vortex-stirring and centrifugation	44.2 µg L ⁻¹	[130]
Cyclic voltammetry	No	Not cited	33 µmol L ⁻¹	[131]
GC-FID-MS	MSTFA	No	0.053 % wt	[132]
GC-FID	MSTFA	No	0.02-0.09 % wt	[133]

CE: Capillary Electrophoresis; DAD: Diode Array Detector; ELSD: Evaporative Light Scattering Detector; FIA: Flow Injection Analysis; FID: Flame Ionization Detector; GC: Gas Chromatography; GPC: Gel Permeation Chromatography; HPAE: High Performance Anion Exchange Chromatography; HPLC: High Performance Liquid Chromatography; HPSEC: High Performance Size Exclusion Chromatography; IC: Ion Chromatography; IR: Infrared Spectrophotometry; PAD: Pulsed Amperometric Detection; RI: Differential Refractive Index Detector; SEC: Size Exclusion Chromatography; SFC: Supercritical Fluid Chromatography; UV-Vis: Ultraviolet Spectrophotometry; BSTFA: N, O-bis (trimethylsilyl)-trifluoroacetamide; MSTFA: N-methyl-N-(trimethylsilyl)-trifluoroacetamide; PTFE: Polytetrafluoroethylene; TMCS: Trimethylchlorosilane.

Table 8. Methods for free glycerol determination in biodiesel.

Method	Derivatization reagents	Sample preparation	Detection limit	Ref.
Enzymatic assay	No	Saponification; Solid phase extraction with C8	Not cited	[101]
Greenhill enzymatic assay	No	Saponification	75 ppm	[108]
GC-FID	MSTFA	No	Not cited	[114]
IC-PAD	No	Saponification; 5 g sample + 45 g distilled water; 5 min shaking, 5 min standing	7×10^{-5} % wt	[117]
UV-Vis-DAD	NaIO_4 , acetylacetone	Saponification; Aqueous extraction, 30 min heating	0.064 % wt	[118]
Enzymatic assay with amperometry	No	Transesterification; 0.1 g sample + 3.9 mL distilled water + 5.0 mL heptane; 2 min vortex-stirring and centrifugation	1×10^{-5} % wt	[121]
Titration	CH_3NaO , NaIO_4 , $\text{C}_2\text{H}_4(\text{OH})_2$	Aqueous Extraction	0.0046 % wt	[123]
HPLC-UV-Vis	9,9-dimetoxifluorene	No	0.05 % wt	[134]
HPSEC-PAD	No	Saponification	$0.5 \mu\text{g kg}^{-1}$	[125]
UV-Vis	NaIO_4 , acetylacetone	Saponification; Aqueous extraction	1.4 mg L^{-1}	[126]
Enzymatic assay with colorimetric detection		Transesterification; 0.5 g sample + 2.0 mL distilled water + 2.0 mL heptane; 1 min vortex-mixing, 2 min centrifugation	7.1×10^{-6} % wt	[129]
GC-FID-MS	MSTFA	No	2.458 % w/w	[132]
GC-FID	MSTFA	No	0.15-0.69 % wt.	[133]

Table 9. Methods for total glycerol determination in biodiesel.

Several GC methods coupled with flame ionization (FID) or mass spectrometric (MS) detectors are listed in Tables 8 and 9 including works published since the early Nineties. The most recent contribution reported a new GC method with reduced sample preparation and analysis time (25 min elution) that can be applied for a wide range of oilseed-derived biodiesels including biodiesel from tallow, babassu, and palm kernel, which contain shorter chain fatty acids and then cannot be accurately analyzed by the EN 14105 method [133]. The EN14105 (and also the ASTM 6584 method) is based on the GC-FID method developed by

Plank and Lorbeer [104], who reported the simultaneous determination of glycerol, mono-, di-, and triacylglycerides in C18 methylic esters produced from vegetable oils by the silylation of the free hydroxyl groups using MSTFA. Analytical methods employing different modes of liquid chromatography coupled with varied detectors were also reported but in less extension. The first HPLC method employed pulsed-amperometric detection and thus it was not necessary the derivatization step [106]. Additionally, this HPLC method allows the determination of residual alcohol (methanol or ethanol) [106]. A high performance size exclusion chromatography method was developed for the simultaneous determination of the total amounts of mono-, di-, and triacylglycerides, fatty acid methyl esters, free glycerol and methanol [110]. The method is simple, robust, relatively fast, and required minimal sample treatment (no derivatization steps); however, the SEC columns are quite expensive. Preliminary results using a supercritical fluid chromatography coupled with three different detectors (MS, UV, and ELSD) were reported [116]. Separation of fatty acid methyl esters, free fatty acids, and glycerol was obtained in less than 5 min [116].

