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Research Article

Characterization of the Fatty Acids Present in Wastewaters from Production of Biodiesel Tilapia

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Biodiesel obtained from oil extracted from the viscera of tilapia is a viable alternative in the replacement of petroleum fuels. However, during the purification step is performed biodiesel washing water is performed, which generates high effluent pollutant loads due to the reagents used and the very composition of the raw material. This study aims to characterize the fatty acids present in water from washing of the process of purifying biodiesel tilapia (*Oreochromis niloticus*). Fatty acid compositions were determined using gas chromatography (GC-FID). The results showed that the fatty acids present in greater quantities in the effluent were lauric (C12: 0), followed by myristic (C14: 0), palmitic (C16: 0), oleic (C18: 1), stearic (C18: 0), linolenic (C18: 3), and linoleic (C18: 2) acids. Therefore, the levels of oil and grease found in the rinse water from washing of the oil biodiesel tilapia are far above the allowed values above; thus they do not comply with Brazilian federal regulations.

1. Introduction

Biodiesel is a biodegradable fuel derived from renewable sources and obtainable through different processes, such as cracking, esterification, or transesterification. It can be produced from animal fat or vegetable oils [1].

In Brazil, biodiesel in the energy matrix was introduced by Law 11,097 from January 13, 2005, determining its mandatory use in blends with fossil diesel at a rate of 2% (B2) beginning in 2008 and 5% (B5) beginning in 2013 [2]. It establishes the National Agency of Petroleum, Natural Gas and Biofuels (ANP) that is responsible for production and commercialization of biodiesel [3].

The transesterification process is now used more and more commercially viable for production of biodiesel [4, 5]. It consists of a chemical reaction of oils or animal fats with short chain alcohols (ethanol and methanol) in the presence of a basic catalyst (sodium hydroxide or potassium hydroxide).

Several feedstocks have been used for biodiesel production [6]. Soy has been a major source of biodiesel production in Brazil [7, 8]. But different raw materials such as castor bean [9, 10], sunflower [11], babassu [12], canola [13], fish oil [14, 15], pork fat [16], frying oils [17], and microalgae [18] have been applied.

The Nile tilapia (*Oreochromis niloticus*) is now the most widely cultivated species of fish in Brazil. The production of tilapia in the state of Ceará is around 150 tons/year. Castanhão dam, located in the Jaguaribara, is responsible for 21% of this production. The use of the viscera of tilapia, waste with high content of lipids, and that which would be wasted emerges as an excellent feedstock for biodiesel production, helping to minimize the problems of pollution being generated by a lack of suitable target for this waste.

Although considered to be environmentally clean, one of the major drawbacks in the production of biodiesel by transesterification (alkaline catalysis) is the generation of

large quantities of wastewater containing soaps, alcohols, and inorganic impurities from the purification by aqueous washing step [19]. Considering that the washing of biodiesel is one of the most important and also one of the most critical issues, the importance of characterization and treatment of waste water resulting from washing process is clear [17].

One of the important parameters to be evaluated is the oil and grease (hexane soluble substances), which may contain compounds of difficult degradation in the environment. Moreover, when it is discarded on the soil, it can reach water sources, surface runoff, or infiltration, forming a dense layer on the surface, which prevents gas exchange and oxygenation, causing the death of species in aquifers, and becoming a problem for rivers and lakes which may also insulate the soil.

Although studies of different processes of production of biodiesel have been growing in recent years, research on the composition of the water washing biodiesel from fish oil is very limited. This study aims to identify and quantify the fatty acids which make up the rinse water generated in the purification step; the aqueous wash of the biodiesel produced with the oil extracted the viscera of tilapia (*Oreochromis niloticus*).

2. Material and Methods

2.1. Preparation. The rinse water was obtained from the process of producing biodiesel on a laboratory scale using oil as a raw material extracted from the viscera of tilapia (*Oreochromis niloticus*). The sodium hydroxide (NaOH) and methanol (CH_3OH) were used as catalyst and transesterification agent, respectively.

The extraction of oil from the viscera of tilapia was conducted monthly in biodiesel plant of the Reference Laboratory Biofuels (LARBIO) in the Fundação Núcleo de Tecnologia Industrial do Ceará (NUTEC), Brazil. The oil obtained from the viscera of tilapia, with the prior removal of bile acids, was extracted following the extraction conditions optimized by means of laboratory experiments by Dias [14], the hot extraction method indirectly.

