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Extraction of bio-oil via wet based from *Nannochloropsis oculata*

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Abstract**—***Current research has shown a great interest in microalgae due to its ability to capture CO2 from the atmosphere, a harmful gas to the environment, and to store a high added value oil. However, the extraction of the oil from microalgae represents a stage of high energy demand, and it is necessary to develop a viable process in this aspect. In this context, the present work aims to extract the bio-oil from the microalga of the species Nannochloropsis oculata via humid base, in order to remove the drying step of the extraction process. For this, an experimental planning of the factorial type (3²) with two central points was used, totalizing 11 experiments for each solvent (ethanol and hexane). The variables studied in the planning were the time of application of the ultrasound and the time of extraction by Soxhlet. As a result, extraction with the application of ultrasound for 60 min, followed by Soxhlet for 8 h with hexane showed better yield (20.29%) of bio-oil. The production of bio-oil under the same conditions with ethanol was 16.83%. The lowest energy expenditure in relation to the production of 1 g of oil occurred under the conditions of application of 60 min of ultrasound and 4 hours of solid-liquid extraction. The energy expenditure was also compared to the dry and wet basis with the two solvents. In the dry base extraction, the yield was lower than the wet basis. By the analysis of GC-MS it was observed that drying influences the profile of fatty acids, reducing the percentage of monounsaturated and polyunsaturated fatty acids. It is concluded that the wet basis extraction is the best option, since drying deteriorates the bio-oil and does not influence significantly the yield of the bio-oil.*

Keywords**—renewable energy, energy, statistic.**

I. INTRODUCTION

Energy security is a concern for an environmentally sustainable economy, hence the need to seek alternative renewable energies that may have the potential to meet human needs and future environmental constraints [1]. Thus, microalgae emerge as a possible solution, due to their ability to reduce atmospheric CO_2, in the face of today's notorious environmental problems, such as the greenhouse effect [2].

They are also more advantageous when compared to terrestrial plants, since they require less water and can even reuse residual water in their growth, thus reducing their overall consumption [3].

This growing interest, due to their attractive potential in the current scenario of reduction of energy and food resources [4], also suggests another relevant feature, that they are important sources of triacylglycerols (TAGs) and high value compounds such as carotenoids and acids long-chain polyunsaturated fatty acids (LC-PUFAs) [5]. These pigments are likely to undergo rapid commercial success in functional foods, cosmetics, aquaculture, pharmaceuticals, or food technology [6].

In relation to production, this can be carried out throughout the year and can grow rapidly in a wide range of habitats under photoautotrophic conditions [7]. However, it is also important to mention that microalgae have high photosynthetic efficiency as a characteristic [8], which provides high efficiency and productivity when analyzing oil produced per unit area [9].

Thus, in light of the above, it is possible to verify that microalgae have characteristics that are very attractive from a productive, socioeconomic and environmental point of view. The specific interest of the genus Nannochloropsis is due to the ease of cultivation, a small size that allows a high growth rate, relatively mature technology for large-scale cultivation systems [10] and its high capacity to synthesize lipids [11].

Let us cite that several species of Nannochloropsis are grown successfully on a large-scale using sunlight by companies such as Solix Biofuels, Aurora Algas, Seambiotice Proviron [12]. *Nannochloropsis oculata* has been widely used because it is resistant, easily accessible and widely distributed in the oceans [13]. However, there are technological challenges to overcome, one of which is

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the optimization of the processes of bio-oil extraction to make it feasible, from an economic, environmental and energy point of view, allowing a large-scale production. It is necessary to develop energy-saving and scalable lipid harvesting and extraction processes so that the biofuel industry and algal bioproducts are prosperous [14]. For example, the extraction of oil from microalgae represents a step that requires high energy demand. For, most of the time, water removal is necessary, which makes the process expensive [15]. It is therefore necessary to develop a viable process from this point of view in order to obtain more economically and environmentally sustainable products.

Achieving the objectives of socioeconomic and environmental sustainability is to demand changes not only in the way energy is supplied, but in the way, it is used [16]. Reducing the quantity required for product delivery is thus an essential complement to the efforts of the set of energy supply and resource technologies aimed at reducing environmental impacts.

The objective of this work is to extract the bio-oil from the microalgae of the species *Nannochloropsis oculata* via humid base in order to remove the drying step of the extraction process to reduce the energy expenditure of the bio- oil from microalgae *Nannochloropsis oculata*.

II. MATERIAL AND METHODS

2.1.Farming

The microalgae strain *Nannochloropsis oculata* was maintained at a mean temperature of 288 K (15ºC) in a greenhouse in the growing room, with culture medium adapted to the methodology of [17] and [18]. In the composition of nitrate, sodium nitrate (150g.L^{-1}), iron chloride (13g.L^{-1}), EDTA (10g.$L^{-1)}$ and traces metal solution (1 mL.L^{-1}); in the composition of the phosphate solution, sodium phosphate (16g.L^{-1}) and vitamin B12 (2mL.L^{-1}); in the composition of the silica solution, sodium silicate (60g.L^{1}).

The cultivation was carried out at the Labor Engineering Laboratory, located at the Federal University of Espírito Santo, in the municipality of São Mateus, state of Espírito Santo, Brazil. The medium was prepared using seawater (water collected at sea) along with the essential nutrients, in a ratio of 1 mL of each nutrient for each 1L of microalgal culture (Figure 1).

