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Direct transesterification of microalgae biomass and biodiesel refining with vacuum distillation

Simonet Torres[a,*], Gabriel Acien[d], Francisco García-Cuadra[d], Rodrigo Navia[b,c]

[a] CEDENNA, Universidad de Santiago de Chile, USACH, Casilla 40, Santiago C.P. 33, Chile
[b] Department of Chemical Engineering and Scientific and Technological Bioresources Nucleus (BIOREN), Universidad de La Frontera, Casilla 54-D, Temuco, Chile
[c] Centre for Biotechnology and Bioengineering (CeBiB), Universidad de La Frontera, Casilla 54-D, Temuco, Chile
[d] Department of Chemical Engineering, University of Almería, 04120 Almería, Spain

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ABSTRACT

The objective of this study was the use of vacuum distillation to increase fatty acid methyl ester (FAME) content and quality of microalgae biodiesel produced through direct transesterification. Microalgae biodiesel obtained from direct transesterification of microalgae (crude biodiesel) has a FAME content of 64.98 ± 2.88%, viscosity of 17.7 ± 0.17 (mm^2/s), and a humidity level of 3.72%. As biodiesel's properties are related to FAME content, to increase FAME content and produce higher quality biodiesel two vacuum distillation experiments were conducted using different vacuum conditions. The best results were obtained in experiment 2 with two consecutive distillations, where FAME content increased from 64.98 ± 2.88% in crude biodiesel to 85.50 ± 2.60% in the D2.2 fraction, while viscosity decreased from 17.70 ± 0.17 (mm^2/s) in crude biodiesel to 3.76 ± 0.01 (mm^2/s) in the D2.2 fraction. Vacuum distillation, therefore, may represent an excellent alternative for the purification of microalgae-based biodiesel.

1. Introduction

Biodiesel is a mixture of fatty acid methyl esters (FAME) synthesized from vegetable fats, animal fat or frying oil [1]. The oil most frequently used to produce biodiesel globally is canola oil, followed by sunflower oil, palm oil, and soybean oil [2]. However, the need for fertile land to meet the demand for biodiesel production has resulted in environmental damage and deforestation in countries such as Malaysia, Indonesia, and Brazil. This situation has stimulated the search for alternative raw materials for biodiesel production, such as non-edible oils including *Jatropha curcas*, *Pongamia pinnata*, and *Madhuca indica* oil, among others. The advantage of these raw materials is that their cultivation doesn't require agricultural land [3–5]. Waste cooking oil is another alternative for biodiesel production, but it cannot be constantly supplied in adequate amounts to meet worldwide demand for biodiesel.

Numerous bibliographical reviews have been published in recent years related to biodiesel production from microalgae [6,7], including microalgae oil extraction methods for biodiesel production [8], microalgae's potential for biofuels production [9], the production process for obtaining biodiesel from microalgae biomass [10], and evaluation of microalgae biodiesel production in the laboratory and in pilot scale projects [11,12]. Microalgae are photosynthetic eukaryotic organisms capable of fixing CO_2 and transforming it into biomass with high lipid content. In addition, they grow rapidly, with varieties that can be cultivated in fresh, marine, and/or wastewater. Compared to biodiesel made from vegetable oils, microalgae do not require agricultural land for cultivation and are highly productive year-round. While crops such as canola, soybean, and Jatropha can produce 446–636 L lipids/ha, 1190 L lipids/ha and 1892 L lipids/ha, respectively, microalgae can generate up to 58,700 L lipids/ha, based on 30% lipid content [6]. Despite this high level of lipid productivity per hectare, microalgae biodiesel is still not commercially available and soybean is the most commonly used feedstock in biodiesel production today.

According to the literature, the lipid content of microalgae varies from 30 to 70% [6,13–14]. Recent studies of strains such as *Nannochloropsis gaditana* grown in large-scale systems have been found to have a lipid content of 20–25% [15–16]. The literature has also demonstrated that microalgae are comprised of a wide variety of lipids including saponifiable lipids (which can be converted into biodiesel) and non-saponifiable lipids. The chemical similarity of saponifiable and non-saponifiable lipids prevents selective extraction, resulting in a crude biodiesel that contains other lipid components such as carotenoids, chlorophylls, phospholipids and waxes, among others [10,8]. Only recently have some efforts been made to selectively extract

* Corresponding author at: CEDENNA, Universidad de Santiago de Chile, USACH, Casilla 40, Santiago C.P. 33, Chile.
E-mail address: simonet.torres@usach.cl (S. Torres).

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esterifiable lipids from microalgae [17]. In that research, the authors found that a higher extraction yield of esterifiable lipids was obtained using both a chloroform–methanol mixture and a petroleum ether–-methanol mixture. At the laboratory scale, scientific research has focused on optimizing processes for producing biodiesel on the one hand by extracting lipids from microalgae, and on the other hand through direct transesterification of wet microalgal biomass.

According to the EN 14214 standard, biodiesel should have a 96.5% FAME percentage, which microalgae biodiesel does not achieve [10]. The objective of the study described in this paper is to apply a vacuum distillation process to refine biodiesel produced by direct transesterification of wet microalgal biomass. The difference in the boiling points of fatty acid methyl esters and other microalgae lipids such as waxes, carotenoids, and chlorophylls was used to refine biodiesel, increasing the methyl ester content of biodiesel and improving the quality of the final product.

2. Material and methods

2.1. Microalgae biodiesel production

Microalgae biomass of *Nannochloropsis gaditana* was provided by Almería University in Spain. It was produced in continuous mode at a 0.3 day^{-1} dilution rate in closed tubular outdoor photobioreactors of 3.0 m^3, using seawater enriched with fertilizers as culture medium. The microalgae biomass was subject to direct transesterification in a 300 L pilot scale reactor build-up at Almería University. For this, 20 kg of wet microalgae (4.0 kg of dry biomass and 16 L of water), then 36.4 L of MeOH were inserted into the reactor and 18.2 L H_2SO_4 98% were slowly added. The reactor was closed and purged with N_2 for 5 min to create a nitrogenous atmosphere (0.5 bars). Agitation and steam heating were applied and a 2-hour reaction time was started once the reactor reached 95–100 °C. After two hours, the water entry valve in the sleeve was opened and the reactor was cooled to 30–34 °C. The reactor was depressurized and opened, and 36.4 L of hexane was added. It was purged again and the N_2 atmosphere was created, and it was then agitated for 30 min. The phases were separated by centrifugation. Then the hexane was cleaned with water to eliminate the acid catalyzer and the phases were separated by centrifugation again. To obtain the biodiesel, the hexane was subsequently eliminated using vacuum evaporation and then the biodiesel obtained was characterized with gas chromatography to determine the fatty acid content, viscosity, and humidity.