The transesterification reaction was conducted by weighing 200 g of oil extracted from the viscera of tilapia and pretreated in a volumetric flask of 1.0 L flat-bottomed with two mouths, which was taken for heating plate heated under magnetic stirring for homogenization of the mixture at a temperature of 60°C, which was added to a mixture of methanol and sodium hydroxide for 45 minutes with molar ratio MeOH/oil (6 : 1) and % NaOH 0.50. At the end of time, the reaction mixture was transferred to a separation funnel of 500 mL for the phase separation methyl ester/biodiesel (upper and lower density) and glycerine phase (lower and higher density).

After the transesterification reaction and separation of the biodiesel and glycerin layer, the washing of biodiesel proceeded. Figure 1 shows the process of biodiesel production and collection of washing samples.

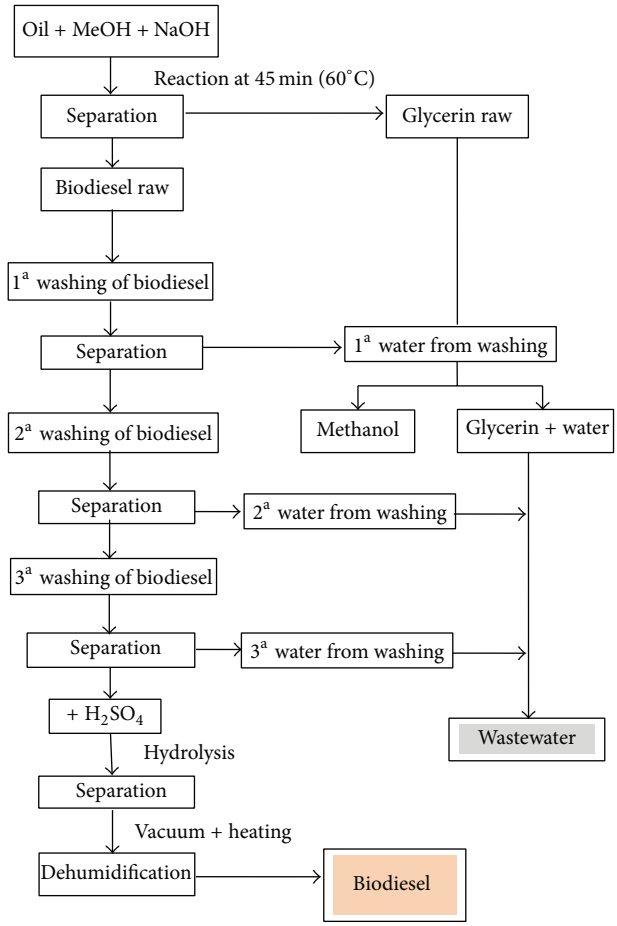

Figure 1: Process for producing biodiesel and the water from washing.

2.2. Determination of Oil and Grease in the Water from Washing. The determination of oil and grease (or substances soluble in hexane) was performed by the extraction-solvent (hexane) at Soxhlet, as recommended by APHA [20]. The method is suitable for determination of biological lipids and hydrocarbons found in natural waters, domestic and industrial discharges.

2.3. Identification and Quantification of Fatty Acids by GC/FID. After the oil extraction of the water was done esterification reaction, according to the procedure described by Instituto Adolfo Lutz [21] for subsequent injection gas chromatography.

Analysis of the fatty acids, present in the washing water from the biodiesel and oil extracted from the viscera of tilapia, was performed by gas chromatography with flame ionization detector (GC-FID, Thermo Scientific, model FOCUS), equipped with a capillary column (Carbowax, 30 m length × 0.25 mm ID; 0.25 μm film thickness), detector temperature at 280°C, injector temperature at 250°C in split mode (1 : 50), and 2.0 μL of the sample volume. The temperature program was as follows: 70°C rising to 240°C at 3°C/min, maintained for 10 minutes. All tests were performed using nitrogen as carrier gas at flow rate of 1.0 mL/min.

The fatty acids quantification was carried out using the area normalization method with the correction factor [21],

Table 1: Physicochemical characterization of the viscera of tilapia oil.