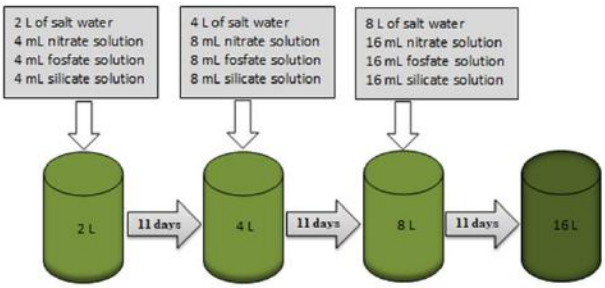

Fig. 1: Flowchart of microalgae cultivation on laboratory scale

In order to elaborate the growth curve, in an automated way, temperature, pH and luminosity sensors were used during 11 days, being the data measured, sent and stored in a spreadsheet, resulting in a database and from the execution and analysis of the growth curve was specifically defined as the 5th day as suitable for the duplication of microalgae.

The experiment was performed in a 4-gallon 16L bookcase, divided into 2 workbenches. Each bench consisted of 2 gallons exposed to 4 LED bulbs, each 18W bulb. The aeration system was carried out in an ascending and constant manner, by an air pump, which aided in the formation of a bubble column. The required power of the pump was 3.5W with a flow rate of 6L.min^{-1} (Figure 2).

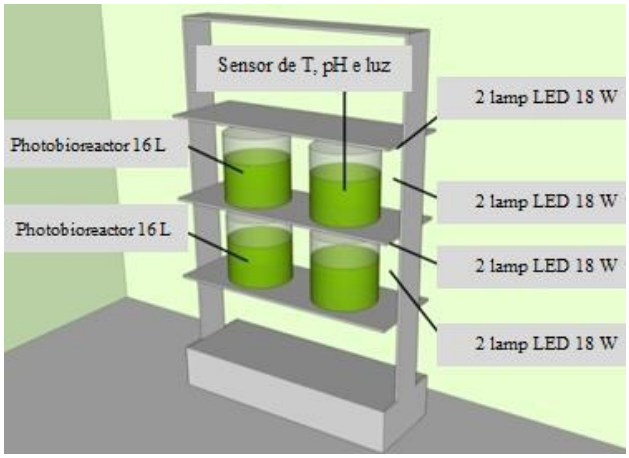

*Fig. 2: Laboratory scale cultivation bench * The increase or decrease of the microalga concentration was observed by the tone of the medium on a scale of 0 to 10, with 10 being more concentrated and 0 being less concentrated*

2.2. Separation of biomass

In order to validate the use and determination of the appropriate concentration of the Tanfloc SG® flocculant, capable of separating the microalgae *Nannochloropsis oculata* from seawater, a previous test was carried out. It was performed in a Jar Test equipment containing acrylic vats with a total capacity of 2L. In each jar of the Jar Test

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was added 1.5L of microalgae and 7.5mL of Tanfloc SG® solution with different concentrations. In the 1st well was added a solution with a concentration of 20g.L^{-1} in the second solution with 30g.L^{-1}, in the 3rd one was added 40g.L^{-1} and in the 4th one was added 50g.L^{-1} (test performed with rotation 100rpm for 10min).

After identifying the concentration of Tanfloc SG® suitable for separation, it was applied in the separation of all cultures and after flocculation a vacuum filtration was applied to remove the algal biomass from the medium.

To measure the moisture variable, the method was used until constant mass. Thus, the mass of 10g was determined and subjected to drying in an oven at 373K (100 ºC) and the masses cleared on an analytical scale until reaching constant values. Finally, these were cooled in a vacuum desiccator [19].

2.3. Extraction of bio-oil by Soxhlet

Lipid extraction was adapted as performed by [20]. 5g of algal biomass were weighed in analytical balance and transferred to a 50ml Erlenmeyer flask. Subsequently, 25ml of solvent was added and then taken to an ultrasonic bath at a frequency of 25kHz and after ultrasonication, the oil was extracted into the Soxhlet extractor.

Soxhlet extraction was performed according to method 920.39 [21], using an experimental design of factorial type (3^2), with two more central points, totaling 11 experiments for each solvent (ethanol and hexane). The variables studied in the planning were the time of application of the ultrasound and the time of extraction by Soxhlet.

After the extraction time, the flask was removed from the extractor and the solvent was evaporated in a rotary evaporator. Subsequently, the flask was transferred to a greenhouse, at 373K (100ºC) and subjected to drying (mass determination was carried out until reaching constant value). The flask was transferred to a vacuum desiccator and cooled [19]. Then, the bio-oil yield was calculated by the percentage difference between the initial and final mass in relation to the initial mass, the results of which were compared to each experimental condition in order to identify the best operational condition for extraction of bio-oil as a function of lower energy expenditure.

Finally, after identifying the operational condition with the highest yield in relation to the energy expenditure, four cultures with 64 L were performed, aiming to analyze the influence of the drying and the type of solvent in the bio-oil extraction.

2.4. Extraction by Bligh and Dyer

The extraction of the oil was also carried out by the method of Bligh and Dyer, in which it was carried out in the cold, using a mixture of chloroform, methanol and water in exact proportions. This mixture provides the extraction of all classes of lipids [22]. 15g of bio-oil was added in a beaker of 250mL was determined on analytical balance. Once determined, 15mL of chloroform and 30mL of methanol were added by stirring for 5 min on a magnetic stirrer. After, 15mL of chloroform was added, stirred for 2min. After stirring, 15mL of water was added by stirring for 5min. A vacuum filtration was then performed. The filtrate was collected on a separatory funnel, and 1mL of a saturated NaCl solution was added thereto. After standing for 24h the chloroform-rich lower phase ($CHCl_3$) was removed, rotating the phase at 33-35°C with a rotation of 50rpm for 15min.

