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FRACTIONAL DISTILLATION OF CRUDE MICROALGAE OIL TO PRODUCE RENEWABLE HYDROCARBONS

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Abstract. *The production of hydrocarbons from microalgae has been an area of great interest, as microalgae are a promising source of renewable biomass that can be used in the production of biofuels and other chemicals. Hydrocarbons derived from microalgae can be used as fuels, including diesel and aviation kerosene. The hydrocarbons produced by microalgae are like those found in fossil fuels and can be extracted and refined to produce renewable and sustainable fuels. For the study, the microalga Tetradesmus obliquus was cultivated in compact photobioreactors (PBRs) for 15 days using pig farming medium. Hot extraction with organic solvents (hexane and ethanol) was used to extract the microalgae oil. After extraction, the solvent present in the sample was recovered for subsequent distillation of the crude oil produced. Three temperature ranges were analyzed: the first ranging up to 150 °C, the second from 150 to 250 °C, and the third from 250 to 350 °C. The first sample obtained a total of 64.45 g of distilled oil with a yield of 51.51% (relative to the initial amount of crude oil), the second sample obtained 64.60 g with a yield of 52.96%, and finally the third sample obtained 57.33 g with a yield of 46.13%. The results of fractional distillation indicated that, on average, for the first temperature range, 4.07 ± 1.5 g of distilled oil were obtained, for the second range, 8.29 ± 1.6 g were obtained, and for the third range, 49.77 ± 3.1 g were obtained. GC-MS analysis revealed an abundance of hydrocarbons (alkanes and alkenes), which can be compared to petroleum diesel. On average, for each fraction analyzed, it was possible to obtain 25.49 ± 2.8% (1), 28.27 ± 4.2% (2), and 21.91 ± 3.5% (3) of alkanes, and 8.30 ± 1.7% (1), 3.62 ± 0.2% (2), and 11.22 ± 6.7% (3) of alkenes, respectively. In summary, this study highlights the importance of producing hydrocarbons from microalgae as a sustainable and viable alternative to produce biofuels and other chemical products. Fractional distillation of hydrocarbons can be an efficient technique for obtaining these compounds, as well as for obtaining a wide variety of products.*

Keywords*: Hydrocarbon, Microalgae, Renewable fuel, Fractional Distillation*

1. INTRODUCTION

The study of new energy sources is one of the critical points of emphasis in the sustainable development goals proposed by the Department of Economic and Social Affairs of the United Nations, aiming to make the global society more sustainable by 2030 (UN, 2015). According to the national energy balance for the year 2020, 53.9% of the energy produced in Brazil comes from non-renewable sources, which release a high amount of CO_2 when used (EPE, 2020).

Given the need to change this scenario, researchers are looking for ways to replace non-renewable sources in the energy matrix with renewable energy and the sustainability of industrial processes (Ortiz *et al*., 2016). One of the main

contenders is the use of green microalgae, mainly due to their ability to capture CO_2 from the atmosphere, their application in industrial waste treatment, and the possibility of extracting hydrocarbons to produce biodiesel, green diesel, and aviation kerosene (Chisti, 2007; Lycourghiotis, 2022).

The possibility of extracting hydrocarbons exists due to the capacity of these organisms to biosynthesize these compounds, as also occurs in other species (Sorigué *et al*., 2016). However, the advantage of employing green microalgae for this purpose is the possibility of cultivating them on an industrial scale in photobioreactors (Vidyashankar *et al*., 2015), as well as treating waste from burning or pig production for their growth (Nwoba *et al*., 2016).

The production of alkanes and alkenes by these organisms occurs from intermediates of lipid metabolism (Sorigué *et al*., 2016). The biomass of microalgae has a variable percentage of hydrocarbons depending on the production conditions, which averages around 2% of the dry matter (McGenity *et al*., 2010). These compounds can be extracted from the cells through organic solvent extraction at high temperatures and separated through fractional distillation of the non-esterifiable fraction of the resulting oil (Miao and Wu, 2006). Despite the low conversion (McGenity *et al*., 2010), their utilization is advantageous due to the scale of production (Chisti, 2007), the use of piggery effluent (Nwoba *et al*., 2016), and the production of co-products from residual biomass (Savio *et al*., 2020).