2.2. Microalgae biodiesel refining with vacuum distillation

Vacuum distillation was used to refine crude microalgae biodiesel. Fig. 1 shows the distillation equipment used.

2.2.1. Experiment 1

Vacuum distillation was performed using 50 g of biodiesel. The system pressure was maintained at 150 mbar, the temperature was increased from room temperature to 300 °C. Fractions of the distillate (D1.1) and residue (R1.1) (see Fig. 2) were collected, and the mass of both products was recorded to compare the yield with the initial mass of the distilled biodiesel. Both the distillate and residue samples were stored for subsequent characterization. The biodiesel distillate (D1.1) fraction obtained in the previous distillation was distilled again, with a system vacuum of 15 mbar (10 times less vacuum in the system). From this second distillation, fractions of distillate (D1.2) and distillation residue (R1.2) were collected. As in the first distillation, the mass of the fraction (D1.2) and the residue (R1.2) obtained in the second distillation was recorded to compare the yield to the distillate mass. Both the distillate and residue samples were stored for subsequent characterization.

2.2.2. Experiment 2

As in experiment 1, 50 g of biodiesel were vacuum distilled, applying 15 mbar of pressure, the temperature was increased from room temperature to 300 °C. Fractions of distillate (D2.1) and residue (R2.1) were collected (see Fig. 2) and the mass of the distillate fraction was recorded to compare the yield with the initial mass of distilled biodiesel (D2.1). Then, a second distillation was performed on the distilled fraction of biodiesel (D2.1) obtained in the previous step, applying a vacuum of 15 mbar to the system. From this second distillation, a fraction of distillate (D2.2) and a distillation residue (R2.2) were collected. The distillate and residue samples were stored for subsequent characterization.

2.3. Physicochemical characterization of biodiesel and refined biodiesel

Kinematic viscosity of the samples was determined according to ASTM Standard D445 using a Koehler KV 1000 viscosity bath. Acidity and iodine values were determined through titration, according to standards EN 14104 and EN 14111, respectively. Humidity was determined according to the EN 12937 standard using a Titroline KF Shott triturator, with chloroform, anhydrous methanol, and standard trituration solution. Carbon residue was determined using an ALCOR MCRT 160 micro carbon residue tester, in accordance with ASTM D 4530. Density was determined according to ASTM Biodiesel standard D6751.

2.4. Chemical characterization of biodiesel and refined biodiesel

Fatty acid methyl ester (FAME) composition was determined through gas chromatography (GC) (Agilent Technologies 6890N Series Gas Chromatograph, Santa Clara, CA, USA). Samples (i.e., crude biodiesel and vacuum distillation fractions) with 10 mL (0.125 mg) of

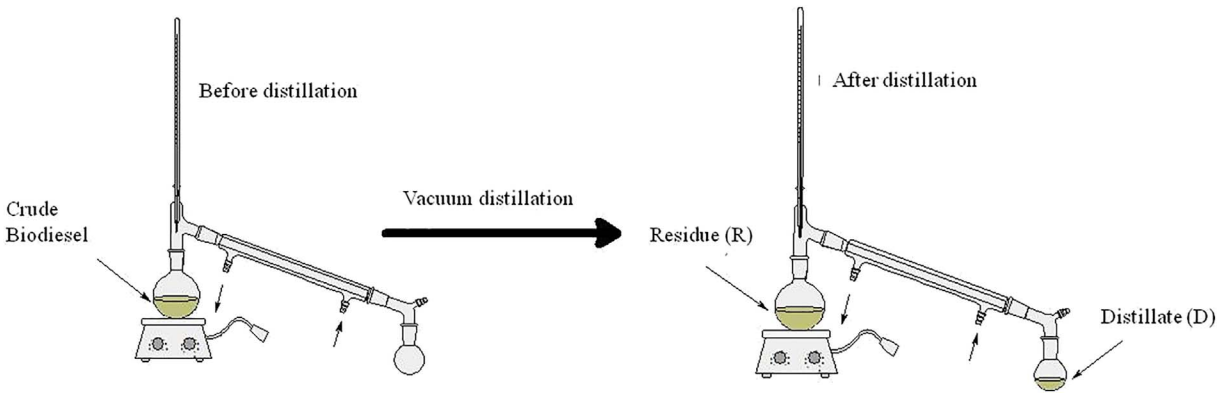

Fig. 1. Distillation equipment of microalgae biodiesel refining.