The oxidation reaction of glycerol with periodate is the basis of the first spectrophotometric method developed for glycerol determination in biodiesel [109]. The oxidation of glycerol results in the formation of formaldehyde, which is reacted with acetylacetone leading to the formation of 3,5-diacetyl-1,4-dihydrolutidine that can be measured at 410 nm [109]. Other spectrophotometric methods using a similar approach were reported in the literature [118,120,126,127]. The great advantage of the spectrophotometric methods is their low-cost, rapidness, accuracy and moderate sensitivity. A capillary electrophoresis method was developed based on the UV detection of iodate generated by the oxidation of glycerol by periodate [115]. Similarly, glycerol in biodiesel was determined by alkaline titration of formic acid generated by the oxidation of glycerol by periodate, which can be considered a very simple method easily assessed by local producers [123].

Electrochemical approaches (cyclic voltammetric, amperometric and pulsed-amperometric detectors) were also developed and coupled with chromatographic techniques, flow-injection methods, and enzymatic reactions. Electrochemical methods present high sensitivity, selectivity, can be easily miniaturized and require portable commercially-available instrumentation. Additionally, glycerol can be electrochemically detected without the use of derivatization reagents. Amperometric detection was often applied due to the easiness of its association with flow techniques such as chromatography or flow-injection methods [106,117,125,128,130]. The selection of amperometry or pulsed-amperometry is more related to the electrochemical oxidation of glycerol at different working electrode materials. Enzymatic assays employed enzymes which specifically convert glycerol to dihydroxyacetone phosphate generating H_2O_2 and consuming oxygen. Then, H_2O generation or oxygen consumption can be easily monitored by electrochemical techniques (similarly to portable glucose-sensors) as well as using colorimetric assays (the commercial-available Greenhill assay provides the formation of a quinoneimine dye that shows maximum absorbance at 540 nm) [108]. The development of such kits for fast and low-cost monitoring of glycerol in biodiesel is an alternative to GC methods which employ bulky instrumentation and organic solvents during the derivatization step. However, enzymes require special condition of storage and

limited shelf-life time. To overcome such a drawback, the development of (electro)chemical sensors based on the direct detection of glycerol is promising.

Analytical methods developed for the determination of total glycerol in biodiesel (Table 9) typically reported the conversion of mono-, di-, and triglycerides into glycerol by saponification or transesterification reactions except GC or HPLC methods which separate and directly quantify each component present in biodiesel. Other analytical methods developed for the determination of free glycerol (listed in Table 8) can also be readily adapted to perform the determination of total glycerol.

6.2. Trace metals

The presence of metals in biodiesel can be arisen from catalyst residues (Na, K, Ca, Mg) and due to corrosion of storage tanks and automotive engine parts (e.g. Al, Cu, Cr, Fe, Mn, Pb, Zn, etc.). Metal ions cause the formation of deposits of insoluble soaps, as well as catalyze polymerization reactions of biodiesel degradation. In this way the different regulatory agencies establishes upper limits as listed in Table 4 for group I (Na and K) and group II (Ca and Mg) cations. However, no limits are established for transition metals which are strong catalysts for biodiesel oxidation even at trace concentrations as previous works have reported [89-94]. Therefore, analytical methods capable of monitoring trace metals in biodiesel are required and this information can be correlated with biodiesel oxidation stability. Table 10 presents a list of analytical methods developed for the determination of metals in biodiesel including sample preparation steps when required and detection limits.

Atomic absorption spectrometry (AAS) and inductively coupled plasma optical emission spectrometry (ICP OES) are the recommended technique by Brazilian and European standards (Table 5). The first analytical methods reported for the determination not only of Na, K, Ca and Mg as well as other metals were the spectrometric methods such as the recommended techniques AAS and ICP OES. AAS coupled to graphite furnace (electrothermal atomization) provides higher sensitivity and for this reason it has been applied for metal determination in biodiesels, although relatively higher cost and longer analysis time are verified. The main advantage of ICP OES is the lower chemical and spectral interferences and multi-element determination; dozens of elements can be analyzed at once after simple sample dilution, including phosphorus and sulfur which are also regulated by European, American and Brazilian norms (Table 4 and 5). However, the overall analysis costs can be so elevated that this method is not available to all analytical laboratories of quality control. ICP MS provides higher sensitivity than ICP OES if sub-ppb levels are required. Most of the spectrometric methods require very simple preparation steps such as sample dilution or sample microemulsion with surfactants, which is an elegant strategy to avoid sample digestions using of concentrated acids. Pre-concentration steps can be applied for AAS with flame atomization which is a low-cost spectrometer but does not provide low detection limits generally required for trace metal determinations. Then pre-concentration steps supplies the low sensitivity of flame atomic absorption (or emission) spectrometers. More details on the use of spectrometric methods to determine metals and metalloids in automotive fuels (including biodiesel) can be found in a recent review [156].