This study aims to demonstrate the importance of using microalgae hydrocarbons as a viable and sustainable option for producing biofuels and other co-products.

2. MATERIALS AND METHODS

The species of microalgae used in the present study was *Tetradesmus obliquus* LGMM0001, also known as *Acutodesmus obliquus* or *Scenedesmus obliquus*. This species belongs to the class Chlorophyta and the family Scenedesmaceae (Miyawaki *et al*., 2021; Dias *et al*., 2023).

The microalga Tetradesmus obliquus was cultivated in a compact 12 m^3 volume Photobioreactor (PBR), with 3.5 km of transparent circular 50 mm Polyvinyl Chloride (PVC) tubes arranged horizontally in a stainless-steel structure, covering an area of only 10 m^2 each, as shown in Figure 1 (Corrêa, 2013; Vargas *et al*., 2019; Severo *et al*., 2022).

Figure 1. The infrastructure of photobioreactors (PBRs) at the NPDEAS facilities.

The study used biodigested swine effluent as a culture medium for microalgae, due to these organisms need for specific chemical compounds such as nitrogen, phosphorus, and carbon to obtain adequate nutrients. The microalgae cultivation process lasted for 15 days, after which they were harvested and subjected to processing procedures. Several authors have presented various methods for breaking the cell membrane and obtaining crude microalgae oil. In this work, we adopted the methodology of extraction with organic solvents, as it demonstrated high selectivity and solubility in extracting lipids from microalgae (Ranjan *et al*., 2010).

The "extractor" assembly is divided into three parts: rotation control, ultra-thermostatic bath, and the extractor itself, as shown in Figure 2. The dried biomass is triturated to increase the mass transfer of lipids during extraction (Halim *et al*., 2011). After triturating, the biomass is placed in the extractor along with a solvent mixture. The rotation control was set to 500 rpm, and the ultra-thermostatic bath was maintained at 50 °C. The extractor has a condenser that prevents solvent evaporation, thereby increasing the final extraction yield (Costa *et al*., 2022). Each experiment required 1.5 kg of dried and ground microalgae biomass, along with 7.5 L of organic solvent, consisting of 5.25 L of hexane and 2.25 L of ethanol (70% hexane and 30% ethanol). The extraction process was carried out over a period of 3 hours.

After the extraction process, the remaining components (solvent, cellular debris, and unextracted lipids) are directed to a solid-liquid separation system to remove the cellular waste. The solid-liquid separation system may involve methods such as centrifugation, filtration, or decantation to remove the cellular debris and obtain the microalgae oil (Harun *et al*.,

2010). Once the biomass residues are separated, the recovery of the solvent is essential to obtain the crude microalgae oil because of the extraction. When the extraction is performed using organic solvents, the water and solvent are removed through liquid-liquid separation methods such as vacuum evaporation, distillation, or solid-phase solvent adsorption (Halim *et al*., 2011).

Figure 2. The microalgae oil extractor at NPDEAS.

Distillation is a type of operation aimed at separating the constituents of a liquid mixture based on their difference in volatility, utilizing heat as the separation agent. The separation of compounds occurs due to various factors such as temperature, pressure, and concentration of the mixture (Roper *et al*., 2005). For the experimental condition (70% hexane and 30% ethanol), a digital multimeter with a temperature sensor (ranging from 0 °C to 750 °C) was employed, along with a Type K Thermocouple (ranging from -270 °C to 1,200 °C), to measure the temperature at the bottom of the flask during the distillation process (Figure 3).

Figure 3. Fractional distillation column.

In this way, three power intervals and consequently three temperature intervals were established to perform fractional distillation. The first temperature range was set up to 150 °C (power varying between 7 and 9), the second interval was from 150 °C to 250 °C (power varying between 10 and 11), and finally, the temperature variation was from 250 °C to 350 °C (maximum power). To collect the distilled fractions, nine 50 ml round-bottom flasks with a 24/40 joint were used. Each of these flasks was designated to receive a specific fraction obtained during the distillation process. All samples were weighed using an analytical balance (GEHAKA, model AG200).