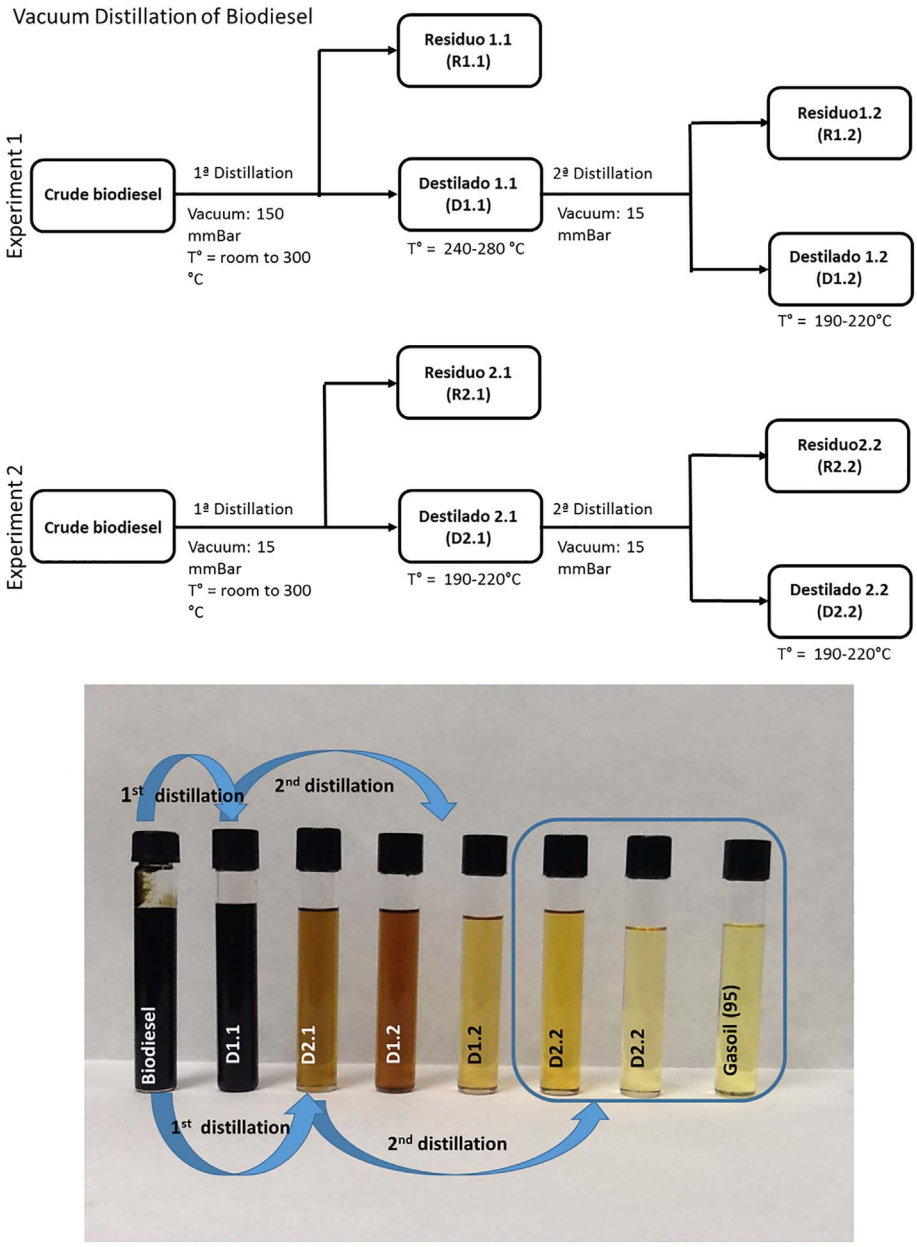

Fig. 2. Microalgae biodiesel refining with vacuum distillation.

internal standard (non-decanoic acid methyl ester, 19:0) in hexane (concentration 1 mg/mL) were analyzed directly with GC. Spectrophotometric properties of the samples were determined using a CM-3500d Minolta spectrophotometer-colorimeter with Spectramagic 3.6 Software (Minolta, Germany). The D1.2 and D2.2 samples were analyzed by ^{1}H NMR and ^{13}C NMR in $CDCl_3$. One-dimensional NMR spectra were recorded at room temperature on a Bruker Avance 400 spectrometer equipped with a z-gradient inverse broadband probe with a diameter of 5 mm, operating at 400.13 MHz (^{1}H) and 100.62 MHz (^{13}C).

2.5. Statistical analyses

All experiments of vacuum distillation were conducted in triplicate. Also, kinematic viscosity, humidity, carbon residue, acidity and iodine value were conducted in triplicate. Results are presented as mean ± standard error.

3. Result and discussion

3.1. Biodiesel production

Microalgae biodiesel (crude biodiesel) was obtained through direct transesterification of wet microalgae biomass using methanol as the esterification agent and sulfuric acid as the catalyst at a semi-industrial (or pilot) scale [18–19]. As shown in Fig. 3, the crude biodiesel has a FAME percentage of 64.98 ± 2.88%. The same biodiesel sample was methylated and analyzed again using GC to determine the total percentage of fatty acids present (which were not methylated in the transesterification process). The FAME percentage in the re-methylated biodiesel sample was 69.77 ± 0.88%, that is, only 4.8% of the fatty acids were not transformed into methyl esters during the biodiesel production process. The reaction conversion percentage was 93.13%, demonstrating that the biodiesel production process was efficient. Jimenez-Callejon et al. (2014) found similar conversion results with sulfuric acid and methanol applied to crude lipid extract of *Nannochloropsis gaditana* [20]. Therefore, the majority of fatty acids present in

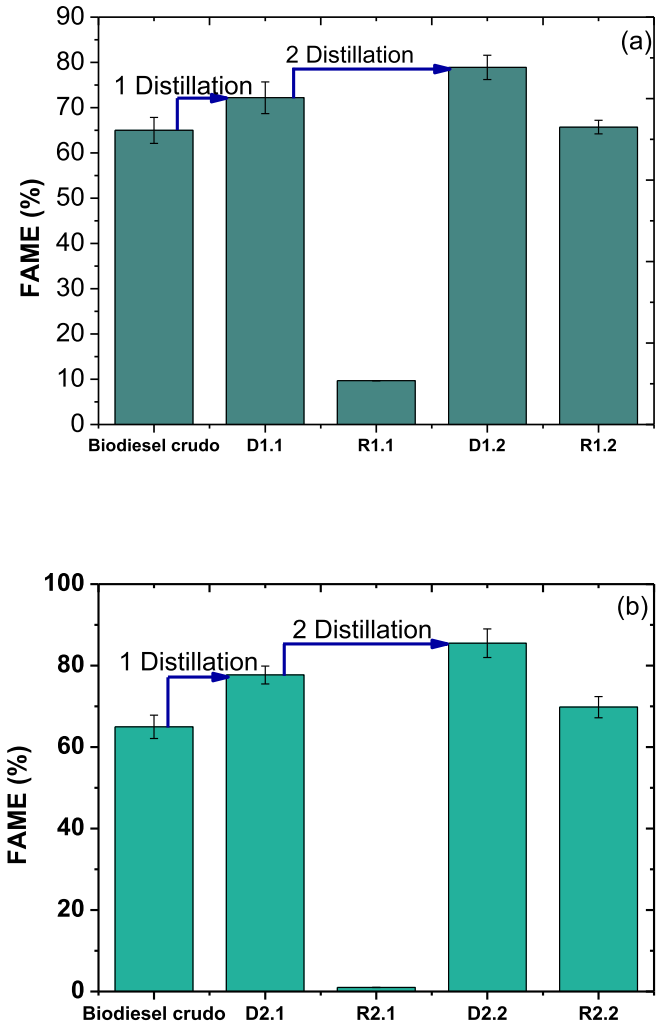

Fig. 3. FAME content in microalgae biodiesel and samples obtained from vacuum distillation processes (experiments 1 and 2).

the microalgae lipids were transformed into methyl esters to produce biodiesel. When using other microalgae such as *Schizochytrium limacinum*, the FAME percentage detected in the oil is 66.37% [18]. According to the EN 14214 standard, biodiesel should have a 96.5% FAME percentage, which microalgae biodiesel does not achieve. Hence, a refining alternative is needed to improve the biodiesel properties.