2.5. Fourier Transform Infrared Spectroscopy (FTIR)

Samples of the obtained oils were analyzed by Fourier Transform Infrared (FTIR) spectroscopy, conducted in the 4000 to 400cm^{-1} waveband to identify the functional groups present in the material. The AgilentCary 630 FTIR Spectrometer model spectrometer [23] was used. The sample was placed directly in the hole of the crystal in which the reading of the ATR accessory occurs.

2.6. Gas Chromatography

The methylation was performed according to [24], about 40-90mg lipid mass was determined in a test tube and 4mL of NaOH (0.5 mol L -1) in CH_3OH was added. The tubes were heated in a boiling water bath until the fat globules dissolve (3-5min). Subsequently, the tubes were cooled in running water and 5mL of the esterifying reagent (NH_4Cl (10g), H_2SO_4 (15mL) and CH_3OH (300mL) were added).

The tubes were shaken and heated again in a boiling water bath for 5min and after cooling in running water, 4ml of saturated NaCl solution was added, then shaken manually. After stirring, 5mL of hexane was added with vigorous stirring and the tubes were allowed to stand for 24h and the supernatant aliquot was evaluated by gas chromatography coupled to mass spectrometer.

The components of the liquid product were analyzed using gas chromatography (GC) coupled to mass spectrometer model GC-MS-OP 2010 (Shimadzu), according to [25], with adaptations. Containing the following specifications: 333K (60°C) oven column temperature, Spitless type injection, injection temperature of 503 K (230°C), split ratio 10, flow control with linear

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velocity, column flow of 1mL.min^{-1}, helium loading gas 99.9995% purity. The furnace column started the temperature of 333K (60ºC), by waiting time of 2 min, followed by a rate of 15 reaching 408K (135ºC), for a time of 1 min, followed by a rate of 3 reaching 488K (215°C), with a holding time of 10 min. The model of the column is the DB-5 with length 30.0 m, diameter 0.25 mm, thickness of the film 0.25µm. A MS conditions, 513K(240°C) ion source temperature and interface temperature 513K(240°C), initial mass band of 40 and final 1000.

III. RESULTS AND DISCUSSION

3.1. Cultivation

It was possible to observe that in the first day the microalgal population was in the adaptation phase or lag phase, with low growth. Between the 1st and 4th day the cellular divisions occurred more rapidly, designating its exponential phase. Between the 4th and 7th day, we can see that there was a reduction of divisions and a certain stabilization between the growth rate. This behavior is characteristic of the stationary phase. Finally, the decline phase began on the 7th day of cultivation, in which it was identified by the brown coloration and decantation of the microalgae (Figure 3).

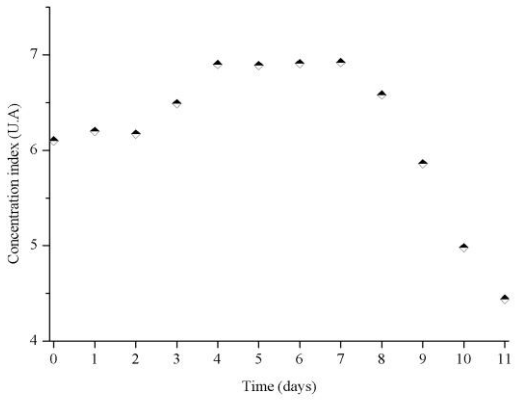

Fig. 3: With the sensing of the culture of the microalgae it was possible to obtain the growth curve from the staining of the culture medium of the species Nannochloropsis oculata

It was noted that the growth of microalgae increases exponentially until some factors begin to deplete. The concentration of nutrients is one of the most important factors, since it decreases over time in a culture medium [26]. Once all the resources (nutrients) are consumed, the cultures end up dying. Observing the growth curve, the duplication of the algal culture took place between the 4th and the 7th day, since the cell concentration reached the limit within this range and from the 7th day, the microalgae began to die. It is concluded that the amount of nutrients used in this work was sufficient for a culture time of up to 7 days. By means of the sensors, the pH was also evaluated during the 11 days of cultivation, with a variation between 7.2 and 7.6 during the whole crop (Figure 4). Therefore, the pH was favorable to the growth of microalgae, as it remained slightly basic throughout the days of cultivation, not reaching cellular enzymes.

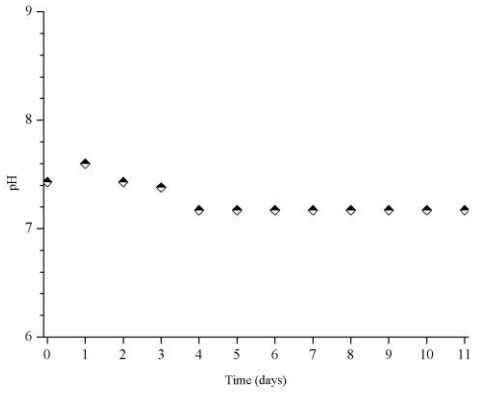

Fig. 4: pH variation as a function of the culture time obtained by the sensor

O pH is an important environmental parameter because it exerts influence on cell growth and product formation [27]. It is directly linked to the good cellular functioning of microalgae, in that sense, its control is of vital importance for the better development of the crops.

Besides the pH and concentration of nutrients, the temperature also has a direct influence on the reproduction of microalgae, as it can cause changes in many biosynthetic pathways [27]. The temperature has significant impacts on the growth rate, cell size and biochemical composition of microalgae [28]. Thus, attention on this parameter is extremely important, since it also has effects on the production of biomass.