The analyses were performed on a Shimadzu gas chromatograph, model GC-2010 Plus, equipped with a VF-5MS capillary column (30 m x 0.25 mm) and a mass spectrometry detector, model GC-MS QP-2010 SE, operating in the ion scan mode from 35 to 500 Da. The injector was set to a temperature of 250 °C, and the injection was done in split mode, with a split ratio of 1:20. Helium was used as the carrier gas, with a flow rate of 0.8 mL/min. The analysis started at a temperature of 100 °C and was held for 1 min, followed by a temperature ramp of 10 °C/min up to 200 °C, where it was held for 2 min. Then, the temperature was increased at a rate of 3.5 °C/min up to 260 °C and held for 20 min. The total program time was 50.14 min. The quantification of compounds was done by normalizing the peak areas based on the total

ion chromatogram. Peak identification was performed using the NIST 11 library. Before injection, the samples were diluted with 20 µL of crude oil in 10 ml of P.A. hexane (Synth®).

3. RESULTS AND DISCUSSION

The results of the extraction and distillation of microalgae oil are presented in Table 1. The experiments were conducted with the aim of determining the efficiency of crude oil extraction and distillation, as well as the conversion rate to oil. Analyzing the results obtained, specifically in Experiment 2, there was an approximate 2.51% reduction in the extracted crude oil compared to Experiment 1, accompanied by a decrease of about 2.52% in the conversion to oil. In Experiment 3, there was a decrease of approximately 0.68% in the extracted crude oil compared to Experiment 1 and a reduction of about 0.72% in the conversion to oil.

Several hypotheses can be considered to explain these differences between the experiments. One possibility is that the chemical composition of microalgae may vary within the same species due to factors such as growth stage and cultivation conditions, as well as the location of the desired compounds, which could be in different parts or compartments of the cells, affecting their extraction. Additionally, experimental variability should also be considered, as even with initially predefined conditions, small differences in experimental procedures, such as extraction time and agitation, can influence the results.

The distillation yield was 62.12 ± 4.2 g, and the final conversion of the distilled oil, i.e., the yield relative to the initial biomass quantity of 1.5 kg used in all experiments, was 4.13 ± 0.3%. These values were below expectations when compared to the results previously obtained by Costa *et al*. (2022), which reported a yield of 6.26 ± 0.46% for a solvent mixture of 70% hexane and 30% ethanol using a smaller amount of biomass, 1.0 kg, for the extraction process.

Several factors can account for these differences in results. Variations in solvent quality can affect extraction efficiency and, consequently, the yield obtained, even if the same solvent is used. Additionally, minor differences in biomass preparation, such as drying time, can also influence the results (Azmir *et al*., 2013).

Table 1. Extraction and distillation of microalgae oil.

Hexane + Ethanol	**Experiment 1**	**Experiment 2**	**Experiment 3**	**Mean + Standard deviation**
Initial mass (g)	1,500	1,500	1,500	1,500 ± 0.0
Extracted crude oil (g)	125.12	121.98	124.27	123.79 ± 1.6
Conversion to crude oil (%)	8.34	8.13	8.28	8.25 ± 0.1
Distilled oil (g)	64.45	64.60	57.33	62.12 ± 4.2
Conversion to distilled oil (%)	51.51	52.96	46.13	50.20 ± 3.6
Final conversion (initial biomass) (%)	4.28	4.31	3.82	4.13 ± 0.3

The Table 2 describes the results of distillation of microalgae oil into three distinct fractions. In the first temperature range, referred to as fraction 1, the experiment values were 5.69 g, 2.86 g, and 3.65 g, respectively. The average of the three experiments was 4.07 ± 1.5 g. In the second temperature range, named fraction 2, the experiment values were 7.25 g, 10.14 g, and 7.47 g, respectively. The average of these results was 8.29 ± 1.6 g. In the last temperature range, from 250 to 350 °C, denoted as fraction 3, the highest results were obtained. The experiments resulted in values of 51.50 g, 51.59 g, and 46.21 g of distilled oil, respectively. The average of these results was 49.77 ± 3.1 g. The last row of the table represents the total sum of microalgae oil quantities obtained in the experiments after distillation, which was 64.45 g, 64.60 g, and 57.33 g, respectively, with a total average of 62.12 ± 4.2 g.