3.2. Biodiesel refining

3.2.1. Experiment 1

Fig. 1 shows the distillation equipment used and where the distillate (D) and residue (R) were obtained. First, the crude microalgae biodiesel was distilled by applying 150 mbar, resulting in two products: D1.1 distillate and R1.1 residue (Fig. 2). Both samples of the first distillation were characterized with GC to obtain the FAME percentage. Then the distillate obtained was distilled a second time with a vacuum of 15 mbar, resulting in a second distillate, D1.2, and residue, R1.2. Table 1 shows the fractions obtained compared to the initial crude biodiesel, as well as the total mass from the two consecutive distillations. The FAME percentage of the crude biodiesel and the distillation products is shown in Fig. 3a. The distillation temperature of D1.1 was 240–280 °C, as can be observed in Table 1. The results show that 56.7% of the biodiesel was distilled, at which point the biodiesel became viscous and distillation stopped. As mentioned previously, the FAME percentage of the crude biodiesel is 64.98 ± 2.88%. When the distillation was conducted under the conditions of experiment 1, the product obtained from distillate D1.1 and residue R1.1 were characterized with GC. Fig. 3a shows the FAME content of crude microalgae biodiesel and the FAME content of the distillate and the residue. According to the results, the distillate product D1.1 has a FAME percentage of 72.2 ± 3.5%, which is higher than the initial crude biodiesel. Meanwhile, the residue contains a very low FAME percentage (9.7 ± 0.008%), confirming that the distillation was stopped in time. Table 2 shows the results obtained from the GC characterization, indicating that there is no significant difference in fatty acid composition between the crude biodiesel and the D1.1 product. When a consecutive distillation was done on D1.1 (experiment 1), reducing the system vacuum to 15 mbar, the temperature of the distillate decreased from 240 to 280 to 190–220 °C. When the vacuum in the system was reduced to 15 mbar, the temperature range was between 190 and 220 °C, which is close to the boiling points of the methyl esters obtained by Cermak et al. (2012) at 13 mbar [21]. The yield of the second distillation was 68.4 ± 1.76 wt%, which was higher than the first, and the overall process yield was 38.7 wt%. It is evident that when D1.1 was distilled a second time, the FAME content increased from 72.2% to 78.9% for D1.2, demonstrating that it is still possible to increase the FAME content in the system.

Both the D1.1 and D1.2 samples were characterized by colorimetric analysis and distilled immediately afterwards (time = 0) and then again every 4, 8, and 24 h thereafter. Fig. 4 illustrates the absorbance obtained for each sample. The absorbance of distillate D1.1 (Fig. 4a) varied notably over time. The absorbance graphic shows the maximum was obtained in the visible around 430 nm and then at 24 h a saturated absorbance curve appeared. This can be seen easily, since the sample obtained has a dark yellow color and its coloration began to change quickly over time, becoming dark brown at 24 h. An analysis of the FAME composition in the sample found no changes in the methyl ester composition. In the characterization of distillate D1.2 (Fig. 4b), the colorimetric analysis showed a lower absorbance at time 0 (the sample has a light yellow color at time 0) but, like distillate D1.1, the absorbance changed over time. This demonstrates that the vacuum in the system is important for maintaining the stability of the sample, avoiding undesired reactions and the appearance of colored compounds caused by decomposition of the sample.

3.2.2. Experiment 2

A second experiment was conducted to obtain a sample with a higher FAME content and greater stability of the refined biodiesel. In this case, 15 mbar were used in the system to distill the crude biodiesel, resulting in distillate D2.1. Fig. 3b shows the FAME content of the initial biodiesel and the D2.1 sample. According to the results, in these conditions a distillate was obtained with a FAME content of 77.7%, that is, an increase of approximately 15% in the FAME content compared to crude biodiesel.

In terms of the process yield, it was very similar to the distillation yield with higher pressure in the system (experiment 1). The stability of the sample over time was analyzed as shown in Fig. 4c, illustrating the absorbance over time for distillate D2.1. Fig. 4c shows that the absorbance intensity is higher compared to D1.2 (Fig. 4a), indicating that the components present in the refined biodiesel don't absorb at that wavelength. Then the curve varies slowly over time for the first 24 h, becoming stable on the fourth day. The maximum absorbance in the visible range was 480 nm with a second peak at 600 nm. After the fifth day of analysis, there were no changes in the sample (Fig. 4c).

There were no significant differences in the FAME composition between samples D2.1 and D1.1. In general, the composition remained within the same ranges. A consecutive distillation of sample D2.1 was performed using the same operational conditions to obtain sample D2.2. The results show that in the second distillation the yield was 92.8 wt%, much higher than the 64.8 wt% obtained in experiment 1. The total yield of the two consecutive distillations was 54.2 wt%,

Table 1
Distillation conditions and yield of distillates obtained.

1st Distillation	T distillate (°C)	Yield (wt%)[a]	2nd Distillation	T distillate (°C)	Yield (wt%)[a]	Total yield (wt%)[b]
Experiment 1						
D1.1	240–280	56.7 ± 2.4	D1.2	190–220	68.4 ± 1.76	38.7
R1.1	–	41.0 ± 0.8	R1.2	–	29.6 ± 0.9	16.7
Loss		2.3	Loss		2.0	
Experiment 2						
D2.1	190–220	58.5 ± 12.7	D2.2	190–220	92.8 ± 5.1	54.2
R2.1	–	41.5 ± 13.1	R2.2	–	6.5 ± 0.8	3.8
Loss		0	Loss		0.7	

[a] Yield based on mass.
[b] Total yield from the 1st and 2nd distillations.