Method	Analyte	Sample preparation	Detection limit	Ref.
Inductively coupled plasma optical emission spectrometry	Ca, K, Mg, Na	Sample dilution in kerosene	0.4 – 0.9 mg kg ⁻¹	[135]
Inductively coupled plasma mass spectrometry	31 elements	Sample dilution in kerosene	0.0109 – 22.7 µg kg ⁻¹	[136]
Inductively coupled plasma optical emission spectrometry (axial viewing)	Ca, Mg, K	Sample dilution in ethanol	0.005 – 0.1 µg g ⁻¹	[137]
Inductively coupled plasma optical emission spectrometry (axial viewing)	Ca, Cu, Fe, Mg, Mn, Na	Sample emulsion with Triton X-100 and water	0.007 – 0.165 µg g ⁻¹	[138]
Flame atomic absorption spectrometry	Na, K	Sample microemulsion with n-pentanol and Triton X-100	0.1 and 0.06 µg g ⁻¹	[139]
Flame atomic emission spectrometry	Na, K	Sample microemulsion with n-pentanol and aqueous acid solution	0.1 µg g ⁻¹	[140]
Graphite-furnace atomic spectrometry	As	Sample microemulsion with n-pentanol and aqueous acid solution	0.3 mg kg ⁻¹	[141]
Graphite-furnace atomic spectrometry	Cu, Pb, Ni, Cd	Focused microwave wet digestion	Not cited	[142]
Cold-vapor atomic fluorescence spectrometry	Hg	Sample microemulsion with n-pentanol and Triton X-100	0.2 µg kg ⁻¹	[143]
Inductively coupled plasma optical emission spectrometry	28 elements	Microwave acid digestion	0.1 – 136.5 µg g ⁻¹	[144]
Inductively coupled plasma optical emission spectrometry and mass spectrometry	19 elements	Microwave acid digestion	0.001 – 0.4 µg g ⁻¹	[145]
Graphite-furnace atomic spectrometry	Ni, Cd	Sample microemulsion with Triton X-100 and acid aqueous solution	0.9 and 0.1 µg L ⁻¹	[146]
Flame atomic emission spectrometry	Cu, Ni, Zn	Pre-concentration by adsorption on chitosan microspheres	Not cited	[147]
Flame atomic emission spectrometry	Na, K	Sample dilution in ethanol	2.16 and 2.00 mg kg ⁻¹	[148]
Inductively coupled plasma mass spectrometry	32 elements	Microwave acid digestion	10 ⁻⁶ mg kg ⁻¹	[149]
Potentiometry	K	Not required	0.01 ppm	[150]
Potentiometry	K	Liquid-liquid extraction	1.9 × 10 ⁻⁵ mol L ⁻¹	[151]
Ion chromatography	Na, K, Ca, Mg	Liquid-liquid extraction, heating, sonication	0.11 – 0.42 mg kg ⁻¹	[152]
Ion chromatography	Na, K	Liquid-liquid extraction	Not cited	[153]
Capillary electrophoresis	Ca, K, Mg, Na	Liquid-liquid extraction	0.07 – 0.14 mg L ⁻¹	[154]
Square-wave stripping voltammetry	Sn	Dry-ashing decomposition	0.14 µg L ⁻¹	[155]
Potentiometric stripping analysis	Cu	Sample dilution in hydroethanolic electrolyte	200 ng g ⁻¹	[81]

Table 10. Methods for trace metal determination in biodiesel.

Separation techniques such as ion chromatography and capillary electrophoresis were applied for simultaneous determination of cations in biodiesel after a simple liquid-liquid extraction [152-154]. The capillary electrophoretic (CE) method presented faster separation of ions, employed very low sample volumes and capillaries presents much lower cost than IC columns. Moreover, the CE method can also be used for glycerol determination after its chemical conversion by periodate as a previous method described [115].