Based on these results, it can be concluded that the highest distillation yields were achieved in the highest temperature range, fraction 3, with average values around 49.77 ± 3.1 g. However, it is also important to consider the standard deviation, which indicates the variability of the results. In this case, the lowest temperature range, up to 150 °C, with fraction 1, showed the smallest standard deviation, suggesting greater consistency in the results. Figure 4 presents the obtained results of distilled oil for each pre-defined fraction, and all analyses were performed in triplicate.

Table 2. Fractions of distilled oil from microalgae.

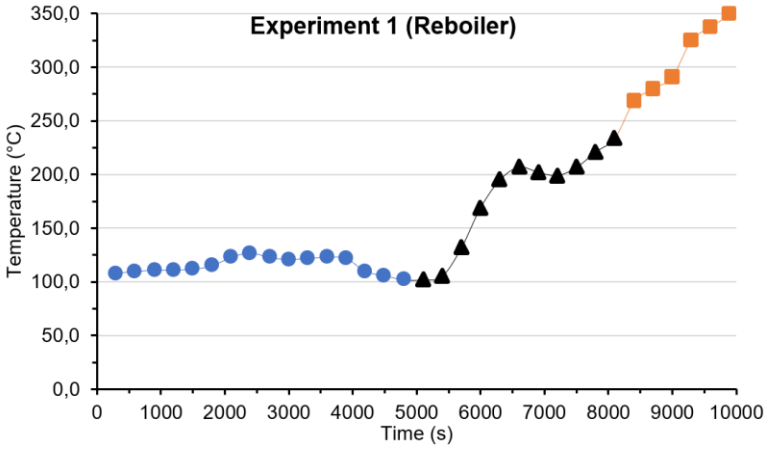
Power	**T (ºC)**	**Fraction**	**Experiment 1**	**Experiment 2**	**Experiment 3**	**Mean + Standard deviation**
[7-9]	Up to 150	1	5.69	2.86	3.65	4.07 ± 1.5
[10-11]	150 to 250	2	7.25	10.14	7.47	8.29 ± 1.6
[Maximum]	250 to 350	3	51.50	51.59	46.21	49.77 ± 3.1
Total (g)			64.45	64.60	57.33	62.12 ± 4.2

Figure 4. The distilled oil was collected in three different fractions.

Three experiments were used to assess the temperature variation over elapsed time, measuring the values at each 5-min interval. It took 60 min for the distillation process to start. The results were recorded in terms of time in seconds and temperature in degrees Celsius as shown in Figure 5, for the temperature variation in the reboiler. The experiments were conducted under controlled conditions to analyze how different power and time values affect the temperature increase of a system.

The results of the three experiments for the first power range [7-9], called fraction 1, show that Experiment 1 had a temperature variation between 108 °C and 127 °C, Experiment 2 ranged from 102 °C to 134 °C, while Experiment 3 ranged from 121 °C to 149 °C. The comparative analysis suggests that Experiment 1 has the smallest temperature variation range, while Experiment 3 presents the largest range. By increasing the power for a range that varied from [10-11], fractions 2 allowed for a more comprehensive analysis. Experiment 1 presented a temperature variation between 102 °C and 234 °C, Experiment 2 ranged from 160 °C to 238 °C, while Experiment 3 ranged from 145 °C to 247 °C. Comparing the results, Experiment 3 has the greatest temperature variation range, while Experiment 1 has the smallest range. With the increase to the maximum power, the temperature analysis expands even further, which is fraction 3. Experiment 1 showed a maximum power ranging from 268 °C to 348.9 °C, Experiment 2 ranged from 258 °C to 347 °C, and Experiment 3 ranged from 263 °C to 349 °C. When comparing the results, it is possible to observe that Experiment 3 presents the largest range of maximum power variation, while Experiment 2 has the smallest range.

Upon observing the data from Experiment 1, it was noticeable that as the heating time increased, the temperature also rose. Additionally, when the system power was low (in the range of 7 to 9), the rate of temperature increase was slower. However, as the power was increased, the temperature rose more rapidly. Analyzing the data from Experiment 2, it becomes evident that the applied power directly impacts the system's temperature. It was found that when the power was in the range of 7 to 9, the temperature increased at a more moderate rate. However, by increasing the power to levels between 10 and 11, the rate of temperature variation was higher. Examining the results of Experiment 3, we noticed that the heating system reached its maximum power at a specific period. At this point, the temperature was recorded as the highest among all experiments, highlighting the significance of maximum power in achieving extreme temperatures.