Indices	Units	Results (average)
Acidity index	mg KOH/g	0.07 ± 0.02
Saponification index	mg KOH/g	133.47 ± 0.12
Peroxide index	meq/kg	3.32 ± 0.06
Iodine index	gI_2/g	70.96 ± 0.12

Table 2: Concentration of oils and greases obtained in the 1^a, 2^a, and 3^a wash waters.

Washing	Oils and greases (mg/L)
1^a	2.075 ± 525
2^a	4.833 ± 215
3^a	3.408 ± 102

which is used to convert the peak areas in mass percentages of the components. The conversion factors were calculated of the chromatogram obtained from mixture of methyl esters (FAME Mix C8–C24, Sigma, Brazil) under the same conditions of the samples analyzed

3. Results and Discussion

3.1. Characterization of Crude Oil Viscera Tilapia. Table 1 shows the average results obtained from the parameters of physicochemical characterization of the viscera of tilapia oil. The transesterification reaction is directly influenced by the quality of the oil. For biodiesel production using basic catalyst it is recommended that the oil has a ratio of less than 2.0 mg KOH/g and containing less than 0.5% moisture acidity.

3.2. Determination of Oils and Greases in Wastewater. In the determination of oil and grease the amount of a specific substance is not measured, but a group of substances with similar physical characteristics that are soluble in hexane. It is considered, therefore, as oil and grease, hydrocarbons, fatty acids, soaps, fats, waxes, oils, and any material extracted by the solvent of an acidified sample.

Table 2 shows the results of the average values of oils and greases obtained for the 1^a, 2^a, and 3^a wash waters. Based on the results (Table 2), it was observed that the levels of oil and grease in ascending order were $1^a < 3^a < 2^a$ at wash water. This can be justified by the drag of the first compounds that have a higher affinity for water (the polar compounds) such as methanol, the residue of the catalyst (sodium hydroxide), and compounds containing carbon-phosphorus (C–P) bonds and carbon-nitrogen (C–N) bonds. The largest value in the 2^a wash water would be due at the beginning of drag by stirring with water washing, organic compounds, for example, fatty acids of longer chain.

Similar results obtained by Grangeiro [17] showed that water washing (1^a and 2^a) of produced biodiesel from soybean oil (1.225; 1.855 mg/L) and frying (1.105; 1.515 mg/L) contain higher levels of oils and greases, respectively.

The high level of oil and grease found in the wash water occurs due to conversion of about 97% transesterification reaction (FFA) during the production of biodiesel, with a small percentage (3%) of unconverted fat. Moreover, the cleaning waters present soap residue (formed during the transesterification reaction), glycerine, and mono-, di-, and triglycerides unreacted.

Gomes [22] used a combination of enzymatic hydrolysis and chemical esterification to produce biodiesel from fish. The authors found a percentage of 56.57% in the final conversion (FFA). Oliveira et al. [23] verified that transesterification of *Moringa* oil in basic means was satisfactory giving a yield of 83.68% biodiesel. This yields approaching other oilseeds such as cotton (92.2%) and sunflower (98.6%) and exceeds the value foundfor palm oil (74.8%).

Table 3: Fatty acid composition from the wash waters (total mixture) of the biodiesel obtained from viscera of tilapia.

Fatty acid	Time (min)	% (w/w)
C8:0 (*caprylic acid*)	5.1	1.3
C10:0 (*capric acid*)	8.5	1.8
C12:0 (*lauric acid*)	12.5	34.6
C14:0 (*myristic acid*)	16.4	24.9
C16:0 (*palmitic acid*)	20.0	14.4
C16:1 (*palmitoleic acid*)	20.4	0.0
C18:0 (*stearic acid*)	23.3	3.2
C18:1 (*oleic acid*)	23.6	7.1
C18:2 (*linoleic acid*)	24.3	2.2
C18:3 (*linolenic acid*)	25.4	7.0
C20:0 (*arachidic acid*)	26.4	0.0
C22:0 (*behenic acid*)	29.3	0.0
C22:1 (*erucic acid*)	29.6	0.0
C24:0 (*lignoceric acid*)	32.0	0.0
∑ Saturated	—	**80.2**
∑ Insaturated	—	**16.3**
Total	—	**96.5**

3.3. Analysis of Fatty Acids of the Biodiesel by GC-FID. Gas chromatography with flame ionization detector (GC-FID) or coupled to mass spectrometry (GC-MS) has been the most common techniques for determination of methyl esters in biodiesel [7, 24].