Electroanalytical methods for metal determination in biofuels were recently reviewed [157,158]. Potentiometry is a well-known technique which allow sensitive detection of K in aqueous solutions and were also applied for biodiesel analysis after a liquid-liquid extraction [151]. An ion-selective electrode sensor associated with cellophane semi-permeable membrane was applied for K determination in biodiesel without a sample preparation step [150]. The elimination of the sample preparation step is a tendency in modern analytical methods since this step may provide sample contamination, analyte losses, and high analysis time, whose characteristics are avoided when developing an analytical method for routine analyses. Electroanalytical potentiometric stripping analysis was applied for Cu determination in biodiesel after its dilution in hydroethanolic electrolyte [81]. This is the first report on the use of electroanalysis for metal determination in biodiesel. Electroanalytical methods provide real advantages for routine analysis such as high sensitivity and selectivity employing a low-cost portable instrumentation. Similar technology applied for gluco-sensors (using disposable sensors) can be extended to on-site analysis of biodiesel, aiming not only the determination of trace metals but also other species in the biofuel [157,158].

6.3. Other trace contaminants

Other trace contaminants can be found in biodiesel such as water (moisture), residual alcohol (typically methanol and ethanol), sterols, phosphorus and sulfur. Table 4 shows the upper limits established for water and residual alcohol and Table 5 lists the recommended methods for the analysis of each parameter. The GC method developed Mittelbach et al [105] can also be applied for the determination of alcohol residues in biodiesel as well as the HPLC-PAD method [106]. A flow analysis method coupled to a membrane extraction was developed for methanol determination in biodiesel [159]. Methanol was detected by spectrophotometry (at 240 nm) after reaction with alcohol oxidase in aqueous solution [159].

Sterols are minor components found in animal fats and vegetable oils and that occur in biodiesel due to their solubility in the biofuel. Sterol glycosides can accelerate precipitate formation in biodiesel even at room temperature and block fuel filters [160-162]. HPLC methods [160,161], mass spectrometry [161], and a GC MS method [162] have been exploited for the determination of sterol glycosides. More information about the use of chromatographic techniques for biodiesel and biodiesel blends (not only including the analysis of sterols) can be found in a recent review [163].

The contents of phosphorus and sulfur in biodiesel are additional parameters regulated by European, American and Brazilian norms. Some ICP OES methods reported for metal deter-

minations in biodiesel (Table 10) were also applied for the determination of phosphorus and sulfur [136,144,145,149]. A graphite-furnace AAS method was developed for the direct determination of phosphorus in biodiesel using a solid sampling accessory [164]. Simpler methodologies using spectrophotometry were developed [165,166]. In the first work biodiesel samples were mineralized (dry ashing) and their residue containing phosphate was reacted with 1-amino-2-naphthol-4-sulfonic acid to form a blue molybdenum complex [165]. In the second work biodiesel samples were digested using an acid mixture and the obtained solution containing phosphate was mixed with ammonium molybdate and potassium and antimony tartrate ion to form phosphomolybdic acid (yellow) [166]. An electroanalytical method was developed for phosphorus determination in biodiesel using a phosphomolybdic modified electrode [167]. X-ray fluorescence [168], and improvements on ICP OES [169] and ICP MS [170] methods for sulfur determination in biodiesel have been reported.

Analytical methods for monitoring of the transesterification reaction and for the determination of fatty mono-alkyl esters in biodiesel-diesel blends or in pure biodiesel as well as other parameters such as biodiesel oxidation and thermal stability are well-addressed in the review by Monteiro et al. [171] and is not discussed in this text.

7. Conclusion and perspective

Much effort has been dedicated to the development of new methods for biodiesel production and improvement of the traditional ones. A large variety of raw materials have been investigated for biodiesel production in the world, and Brazil has favourable environment and climate conditions in this scenario for the development of biodiesels from new oilseeds although the large Brazilian soybean biodiesel production. The production of biodiesels from new oilseeds has economic impact since small local producers can contribute for biodiesel production and the special characteristics of these biodiesels may improve the physical-chemical properties (e.g. acidity, oxidation stability, viscosity, etc.) of other biodiesels by using blends of biodiesels. Microalgae for biodiesel production is a promising sustainable source which has received increasing interest from researchers worldwide. Metallic corrosion is a real problem in storage stability of biodiesels and inside diesel engines and thus requires constant investigation. The monitoring of trace metals in the biofuel may provide information on how trace metals really affect biodiesel oxidation stability. Moreover, the presence of antioxidants and corrosion inhibitors in biodiesel plays key role on the metallic corrosion and deeper investigations would indicate the real need of additives in the biofuel and their required concentration. Modern analytical methods developed for monitoring contaminants in biodiesel have been reported and the creation of portable devices for the quality control of biofuels is a promising tendency. In the future, any local producer would have access to fast and reliable technologies to certificate the quality of its biodiesel during the production process. Additionally, the real-time and on-site analysis of biofuels is desirable at local gas stations which receive large volumes of biodiesel and need to quickly check the real quality of the biofuel.

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