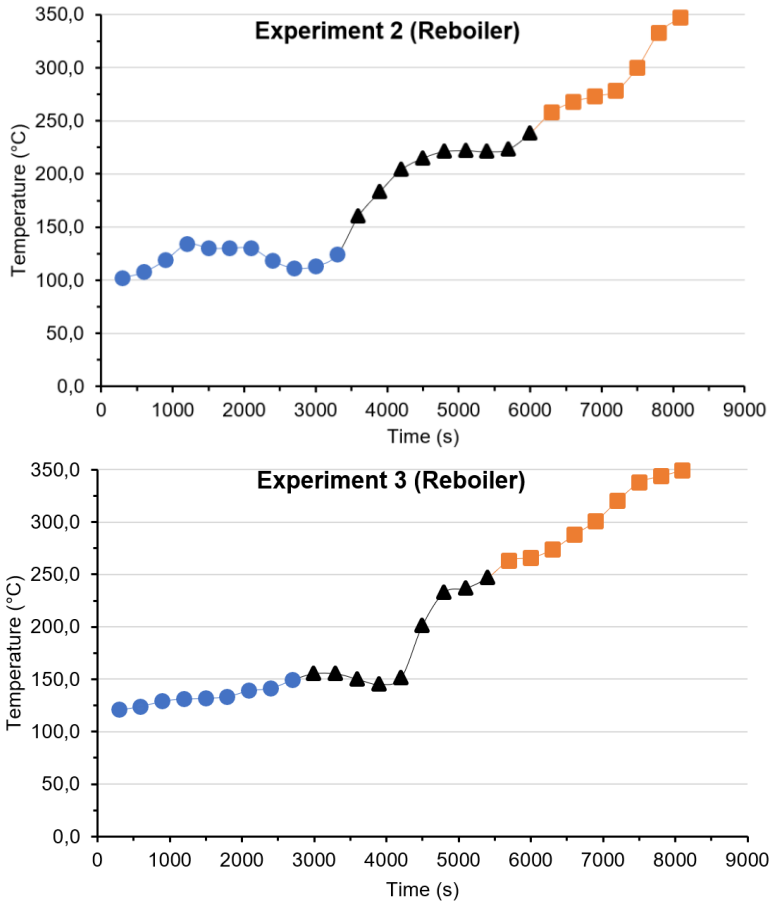
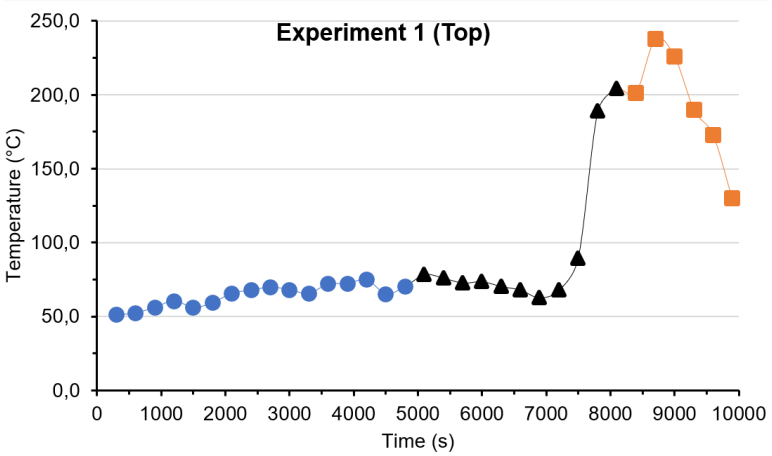


Figure 5. Variation of temperature in reboiler for the three experiments.