The identification and determination of the fatty acids in the composition of the biodiesel were determined in the mixture of the third wash waters. These samples have been subjected to the esterification process and subsequent injection into the GC-FID system. Figure 2(a) shows the chromatogram of the fatty acid standards (C8–C24) and Figure 2(b) the fatty acids found in the mixture of water washing. It is observed that the fatty acids present in larger quantities were lauric (C12: 0), followed by myristic (C14: 0), palmitic (C16: 0), oleic (C18: 1), stearic (C18: 0), linolenic (C18: 3), and linoleic (C18: 2) acids.

Table 3 shows the fatty acid composition of the mixture obtained in the first washing of biodiesel tilapia. According to the results, it is observed that lauric acid (C12: 0) is the major component with 34.6% of the composition. Saturated fatty

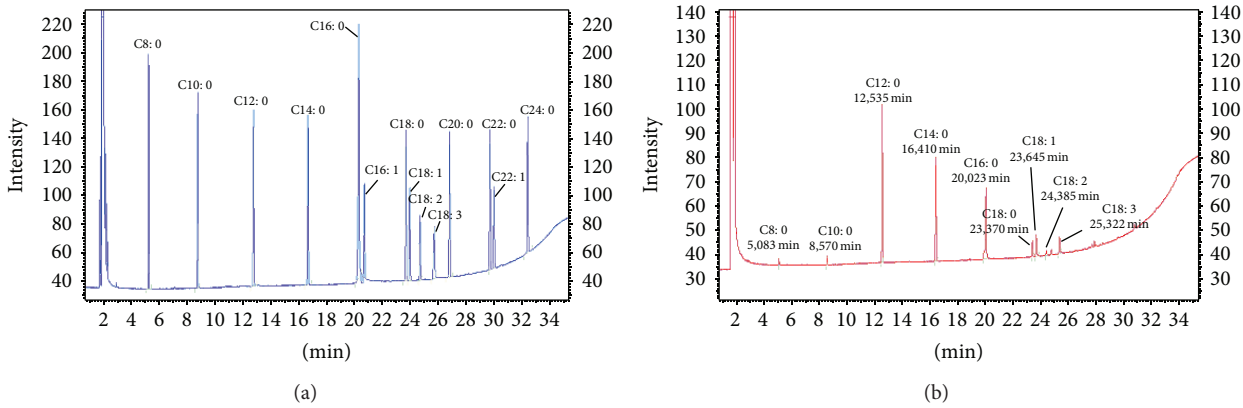

Figure 2: Chromatogram: (a) standards of fatty acids (C8–C24), (b) mixture of 1^a, 2^a, and 3^a wash waters from biodiesel.

acids showed higher content (80.2%), characteristic of the composition of animal oils [16].

According to Dias [14] oleic acid (C18: 1) is a major fatty acid present in the composition of oil extracted from the viscera of tilapia. Regarding the washing, it is observed that oleic acid is present but in smaller proportions (7.1%). This can be explained by the saponification index (IS), which is the mass of potassium hydroxide (KOH) required to saponify 1.0 g of fatty material (oil), which is inversely proportional to molecular weight of the glyceride. That is, the higher the molecular weight of the glyceride, the lower its rate of saponification (IS).

The number of carbons of the fatty acid has great influence on the IS, with the same one not being checked against the unsaturation for the same number of carbons. There is a relationship between IS and sodium hydroxide (NaOH) which is also present in the washing water.

Grangeiro [17] verified that the majority of fatty acids coming from oils and grease present in the washing water of soy biodiesel and frying were the linoleic acid and the lowest concentrations of palmitic acid. Water of soy biodiesel and frying was the linoleic acid and the lowest concentrations of palmitic acid.

3.4. Effluent Emission Standards and Treatment. The analysis of the content of oils and greases is widely used as a parameter of water quality. Disposal control of oil and grease present in the water which originated from the biodiesel production process is of great importance, because it is a parameter required by Brazilian law.