The temperature was monitored, presenting variations between 298.5K (25.5ºC) and 290.4K (27.4ºC), such variation being not significant (Figure 5). It is observed that temperatures below 291K (18ºC) can decelerate growth, whereas above 308K (35ºC) may be lethal for most species [28]. Therefore, this culture parameter was considered adequate, since it was within the range appropriate to microalgal growth (Figure 5).

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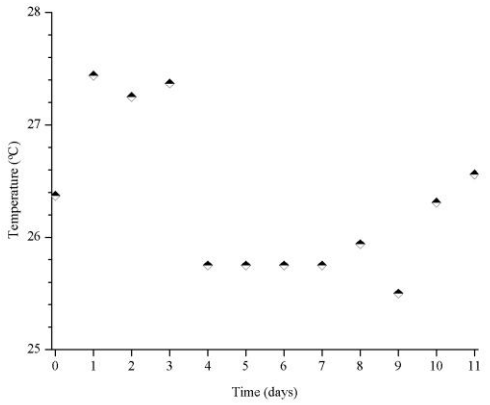

Fig.5: Temperature variation as a function of the culture time obtained by the sensor

From these analyzes it was concluded that the pH and temperature did not limit the growth, fact occurring with the decrease of the concentration of nutrients in the culture medium over time.

3.2. Separation and production of biomass

The flocculation, dedicated to the separation of the biomass from the medium, only occurred with the concentration 50g.L^{-1}. This concentration was adopted as a satisfactory condition for separation of the whole crop, and for each 64L culture, about 0.48L of Tanfloc SG® solution was consumed.

The moisture content of the sample was approximately 60% and this parameterization was performed to estimate the biomass production and to avoid errors of biomass mass determination in the experiments.

As a result, the yield of algal biomass was 88 g in 64L of culture, equivalent to a growth rate of 0.09g.L^{-1}.day. This measure achieved a productivity higher than that of other species comparatively. This is a possible conclusion from the data obtained by [29], which obtained a growth rate of 0.04g.L^{-1} day for the species (Chlorella sorokiniana, Bracteacoccus minor, Radiosphaera negevensis, Chlorosarcinopsis negevensis and Chlorococcum novae-angliae). in a photobioreactor with high levels of CO_2.

Biomass production in this work was also higher than that reported by [30], in which they cultivated the species *Nannochloropsis oculata* in open ponds during winter and spring reaching a concentration of 0.05g.L^{-1}.d^{-1} biomass.

It is important to mention that the extraction and processing of biochemical microalgae compounds requires the handling of large volumes of raw material, largely due to the small biomass / liquid ratio, typically <0.1% solids [31].

3.3. Extraction of bio-oil

In Table 1 it is possible to note the data on the bio-oil yield and energy expenditure referring to the extraction with the solvent ethanol and hexane under experimental planning conditions.

Table 1: Factorial Planning 3² Experiment planning matrix for the extraction of bio-oil by ultrasound and Soxhlet with ethanol solvent.

Experiment	Exhibition Ultrasound(min)	Extraction Soxhlet (h)	R (%)	Energy Expenditure (kWh.g^{-1}oil)	R (%)	Energy Expenditure (kWh.g^{-1}oil)
			Ethanol		Hexane	
1	30	4	11.45	7.14	17.21	4.76
2	30	6	11.62	11.11	17.25	7.14
3	30	8	11.82	14.28	17.32	9.09
4	45	4	13.32	6.25	18.35	4.54
5	45	6	13.52	9.09	18.42	6.67
6	45	8	14.15	11.11	18.51	9.09
7	60	4	15.72	5.26	20.18	4.17
8	60	6	16.21	7.69	20.26	6.25
9	60	8	16.83	10.00	20.29	8.33
10	45	6	13.58	9.09	18.41	6.67
11	45	6	13.61	9.09	18.45	6.67

For the statistical analysis of the result, the effects of each independent variable (linear and quadratic terms) were calculated, as well as the possible interactions, in the response. The determination of the significant parameters of the regression model was performed by means of a hypothesis test using the t-student distribution (significance level 5%) (Table 2).

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Table 2: Time effects of exposure to ultrasound and Soxhlet extraction on the yield of bio-oil extracted with ethanol.

Variable Response	Factor	Effect		t(5)	p-value	Effeito		t(5)	p-value
		Ethanol				Hexane			
R (%) R²= 0.99	Average	13.85	.02	740.26	0.00	18.64	.01	2770.71	0.00
	X_1	4.62	.05	95.43	0.00	2.93	.02	171.21	0.00
	X_1^2	-0.28	.04	-7.49	0.00	-0.32	.01	-24.16	0.00
	X_2	0.77	.05	15.89	0.00	0.12	.02	7.27	0.00
	X_2^2	-0.09	.04	-2.66	0.04	0.00	.01	0.08	0.94
	X_1X_2	0.37	.06	6.24	0.00	0.00	0.02	0.00	1.00

For the yield response with ethanol, it was verified that the linear and quadratic terms of the time of exposure to the ultrasound (X_1) and time of extraction by Soxhlet (X_2), as well as the term of interaction between the variables are significant for the model, since they had a p-value lower than 0.05 (red terms). The linear factor of the time of exposure to the ultrasound (X_1) has the greatest effect and the quadratic factor of the time of exposure to the ultrasound (X_1^2) has the smallest effect on the yield of the bio-oil extracted via the wet basis with ethanol. Evidencing that depending on the type of biomass, it is desirable to pretreat the biomass prior to the extraction of the lipid.

That is, pre-treatment or disruption of microalgae cells induces the extraction of lipids, releasing them from the encapsulated cellular structures [32].