Figure 6 shows the behavior of fractional distillation at the top of the column, and it is possible to observe that, for all experiments, fraction 1 reached a maximum temperature of 75 °C using a power range of [7-9]. After this period, when further distillation was no longer possible, the power was increased to [10-11], and distillation cuts of fraction 2 were performed. In this fraction, a more abrupt temperature variation at the top of the column is observed, indicating the presence of heavier compounds compared to the previous fraction. The maximum temperature reached for fraction 2 was 205 °C at the top of the column. Finally, the power was set to the [maximum] for fraction 3, which resulted in a higher quantity of distilled oil in all experiments. The temperature in the reboiler reached up to 350 °C, but at the top of the column, the maximum temperatures obtained in each experiment were 237.7 °C, 235.1 °C, and 225.6 °C, respectively. As the top temperature started to decrease, it became evident that the distillation yield was significantly decreasing, indicating the process was concluding. Even if the bottom temperature was increased, it would not be possible to obtain more oil fractions.



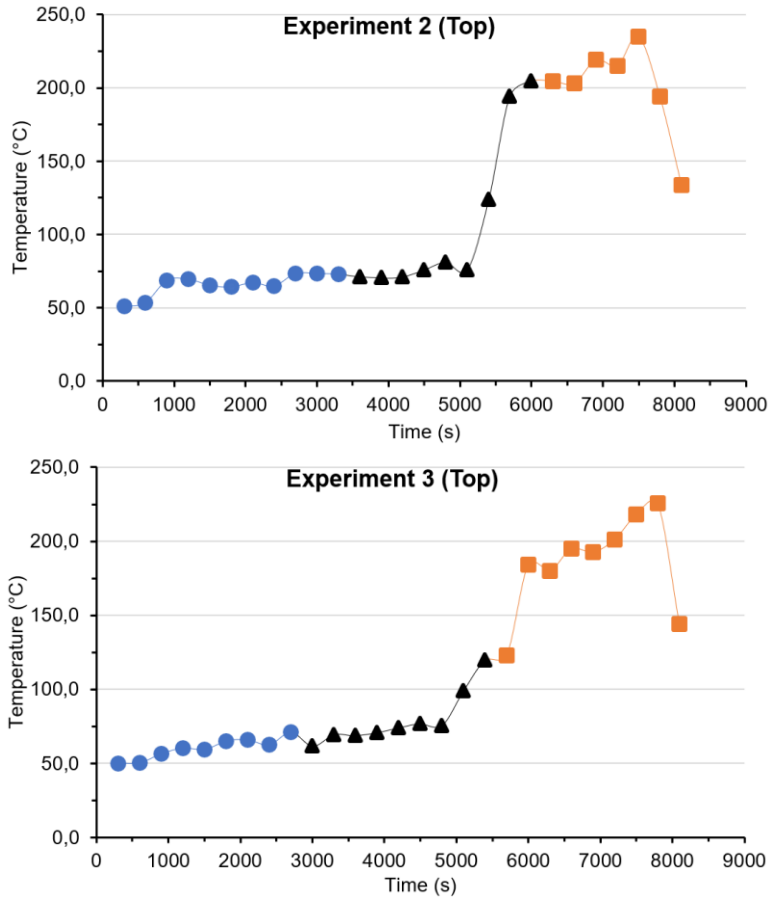


Figure 6. Variation of temperature at the top of the column for the three experiments.

Table 3 presents the GC-MS results, allowing the observation of compound distribution in different fractions. The hydrocarbons (alkanes) are the most predominant, representing the highest proportion in all fractions, ranging from 21.91 ± 3.5% to 28.27 ± 4.2%. The most identified alkanes are tridecane, tetradecane, hexadecane, and pentadecane. Alkenes are also present but in smaller quantities, with a percentage distribution ranging from 3.62 ± 0.2% to 11.22 ± 6.7%. The identified alkenes are 3-tetradecene and 3,7,11,15-tetramethyl-2-hexadecene. Regarding esters, ethyl docosanoato, ethyl behenate, ethyl oleate, and ethyl stearate were found, with a percentage distribution ranging from 6.58 ± 1.6% to 23.92 ± 5.6%. Carboxylic acids are present in smaller amounts, with a percentage distribution ranging from 0.46 ± 0.4% to 4.16 ± 1.5%. The most identified acids are dodecanoic acid, 5,8,11,14,17-eicosapentaenoic acid, and 8,11,14-eicosatrienoic acid.

In summary, Table 3 provides a detailed overview of the compounds identified in the GC-MS analysis of Tetradesmus obliquus microalgae oil. This analysis shows that hydrocarbons, mainly alkanes, are within the expected range when found in a higher percentage area.