Table 2
Chemical composition of biodiesel and fractions obtained and their boiling points.

				Boiling point (13 mbar)[a]
Content (%)	Biodiesel	D1.1	D1.2	Acid
C14	1.98 ± 0.20	3.57 ± 0.40	2.89 ± 0.30	212
C16	14.08 ± 1.50	19.71 ± 2.10	18.89 ± 1.99	193
C16:1n7	10.23 ± 1.30	12.24 ± 1.50	11.70 ± 1.30	180
C16:2n4	3.17 ± 0.20	4.22 ± 0.60	4.06 ± 0.54	–
C16:4n1	4.44 ± 0.50	4.16 ± 0.50	4.08 ± 0.53	–
C18:1n9	2.82 ± 0.30	2.93 ± 0.35	3.05 ± 0.21	223
C18:1n7	0.44 ± 0.50	0.00 ± 0.00	0.00 ± 0.00	–
C18:2n6	8.84 ± 0.90	8.42 ± 1.16	8.35 ± 0.90	224
C18:3n3	7.21 ± 0.85	5.17 ± 0.42	5.05 ± 0.65	225
C20:4n6	1.61 ± 0.20	0.56 ± 0.00	0.69 ± 0.00	163
C20:5n3	5.37 ± 0.60	1.47 ± 0.23	1.46 ± 0.20	163
Unidentified	0.00 ± 0.00	10.11 ± 1.20	7.47 ± 0.85	–
Content (%)	D2.1	D2.2	R2.2	Methyl
C14	4.16 ± 0.62	4.12 ± 0.05	0.00 ± 0.00	184
C16	22.01 ± 2.50	25.66 ± 1.75	16.17 ± 1.78	161
C16:1n7	13.99 ± 1.60	17.26 ± 1.65	10.44 ± 1.20	182
C16:2n4	4.96 ± 0.55	5.39 ± 0.07	2.63 ± 0.21	–
C16:4n1	4.90 ± 0.59	5.13 ± 0.06	3.07 ± 0.35	–
C18:1n9	2.45 ± 0.03	3.23 ± 0.04	5.30 ± 0.60	201
C18:1n7	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	–
C18:2n6	6.69 ± 0.75	9.27 ± 1.10	13.44 ± 1.45	200
C18:3n3	3.90 ± 0.51	4.84 ± 0.35	5.38 ± 0.45	202
C20:4n6	0.38 ± 0.12	0.00 ± 0.00	0.43 ± 0.03	194
C20:5n3	0.66 ± 0.15	0.68 ± 0.01	0.51 ± 0.04	194
Unidentified	7.16 ± 0.95	12.50 ± 1.5	12.85 ± 1.45	–

[a] Cermak et al. (2012) [21].

enabling this process to refine 50 wt% of the biodiesel. The FAME content of sample D2.2 was 85.5%; in other words, reducing the pressure in the distillation system improved the yield. In addition, FAME content improved in the refined biodiesel from 64.98% to 85.50%, a 20% increase (Fig. 3b). Fig. 4d shows the absorbance of sample D2.2, indicating that distillate D2.2 had a more stable color, with no variation in the first 24 h. The absorbance graphic shows no peak, unlike samples D1.1 and D1.2. Clearly, decreasing the pressure (increasing the vacuum) in the system results in a higher-quality product with fewer impurities. By achieving a product with 85% FAME, the vacuum distillation process substantially increases FAME content to a value that is much closer to the ASTM standard EN 14214 standard.

3.3. Characterization of distilled biodiesel samples by ^{1}H-RMN and ^{13}C-RMN

The final products of each experiment–distillates D1.2 and D2.2–were characterized by ^{1}H-RMN and ^{13}C-RMN. Fig. 5a and b show the ^{1}H-RMN spectra for the D1.2 and D2.2 distilled biodiesel samples, respectively. In both spectra there is a signal at 3.66 that corresponds to the proton of the methyl ester group, but the signal is much more intense in the distillate D2.2 spectrum. Previous studies characterizing biodiesel by ^{1}H-RMN have shown that this signal is characteristic of the methyl ester in FAME. Nautiyal et al. (2014) reported similar results for biodiesel from karanja and *Spirulina* [22]. The signal's intensity is related to the FAME content of the sample, as has been demonstrated by Mello et al. (2008) [23], making it possible to estimate the FAME content of biodiesel samples. The ^{1}H-RMN spectra of the samples, therefore, confirm that sample D1.2 has a lower FAME content than sample D2.2. The signal at 2.80 ppm (in both spectra) corresponds to the protons in the aliphatic chain of fatty acid methyl esters present in the samples and the triplet at 2.29 ppm corresponds to the ester proton.

Another series of signals can also be observed in the ^{1}H-RMN spectra. The multiplet at 5.35 ppm corresponds to the olefin protons of the alkyl chain and the signals between 0.87 ppm and 2.05 ppm correspond to the protons of the alkyl chain of the fatty acid methyl esters. The spectra obtained for both samples are very similar and don't show any signals above 6 ppm associated with aromatic protons, or signals below 0.7 ppm related to epoxide protons. In the ^{13}C-RMN spectrum (Fig. 6a) we observe signals at 174.73 ppm and 51.84 ppm for sample D1.2. These signals are related to the carbon from carboxylic acid esters and the carbon from the methoxide group, respectively. Likewise, for sample D2.2 (Fig. 6b) these signals appear at 174.69 ppm and 51.78 ppm, respectively. Hence, the ^{1}H-RMN and ^{13}C-RMN spectra confirm the presence of protons and carbons for the methyl group and the remaining signals correspond to the aliphatic chain. This in turn confirms the presence of FAME and that the sequential distillation process produces biodiesel with a higher FAME concentration, greatly reducing the impurities in the microalgae biodiesel. In addition, sequential distillation with a lower vacuum resulted in a better quality final product.