With respect to the yield of bio-oil extracted with hexane, it is noted that the quadratic effect of the extraction time by Soxhlet (X_2^2) and the time interaction of exposure to ultrasound and extraction by Soxhlet X_1X_2 were not significant for the range of determined conditions by the planning of experiments (30 min $<X_1<$60 min and 4h$<X_2<$8h). The other terms had a p-value of less than 0.05 and were significant in this study.

The linear factor of time exposure to ultrasound (X_1) has the greatest effect and the quadratic factor (X_1^2) has the smallest effect on the yield of the bio-oil extracted via wet basis with hexane. Since the statistical models for predicting the time of exposure to ultrasound (X_1) and time of extraction by Soxhlet (X_2), with significant terms, are exposed in Eq.1 (ethanol) and Eq.2 (hexane). models $30<X_1<60$ and $4<X_2<8$ and $R^2 = 0.99$ are valid.

$$\text{Yield } (\%) = 10.58 + 0.01\ X_1 - 0.38X_2 + 0.01X_1^2 + 0.01\ X_1X_2 + 0.02\ X_2^2 \quad (1)$$

$$\text{Yield } (\%) = 16.67 - 0.03\ X_1 + 0.035\ X_2 + 0.01\ X_1^2 \quad (2)$$

Analyzing Figure 6 below it is verified that the higher yield of bio-oil via wet basis with ethanol (greater than 16% relative to wet biomass) is achieved when using biomass exposure to the ultrasound for 60min and extraction in Soxhlet for 8h, being the optimal region for the production of bio-oil with ethanol from the microalgae *Nannochloropsis oculata*. And the highest yield of wet-based bio-oil with hexane (greater than 20% relative to wet biomass) is achieved when using biomass exposure to the ultrasound for 60min and extraction time in Soxhlet for 8h, being also the optimal region for the production of bio-oil with hexane.

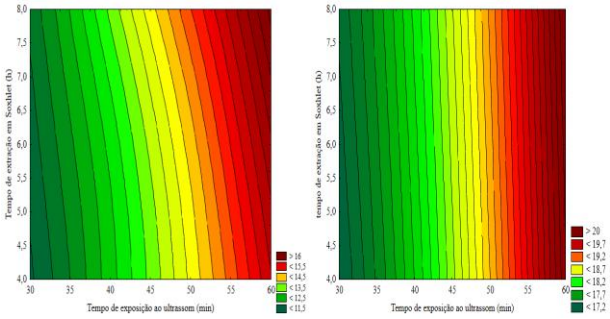

Fig. 6: Contour surface for the yield of the bio-oil wet basis using ethanol solvent as a function of the time of exposure to the ultrasound and extraction time in Soxhlet extractor.

Statistically considering the result obtained, the effects of each independent variable (linear and quadratic terms) were calculated, as well as possible interactions in the response, energy expenditure per g of oil ($kWh.g^{-1}$). The determination of significant parameters of the regression model was performed using a hypothesis test using the t-student distribution (significance level of 5%) (Table 3).

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Table 3: Time effects of exposure to ultrasound and Soxhlet extraction on energy expenditure per gram of bio-oil extracted with ethanol.

Variable Response	Factor	Effect	σ	t(5)	p-value	Efeito	σ	t(5)	p-value
		Ethanol				Hexane			
R (%) R^2= 0.99	Average	9.11	0.09	96.56	0.00	6.66	0.04	160.40	0.00
	X_1	-3.19	0.24	-13.07	0.00	-0.75	0.11	-6.94	0.00
	X_1^2	-0.40	0.19	-2.15	0.08	0.11	0,08	1,30	0,25
	X_2	5.58	0.24	22.84	0.00	4.35	0.11	40.43	0.00
	X_2^2	0.31	0.19	1.68	0.15	-0.01	0.08	-0.15	0.89
	X_1X_2	-1.20	0.30	-4.00	0.01	-0.09	0.13	-0.64	0.55

As a response to the energy expenditure per gram of oil, it was found that the linear terms of the time of exposure to ultrasound (X_1) and time of extraction by Soxhlet (X_2), as well as the term of interaction between the variables, are significant for the model, since they presented a p-value inferior to 0.05 (terms in red). It was also found that the quadratic terms of the exposure time to the ultrasound (X_1^2) and extraction time by Soxhlet (X_2^2), are not significant for the model, since they had a p-value greater than 0.05 (terms in black).

The linear factor of the extraction time in Soxhlet (X_2) has the greatest effect and the linear factor of the time of exposure to the ultrasound (X_1) has the smallest effect on the energy expenditure per gram of the bio-oil extracted via the base with ethanol.

For the energy expenditure per gram of oil extracted with hexane, it was verified that the linear terms of the time of exposure to the ultrasound (X_1) and time of extraction by Soxhlet (X_2) are significant for the model, since they presented a p-value less than 0.05 (terms in red). It is observed that the quadratic terms of the time of exposure to the ultrasound (X_1^2), time of extraction by Soxhlet (X_2^2) and interaction between the linear terms are not significant for the model, since they presented a p-value superior to 0.05 (terms in black).

The linear factor of the extraction time in Soxhlet (X_2) has the greatest effect and the linear factor of the time of exposure to the ultrasound (X_1) has the smallest effect on the energy expenditure per gram of the bio-oil extracted via the base with ethanol.