Table 3. GC-MS of the distilled oil.

Compound (IUPAC)	Molecular formula	Polarity	Fraction 1 (% area)	Fraction 2 (% area)	Fraction 3 (% area)
Hydrocarbons (Alkanes)					
3-Methyl-decane	$C_{11}H_{24}$	NP	5.51 ± 4.9	0.19 ± 0.1	0.65 ± 1.1
Tridecane	$C_{13}H_{28}$	NP	2.30 ± 0.3	3.02 ± 1.1	1.94 ± 0.7
Tetradecane	$C_{14}H_{30}$	NP	5.67 ± 1.1	3.70 ± 1.3	3.28 ± 1.6
2,6,10,14-tetramethyl-heptadecane	$C_{21}H_{44}$	NP	2.81 ± 0.2	1.91 ± 1.3	2.49 ± 0.4
Eicosane	$C_{20}H_{42}$	NP	0.82 ± 1.4	ND	1.36 ± 2.3
Pentadecane	$C_{15}H_{32}$	NP	ND	11.16 ± 3.5	1.31 ± 2.3
Hexadecane	$C_{16}H_{34}$	NP	8.38 ± 1.9	8.29 ± 2.5	10.88 ± 0.9
Total (% area)			**25.49 ± 2.8**	**28.27 ± 4.2**	**21.91 ± 3.5**
Hydrocarbons (Alkenes)					
3-Tetradecene	$C_{14}H_{28}$	PP	5.37 ± 0.7	1.98 ± 0.7	0.84 ± 0.7

Compound (IUPAC)	Molecular formula	Polarity	Fraction 1 (% area)	Fraction 2 (% area)	Fraction 3 (% area)
3,7,11,15-tetramethyl-2-hexadecene	$C_{20}H_{40}$	PP	2.93 ± 2.3	1.62 ± 0.4	10.38 ± 2.7
Total (% area)			**8.30 ± 1.7**	**3.62 ± 0.2**	**11.22 ± 6.7**
Ester					
Ethyl docosanoato	$C_{24}H_{50}O_2$	P	0.82 ± 0.7	1.04 ± 0.2	0.99 ± 0.9
Ethyl behenate	$C_{24}H_{50}O_2$	P	0.54 ± 0.9	0.38 ± 0.3	6.35 ± 6.4
Ethyl oleate	$C_{20}H_{38}O_2$	P	3.69 ± 1.7	1.23 ± 0.8	2.86 ± 2.8
Ethyl stearate	$C_{18}H_{36}O_2$	P	9.30 ± 1.2	3.93 ± 1.4	13.72 ± 2.9
Total (% area)			**14.35 ± 4.1**	**6.58 ± 1.6**	**23.92 ± 5.6**
Carboxylic acids					
Dodecanoic acid	$C_{12}H_{24}O_2$	P	3.08 ± 0.8	0.46 ± 0.4	2.20 ± 0.5
5,8,11,14,17-Eicosapentaenoic acid	$C_{20}H_{36}O_2$	P	0.55 ± 0.9	ND	ND
8,11,14-Eicosatrienoic acid	$C_{20}H_{32}O_2$	P	0.53 ± 0.9	ND	ND
Total (% area)			**4.16 ± 1.5**	**0.46 ± 0.4**	**2.20 ± 0.5**

ND: Not detected
NP: Non-polar
PP: Partially polar
P: Polar

4. CONCLUSIONS

The production and processing of biomass from the microalgae *Tetradesmus obliquus* in compact photobioreactors (PBRs) were investigated to obtain a hydrocarbon-based fuel. After crude oil extraction, fractional distillation was employed as a purification method for the compounds. GC-MS analysis revealed an abundance of hydrocarbons (alkanes and alkenes) comparable to petroleum diesel. The extraction and distillation yields were evaluated under different experimental conditions, resulting in process yields of 123.79 ± 1.6 g and 50.20 ± 3.6%, respectively. Regarding the final conversion of the distilled oil, i.e., the yield relative to the initial biomass quantity of 1.5 kg used in the extraction, a value of 4.13 ± 0.3% was obtained. In summary, the pilot-scale production and extraction process of microalgae oil for hydrocarbon production appear promising, as it is possible to purify these compounds and obtain relevant information for future use.

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