3.4. Physicochemical characterization of the distilled biodiesel samples

Table 3 shows the results obtained for the characterization of crude microalgae biodiesel and the distilled biodiesel samples in each experiment. Humidity, density, viscosity, acid value, iodine index, and carbon residue were analyzed for most of the samples. The results indicate that in general, all of the biodiesel fractions obtained from vacuum distillation in experiment 1 (D1.1 and D2.2) and in experiment 2 (D2.1 and D2.2) meet biodiesel standard EN 14214 for density, viscosity, iodine index, and carbon residue; however, they are outside the ranges for humidity and acidity. Comparing the results with the specifications of the ASTM D6751 standard, we observe that the biodiesel fractions are outside the ranges for acid value and carbon residue. As shown in Table 3, the humidity of the crude biodiesel is 3.72% and it decreases in the distillate samples; the only sample that meets the humidity standard is D2.1. In this work, wet microalgae was used to produce biodiesel, an alternative to reduce the humidity is use dry microalgae to reduce the water content in the process.

The density of crude biodiesel is within the parameters of the EN 14214 standard, with lower density for the D2.2 distillate biodiesel

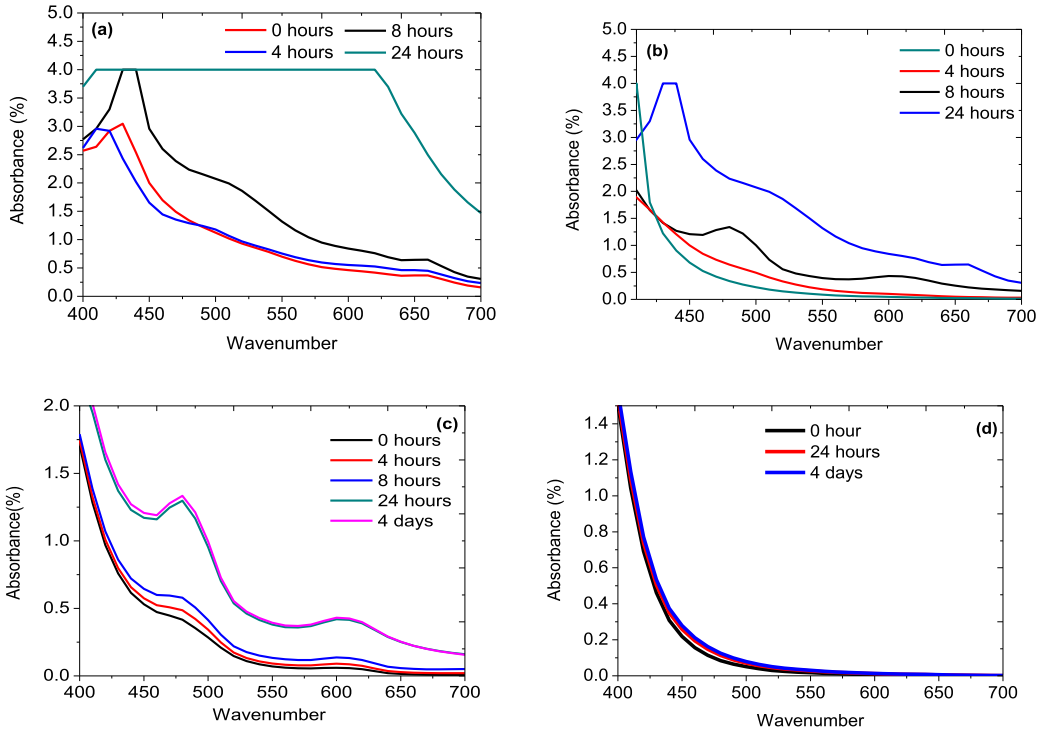

Fig. 4. Colorimetric characterization of D1.1 (a); D1.2 (b); D2.1 (c) and D2.2 (d).

fraction. The density results obtained for the crude biodiesel and the samples of distilled biodiesel are lower than those obtained by Chongkhong et al. (2007) for palm oil biodiesel (879.3 kg/m^3) [24] and Cunha et al. (2013) [25] for animal fat biodiesel (870 kg/m^3), although the density results for the biodiesel distillates are very similar to those obtained by Nautiyal for *Spirulina* biodiesel (860 kg/m^3) and marine algae biodiesel (872 kg/m^3) [22].

According to Knothe et al. (2005) viscosity is one of the most important characteristics of biodiesel. Viscosity is also closely associated with the chemical structure of the samples. Thus, free fatty acids or compounds that contain hydroxyls may have high viscosity levels; this is one of the reasons why oils can't be used as fuels [26]. The viscosity value at 40 °C for crude biodiesel from *Nannocloropsis gaditana* microalgae is high (17.70 mm^2/s) (Table 3). Similar viscosity values have been obtained in other studies for biodiesel produced from non-edible raw materials such as *Lesquerrella fendleri* (10.02 mm^2/s), *Ricinus communis* (castor) (15.25 mm^2/s), and *Simmondsia chinensis* (jojoba) (19.2 mm^2/s) [27].

For biodiesel from microalgae such as spirulina (microalgae oil transesterification process), the viscosity values are 5.66 mm^2/s, while for algae they are 5.82 mm^2/s, outside the range of the EN 14214 standard. But the transesterification process involves extraction of microalgae oil and then oil transesterification, which requires processing dry microalgae. In this study, transesterification was performed at pilot scale with a direct transesterification process. In addition, Predojevic et al. (2008) obtained high viscosity values for unpurified frying oil biodiesel (32.20–48.47 mm^2/s) and then conducted three different biodiesel purification processes to obtain values of 3.7–5.0 mm^2/s [28]. According to the EN 14214 standard, viscosity values should be in the range of 3.5–5.0 mm^2/s. An analysis of the results for the distilled biodiesel samples in experiment 1 and experiment 2 reveals that the viscosity in all of the samples declines considerably and is within the standards established for biodiesel. The lowest viscosity value was obtained for D2.2 (3.76 mm^2/s). This demonstrates that when using vacuum distillation as a purification process for microalgae biodiesel, viscosity decreases, FAME content increases, and the resulting values are within the current standards for biodiesel.