The mathematical models for predicting the time of exposure to ultrasound (X_1) and time of extraction by Soxhlet (X_2) with the significant terms are shown in Eq.3 and Eq.4. The model is valid only $30<X_1<60$ and $4<X_2<8$ and $R^2 = 0.99$.

$$\text{Energy Expenditure (kWh.g}^{-1}\text{ oil)} = 0.88 - 0.15\ X_1 + 3.24\ X_2 - 0.02\ X_1X_2 \quad (3)$$

$$\text{Energy Expenditure (kWh.g}^{-1}\text{ oil)} = 0.08 + 0.03\ X_1 + 1.11\ X_2 \quad (4)$$

In the Figure 7 shows the behavior of the estimated contour surface for the energy expenditure of biomass production, separation and extraction of the bio-oil via wet basis with ethanol and hexane. The lowest energy expenditure per g of oil extracted with ethanol was in the condition of the experimental design, which exposed the biomass for 60min and the extraction via Soxhlet for 4h, being the optimal region. And for the production of bio-oil extracted with hexane was in the condition of the experimental planning that exposed the biomass by 60min and the extraction by Soxhlet, by 4h, being the optimal region.

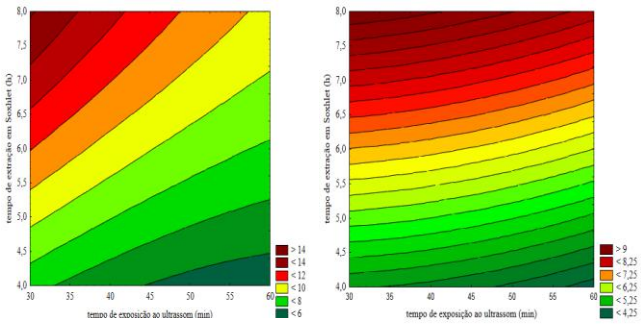

Fig. 7: Response surface of energy expenditure in the production of bio-oil via wet basis with ethanol and hexane

Thus, as in the extraction via wet basis with hexane, the lowest energy expenditure per g of oil for the production of bio-oil extracted with ethanol was in the condition of the experimental design, which exposed the biomass for 60min and the extraction via Soxhlet for 4h , therefore, it was adopted as operational conditions for comparison between wet and dry extraction with the different solvents (Table 4).

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Table 4: Energy expenditure in the production of bio-oil for each extraction method.

Extraction method	Biomass	Solvent	R (%)	Energy expenditure
Ultrasound and Soxhlet	humid	ethanol	15.72	5.25 kWh.g^{-1}oil
Ultrasound and soxhlet	dry	ethanol	14.58	141.55 kWh.g^{-1}oil
Ultrasound and Soxhlet	humid	hexane	20.18	4.25 kWh.g^{-1}oil
Ultrasound and soxhlet	dry	hexane	19.92	101.09 kWh.g^{-1}oil
Bligh and Dyer	dry	chloroform/methanol	17.82	122.56 kWh.g^{-1}oil

In the extraction method that used the ultrasound in conjunction with Soxhlet, the yield varied between 14.58 and 20.18%. This variation in yield was due to the condition of the biomass (dry or wet) and the type of solvent. In this method the best yield occurred with and extraction via wet basis using hexane as solvent.

It may be noted that hexane is the most popular apolar solvent in oil extraction because it is relatively inexpensive and has a high affinity to the oil, exhibiting a better extraction efficiency compared to the use of non-polar solvents such as ethanol [33]. However, factors such as toxicity and damage to the environment caused by products from fossil sources need to be better investigated.

The yield of the method using the Soxhlet ultrasound was much higher than the yield [34] obtained when using only ultrasound with fresh biomass (5.7%) for 30min. They highlighted the importance of Soxhlet in the bio-oil yield. These authors also evaluated the extraction by Bligh and Dyer with fresh biomass and obtained a yield of 0.48%. Such yield was lower than that found in this work by the same method. Already [17], they obtained a superior yield (22.75%) to that found in this work by the extraction by Bligh and Dyer. It should also be noted that, in addition to the extraction methods, the culture conditions influence the storage of lipids in microalgae.

The yield of bio-oil was also compared to other methods, since [35] obtained a yield of 71.13% by supercritical CO_2 extraction and [36] by hydrothermal liquefaction (HTL), using water under high temperatures and pressures (200-350°C, 5-20MPa) yielded between 23-59% lipids. It should be noted that the yields found in this study are close to those of the authors cited.

By analyzing the energy expenditure and the yield of the methods, it can be seen that drying does not have a significant influence on the yield of the bio-oil, since the production of bio-oil was very close when using dry and wet biomass. Also, among the methods, no significant variations in yields were observed.

3.4. Analysis of the products by Spectroscopy in the Infrared Region (FTIR)

The FTIR spectra of the bio-oil samples via wet and dry basis extracted with ethanol can be seen in Figure 8a and hexane in Figure 8b. FTIR analysis was performed to compare dry base wet extraction with different solvents and to certify that the effect of ultrasound waves ruptured the cell wall.

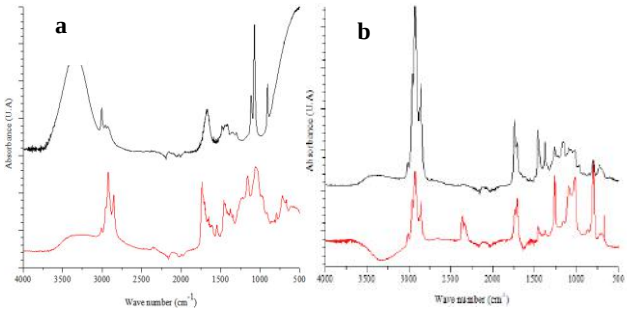

Fig. 8: FTIR spectra of the bio-oil samples via wet and dry basis a) extracted with ethanol b) extracted with hexane.