The variation of oil and grease content for 1^a, 2^a, and 3^a wash water for one year is illustrated in Figure 3. It is observed that there is a greater variability for the 2^a and 3^a wash waters. The variability can be explained by the presence of less soluble compounds which are less extracted by water washing.

CONAMA Resolution N° 430/2011 establishes standards for effluent discharge concentrations of oil and grease less than 20.0 mg/L and 50.0 mg/L for vegetable oils and animal fats, respectively [25]. Therefore, the levels of oil and grease (2.075–4.833 mg/L) found in the rinse water from washing the oil biodiesel tilapia are far above the allowed values above;

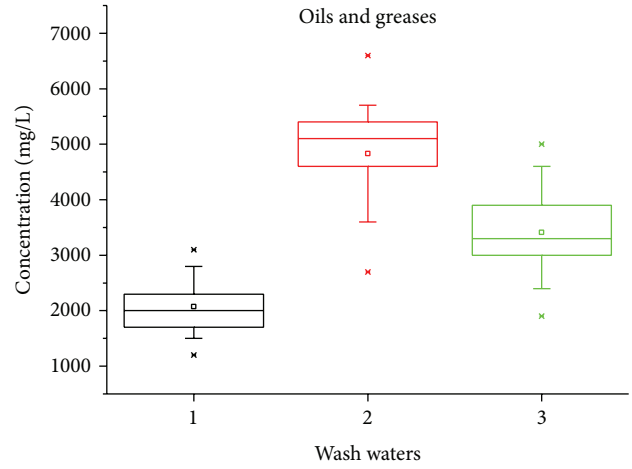

Figure 3: Box-plot variability for levels of oil and grease present in 1^a, 2^a, and 3^a wastewaters from biodiesel.

thus, they do not comply with federal regulations. These results show that despite the low solubility of oils and greases, they appear as wastewater generated in the purification of biodiesel, regardless of type of raw material. In the decomposition process the presence of oils and greases reduces the dissolved oxygen raising the biochemical oxygen demand (BOD) and chemical oxygen demand (COD), changing the aquatic ecosystem.

Several types of treatment have been used to minimize the impact of these wastewaters. Meneses et al. [26] used electrocoagulation/flotation for the treatment of effluents from biodiesel. According to research, about 99.23% of oil and grease present in the effluent were removed after treatment. Other studies indicated a possibility of using bacteria "biofixed" (concentrated inoculum) to improve the operation of grease traps and biological treatments to relieve over loaded; however this technology is poorly developed in Brazil [27]. Research conducted by Jaruwat et al. [27] showed that the combined treatment using chemical recovery and electrochemical treatment completely removed COD and oil and grease and reduced BOD levels by more than 95.0%.

The combination of physical-chemical and biological process can increase the efficiency of the wastewater treatment. According to Siles et al. [28], the combination of acidification-electrocoagulation with anaerobic digestion might be a good alternative to improve the quality of the effluent derived from biodiesel manufacturing.

According to Veljković et al. [29], proper acidification and chemical coagulation/flocculation or electrocoagulation remove grease and oil successfully but they are unsuccessful in removing COD.

The obtained results by De Gisi et al. [30] after the treatment of wastewater derived from a biodiesel fuel (BDF) production plant with alkali-catalyzed transesterification showed a COD removal percentage of more than 90% for the wastewater considered. The investigated wastewater treatment plant consisted of the following phases: primary adsorption/coagulation/flocculation/sedimentation processes, biological treatment with the combination of trickling filter and activated sludge systems, secondary flocculation/sedimentation processes, and reverse osmosis (RO) system with spiral membranes.

4. Conclusions

The results obtained showed that the wastewater showed high levels of oils and greases, which means they are effluents with high polluting load. The oil and grease present in the washing water come from the biological lipids (fats of tilapia), which are organic compounds consisting of fatty acids. The rinse water showed values of oils and greases in violation of state environmental laws (CONAMA 430/2011) and therefore cannot be discharged into any receiving body.

Disclosure

The authors confirm that the paper has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. They further confirm that the order of authors listed in the paper has been approved by all of them.

Conflict of Interests

The authors wish to confirm that there is no known conflict of interests associated with publication of the paper. They confirm that they have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing they confirm that they have followed the regulations of their institutions concerning intellectual property.

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