The acid value is fundamental to obtaining a non-corrosive product and maintaining the stability of the biodiesel over time. In this study, the acid value for all of the distilled biodiesel samples is outside the standards' range. The results, shown in Table 3, indicate that in both experiments 1 and 2 the samples obtained in the first distillation have higher acid values than the samples obtained in the second distillation. The acid value is generally associated with quantification of the carboxylic acid group content of fatty acids that did not react in the transesterification process. But in this case, because H_2SO_4 was used as a catalyst, the high acid values are also associated with catalyst residue that was not extracted in the biodiesel washing process. At an industrial scale, the washing process to eliminate acid catalyst is crucial for obtaining the desired quality in the final product. At industrial scale it is necessary improve the washing process and may be incorporate neutralization step before distillation.

Table 3 also shows results obtained for the iodine index and carbon residue. The iodine index value is used to quantify unsaturation (double bonds) of FAME in the biodiesel samples. Vegetable biodiesel samples have low unsaturation indices since they are largely comprised of oleic acid methyl esters. Biodiesel produced from microalgae may have high iodine index values since it is mostly made up of unsaturated fatty acid methyl esters. Nautiyal et al. (2013) did not report the iodine index and carbon residue results for spirulina and marine algae biodiesel [22]. The results obtained in the present study show that the iodine index values for all of the distilled biodiesel samples meet the EN14214 standard. The results are in the range of 85.7–89.8 g I_2/100 g, and the maximum value for the standard is 120 g I_2/100 g. Close values were obtained for biodiesel from animal fat and for biodiesel produced from waste frying oil [25,28]. The carbon residue results generally decline in the second vacuum distillation and are within the EN 14214 standard.

4. Conclusions

This study has demonstrated that it is possible to produce biodiesel at a semi-pilot scale with a direct transesterification process of wet microalgae biomass, without the need for prior oil extraction. Despite the high conversion percentage of the reaction, the FAME content of crude biodiesel samples is well below the standard and has also high viscosity values. Vacuum distillation was used as the purification

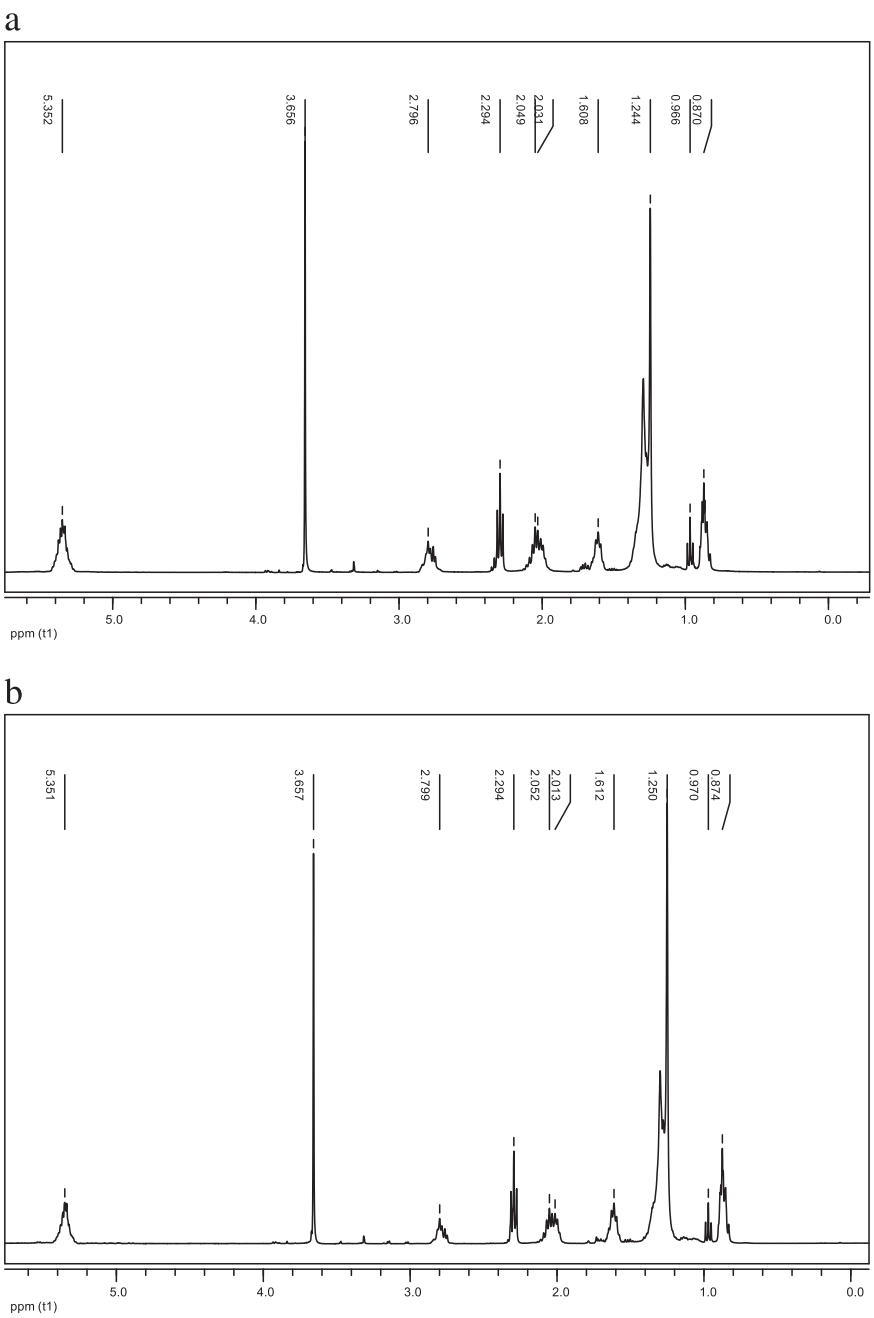

Fig. 5. ^{1}H-RMN-spectrum for distilled biodiesel sample (D1.2) (a) and distilled biodiesel sample (D2.2) (b).

process for microalgae biodiesel and achieved much higher FAME values. Two experiments were conducted with different vacuum levels in the system and the best results in terms of product yield were achieved with two consecutive distillations with low vacuum in the system. The FAME characterization in the distillate samples showed that when two consecutive distillations are performed with low vacuum, the FAME percentage increases considerably. In addition, the process stabilizes the color of the product and improves the viscosity of the biodiesel produced. Sample D2.2 of distilled biodiesel is the best product obtained in this study, and therefore, vacuum distillation could be considered an effective purification process for microalgae crude biodiesel. While this study demonstrates that low vacuum distillation can improve the FAME percentage and quality of biodiesel, a future objective consists of improving the process to achieve higher FAME percentages in the final product.