The spectra were divided in the regions of 4000 to 1300cm^{-1} (region 1), from 1300cm^{-1} to 900cm^{-1} (region 2) and from 900 to 500cm^{-1} (region 3), the 1 was pointed as a region of functional groups, where there are generally few absorption bands, which refer to the stretches of the main functional groups, such as OH, NH and C = O [37]. Thus, by the analysis of the spectra, it was possible to observe that the effect was positive due to the presentation of bands characteristic of carboxylic acids and ester. This is because the oil is a mixture of fatty acid esters of glycerol, and the fatty acids are long chain carboxylic acids [38].

In region 1 it was possible to analyze the spectrum and to identify a broad band in 3326cm^{-1} superimposed on other bands, indicating the presence of OH in the bio-oil sample and indicating the presence of carboxylic acid, water and ethanol. The OH band in the samples that have carboxylic acids always appears superimposed on the

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stretching bands of CH bonds, both those involving sp^3 carbon and those associated with sp^2 carbon. Also, in region 1 of the spectrum, a band at 1622cm^{-1}, which is attributed to the stretching of the C=O bond, confirms the presence of oil in the sample [37].

Also, in region 1, two bands are identified, one at 1439cm^{-1} and the other at 1368cm^{-1}, referring to the asymmetric and symmetrical stretching of CH_3 and also, a band at 1328cm^{-1}. [38] also cites that the CH_2 band is common in long-chain fatty acid spectra in the wavelength range at 1345-1118cm^{-1}.

Analyzing the region 2 of the sample studied, known as fingerprint or fingerprint [39], it is found that it is between 1,300-900cm^{-1}. In this region a band is observed in 1247cm^{-1} referring to C-CO-O and one in 1095cm^{-1} referring to O-C-C. In region 3, only one band at 881 cm^{-1} of the absorption of the angular deformation outside the CH plane was identified.

With the above, comparing the spectrum of the extracted bio-oil from the wet biomass with the drought, an absorption band between 3400-2800cm^{-1} higher when extracted via humid base is observed. The higher OH band, in the bio-oil sample extracted via wet basis, is due to the OH pools of water from the biomass moisture. Also, analyzing the bands in the spectra, it is noticed that the moisture of the biomass during the extraction does not alter the bonds of the molecules of the final product, since the bands referring to the groupings are identical.

Examining the spectra of Figure 10 it will be seen that the absorption bands are located at the same wavelength. This indicates that the two samples have the same functional groups. It was observed that the extractions by wet basis with both ethanol and hexane were satisfactory, since the samples had bio-oil, so an extraction was carried out following the Bligh and Dyer method as well (Figure 10).

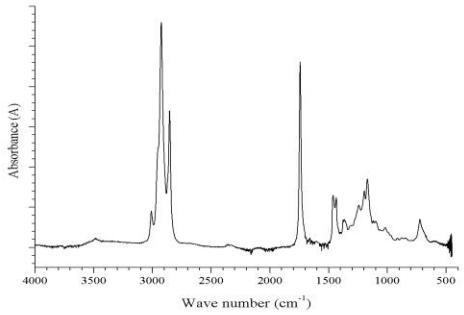

Fig. 10: FTIR spectrum of the bio-oil sample extracted by Bligh and Dyer

It is observed that it is possible to note that the groups in the sample extracted by the Bligh and Dyer method are bio-oil clusters, as well as in the hot extractions. However, the C-H uptake peaks between 3000-2800cm^{-1} and C=O at 1750cm^{-1} are very high compared to Soxhlet extraction with both ethanol and hexane.

Bligh and Dyer extraction or cold extraction was performed and analyzed by FTIR to identify possible changes in the bio-oil chains, since Soxhlet extraction is used at an elevated temperature. However, no change in the structure of fatty acid chains was observed by the FTIR spectra. It is verified that they have the same bands in all the extractions executed in this work.

3.5. Gas Chromatography

The chemical composition of the oils and fats is expressed by the fatty acids present [40]. The bio-oil obtained was analyzed by gas chromatography coupled to a mass spectrometry detector (CG / MS), in order to evaluate the fatty acid profile present in the samples in the different methods studied. Thus, considering 90% similarity for the identification of the compounds compared to the mass spectra obtained from the samples with the NIST 08 library of the equipment worked, it is noted that all products are composed of fatty acids, such as expected [41]. It is known, however, that the products differ in composition and fraction of fatty acids (Table 5).

In the composition of the extracted bio-oil wet basis with ethanol the linoleic acid (C18:2) stands out, while in the composition of the bio-oil extracted via dry base, with the same solvent, the predominance is oleic acid (C18:1) . However, the composition of the extracted bio-oil wet basis with hexane predominates the C16: 1, whereas in the composition of the bio-oil extracted by dry basis with the same solvent the predominance is C16:1.

With similarity of 90% no fatty acid C22:6n-3 (DHA) was identified. As for drying, it is responsible for the removal of a large amount of water present in the biomass of microalgae [42]. However, it should be noted that this operation may affect the quality of the product and cause lipid oxidation. In their work, [43] also showed that drying may cause changes in the fatty acid profiles of microalgae, with the double bonds breaking when the biomass is subjected to dry base extraction.

According to [44] and [45], low levels of polyunsaturated fatty acids are desired for the production of biodiesel because it reduces the need for treatments such as catalytic hydrogenation. The reverse trend is observed with EPA, whose content increases with temperature. Therefore, it is possible to obtain the content

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of the FAME classes according to the extraction process used.

Table 5: Profile of fatty acids for different methods and solvents

Tipo de biomassa	Tempo de retenção	%	Fórmula
Biomass humid Ethanol	25.03	2.10	C20:4
	25.23	3.48	C20:5
	25.82	1.14	C16:1
	26.55	17.21	C16:0
	31.77	41.34	C18:2
	31.95	27.45	C18:1
	32.55	6.78	C17:0
Biomass dry Ethanol	26.461	11.19	C16:0
	31.54	35.47	C18:2
	31.723	50.51	C18:1
	32.486	2.83	C18:0
	32.629	0.80	C17:0
Biomass humid Hexane	25.216	2.39	C20:4
	26.532	26.99	C20:5
	26.532	53.63	C16:1
	32.224	1.04	C16:0
	32.538	13.08	C18:2
	37.851	0.94	C18:1
	38.858	1.93	C17:0
Biomass dry Hexane	25.82	4.89	C16:1
	32.54	61.86	C16:0
	37.85	4.16	C18:1
	38.86	9.82	C17:0
Biomass dry Methanol e Chloroform	26.48	10.94	C16:0
	31.58	51.46	C18:2
	31.75	34.55	C18:1
	32.51	3.05	C18:0

Analyzing the fatty acid profile previously discussed in Table 5, only long-chain polyunsaturated fatty acids, such as arachidonic acid (AA 20:4), which originates from linoleic acid (C18:2) and eicosapentaenoic acid (EPA C20:5), in wet basis extraction with different solvents. Noting therefore that the biomass drying has a significant influence on the production of polyunsaturated fatty acids.

For example, the values found with wet basis extraction are close to those of [46], with a yield of 19.13±0.08 to 37.83±0.37% eicosapentaenoic acid (C20:5) of *N. gadinata*.

However, when analyzing the thermal effect on the yield value, [34] concluded that the distribution of fatty acids depends on the treatment of the sample. It is notable that the profiles are different depending on the temperatures. Generally, when the temperature increases, the amounts of polyunsaturated fatty acids tend to decrease [42].

By the composition of the samples it is also noticed that the solvent is important in the extraction of lipids, especially long chain polyunsaturated. Figure 11 shows the mass spectra obtained by the mass spectrometer model GC-MS-OP 2010 (Shimadzu), which confirms the identification of the methyl ester substance of eicosapentaenoic acid.

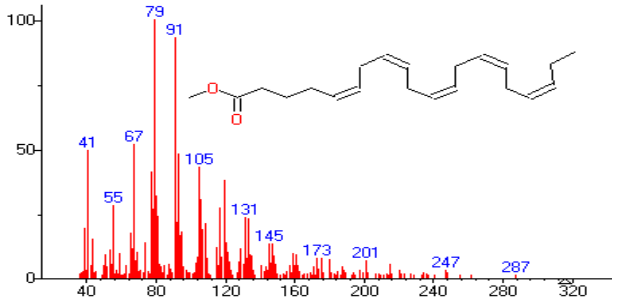

Fig. 11: Espectro de massas do metil éster do ácido eicosapentaenoico (C20:5)

However, it is noteworthy that the solvent-based extraction processes ideal for microalgae vary according to the cellular construction and chemical interactions of lipids and solvents used for extraction [47]. These techniques are capable of quantitatively extracting lipids from countless different samples. The only possible problem is the use of chloroform (toxic and suspected carcinogen [48]. Therefore, it is not feasible for large-scale applications, and some studies have investigated the use of other organic solvents such as hexane, ethanol, these solvents are highly flammable [44].

In the work of [34], they also extracted lipids with fresh biomass by the Bligh and Dyer method and ultrasound with low frequency (20kHz) without solvent for a time of 30min, which obtained 3.4 and 33.8% of C20:4 and C20:5, respectively in Bligh and Dyer extraction.

They also extracted the polyunsaturated fatty acids of type C20:4 and C20:5 with fresh biomass, with supercritical CO_2, having a yield of 0.58 and 3.59%,

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respectively [35]. Corroborating, in their study, [45] obtained with supercritical CO_2 0.60 and 4.53% of C20:4 and C20:5, respectively. Quantities lower than that found in the present study by the use of ultrasound with Soxhlet, independent of the solvent.

Comparing the profile of the fatty acids in Soxhlet extraction and Bligh and Dyer extraction, it was observed that in Soxhlet extraction with ethanol 35.47% of C18:2 was obtained, while in cold extraction the content of linoleic acid was 51.46%. Therefore, Soxhlet extraction, as well as drying influence the profile of fatty acids, breaking the bonds, and decreasing the percentage of monounsaturated and polyunsaturated fatty acids.

IV. CONCLUSION

The approach used in the present study was technically feasible for lipid extraction from microalgae cultivated via wet basis, with a significant reduction in energy expenditure. As explained, hexane allowed a better extraction of the oil when compared to ethanol under the same conditions.

The experimental study clearly demonstrated that the application of ultrasound waves in biomass, followed by Soxhlet extraction, can be used to extract lipids from moist Nannochloropsis oculata microalgae with an extraction yield similar to dry basis.

The yield of the wet basis extraction using hexane as the solvent was the largest of the other solvents and this efficiency can be justified by its affinity with oil, since both are apolar. However, the yield for the two types of solvent under the same operating conditions was close, not justifying the use of hexane over ethanol because of its toxicity.

The efficiency of the wet basis extraction with both solvents was considerably higher than the conventional lipid yield procedure if we consider the costs, the energy expended and the shorter distillation and extraction times needed used in the process the poly- unsaturated biomass cannot be subjected to excessive heat.

On the other hand, the methods that dry the biomass produce bio-oil with a higher percentage of saturated and monounsaturated fatty acids, being an important profile for biodiesel production, since the polyunsaturated chains affect the stability of the fuel, making it less resistant to oxidation.

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