Author contributions

All authors have approved the final version of this manuscript. S.T., G.A and R.N conceived and designed the experiments; S.T. and F.G.-C. performed the experiments; S.T. and F.G.-C. analyzed the data; S.T., G.A. and F.G.-C. contributed reagents/materials/analysis tools; S.T, R.N and G.A. wrote the paper.

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The authors declare no competing financial interest.Statement of informed consent

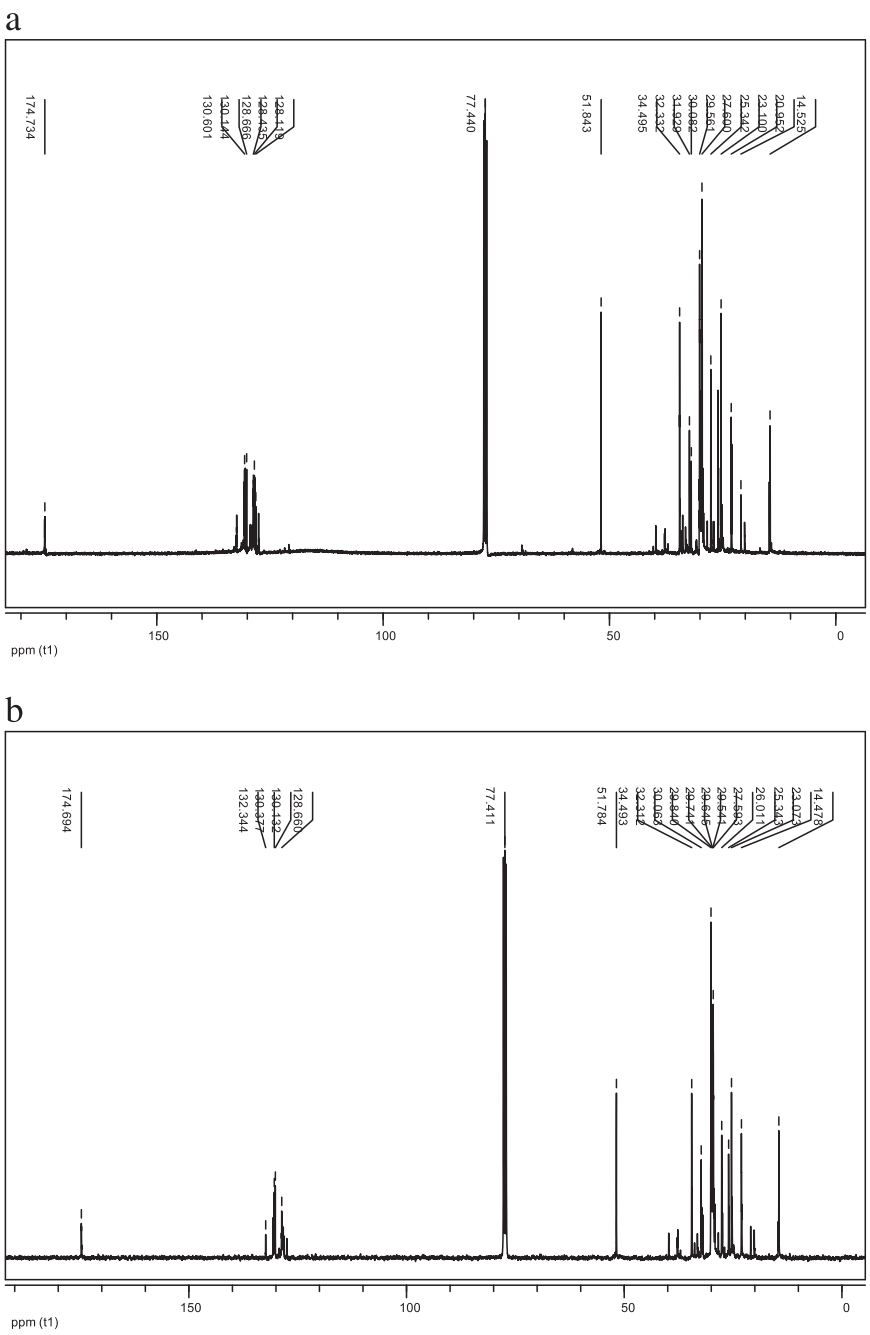

Fig. 6. ^{13}C-RMN for distilled biodiesel sample (D1.2) (a) and for distilled biodiesel sample (D2.2) (b).

Table 3
Characterization of biodiesel and distillate fractions obtained in each process.

Sample	Humidity (%)	Density (at 15 °C) (kg/m^3)	Viscosity (40 °C) (mm^2/s)	Acid value (mg KOH/mg oil)	Iodine value (g I_2/100 g)	Carbon residue (%)
Crude biodiesel	3.72 ± 0.03	862 ± 0.01	17.70 ± 0.17	10.0 ± 0.40	UND	UND
D1.1	0.36 ± 0.01	874 ± 0.01	3.97 ± 0.03	6.4 ± 0.40	87.9 ± 0.20	0.26 ± 0.05
D1.2	0.43 ± 0.01	864 ± 0.00	3.88 ± 0.04	4.5 ± 0.10	89.8 ± 3.90	0.01 ± 0.05
D2.1	0.00 ± 0.00	877 ± 0.00	4.38 ± 0.06	9.4 ± 0.30	85.7 ± 0.40	NA
D2.2	0.34 ± 0.02	852 ± 0.01	3.76 ± 0.01	2.7 ± 0.10	86.5 ± 1.60	0.09 ± 0.04
Biodiesel standard EN 14214	Max. 0.05	860–900	3.50–5.00	Max. 0.50	Max. 120	Max. 0.30
ASTM biodiesel standard D6751	–	880	1.90–6.00	Max. 0.50	–	Max. 0.05

UND: undetermined.

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