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Experimental investigation on fast pyrolysis of freshwater algae. Prospects for alternative bio-fuel production

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ABSTRACT

Several technologies have been developed with the aim of obtaining fuels from algae. In the present work, the fast pyrolysis of three different types of microalgae (*Botryococcus braunii*, *Spirulina platensis*, and *Pithophora* sp.) is investigated focusing on the quality and yield of the liquid product as a potential biofuel. The characterization of microalgae shows an elevated content of lipids in *Botryococcus braunii*, higher levels of proteins in *Spirulina*, and an equal number of proteins and carbohydrates in *Pithophora* sp. A fixed bed reactor, connected to a vacuum system, and nitrogen as inert gas flow are used in the pyrolysis experiments at 300, 400, 500, and 600 °C. At 500 °C, *Botryococcus braunii* produces the maximum amount of pyrolytic oil (65% yield) while *Spirulina* and *Pithophora* sp. affords the greatest amount at 600 °C, in 45% and 28% yield, respectively. Gas chromatography-mass spectrometry (GC–MS) analysis of the *Botryococcus braunii*-derived oils shows a high content of long-chain derivatives of alcohols, carboxylic acids, and unsaturated hydrocarbons. On the other hand, *Spirulina*-derived oil consists mostly of nitrogenated compounds while oil from *Pithophora* sp. is composed of oxygenated and/or nitrogenated products, depending on the reaction temperature. The measured higher heating value (HHV) of *Botryococcus braunii*-derived oil produced at 500 °C is 45 MJ/kg and this bio-oil could be used as a feedstock for fuel production after chemical upgrading to decrease the oxygen content (6.59 wt%).

1. Introduction

Among the thermochemical processes, pyrolysis has emerged as a promising front-end processing technology on the path to renewable fuels [1–3]. In the pyrolysis process, the decomposition of biomass is carried out in absence of oxygen giving three mains products: a solid or bio-char, a liquid or bio-liquid, and a non-condensable or gas phase. Particularly, fast pyrolysis had moderate working temperature and very short vapor residence time, which favors the liquid fraction [4]. These short residence times can be achieved due to the introduction of vacuum and the presence of inert gas flow in the reactors [5–7]. For algae treatment, fast pyrolysis has become of great interest not only when searching for new fuel sources but also when leveraging a micro or macroalga that growths rapidly as a result of watercourses eutrophication in order to generate bio-oils or other products [8,9].

The pyrolysis oil from microalgae can contain different compounds such as straight-chain alkanes that are similar to diesel and nitrogenated species, which result from degradation of lipids and proteins, respectively. It is believed that these differences between lignocellulosic feedstocks may lead to improved properties in the resulting bio-oil, such as higher heating values (HHV), minor sulfur content, and reduced tar formation. However, some features of microalgae pyrolysis oil such as low viscosity, elevated nitrogen and oxygen content, high moisture, corrosivity, and low thermal stability, among others, should be improved before its direct application as a diesel or fuel oil [10].

To date, there have been different studies concerning the pyrolytic characteristics of microalgae. Miao et al. investigated the production of bio-oil from *C. prototheccoides* and *Microcystis aeruginosa* using a fluidized bed fast pyrolysis system. In this case, a high-quality bio-oil can be generated in 17–23% yield, with a HHV of 30 and 29 MJ/kg for *C. prototheccoides* and *Microcystis aeruginosa*-derived oils, respectively, which are about 1.4 times higher than those of wood sources [1]. Blue-green algae blooms (BGAB) mainly composed of *Microcystis* species could also be a potential source of renewable fuel with a HHV of 32 MJ/

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kg. In this study, the maximum oil yield (~55%) was obtained at a pyrolysis temperature of 500 °C in a fixed bed reactor [8]. Fast pyrolysis of *C. vulgaris* remnants obtained after lipid extraction produced high yield of pyrolytic oil (53 wt%) at 500 °C by using an atmospheric-pressure fluidized bed reactor. Results showed that 52% of the carbon in the algal remnants was recovered in the oil, while 43% of the carbon was recovered in the char [11]. Du et al. studied the pyrolysis of *Chlorella* sp. in a microwave oven, obtaining a maximum bio-oil yield of 29% at 750 W. This algal bio-oil had a density of ~1 kg/L and a HHV of 31 MJ/kg and was characterized by low oxygen content with high content of aliphatic and aromatic hydrocarbons [12]. According to Du's studies, *Scenedesmus* sp. is also a renewable fuel source. The fast pyrolysis of this microalgae at 480 °C afforded a high yield of pyrolysis oil (55%) showing an average calorific content of 18.4 MJ/kg. Moreover, the oil had a high nitrogen content and the average total acid number was lower than typical wood-derived bio-oils [13]. Aysu and Sanna [14] pyrolyzed *Nannochloropsis* sp. with and without various ceria-based catalysts at different temperatures. The highest bio-oil yield (23 wt%) and deoxygenation efficacy were obtained in the presence of Ni–Ce/Al_2O_3 at 500 °C. They found that HHVs of bio-oils were greater than the ones obtained in the non-catalyzed reactions as well as in zeolite-catalyzed processes.

Botryococcus braunii is considered as a promising green alga for fuel production since it has high lipid content (~75% of dry biomass weight) [15]. The pyrolytic behavior and kinetics of this alga were investigated by thermogravimetric analysis [16], analytical pyrolysis [17,18], fast pyrolysis [17], and laser micropyrolysis [19]. High yields of bio-oils were obtained in pyrolytic preparative processes between 460 and 600 °C. These oils were characterized by the dominant presence of hydrocarbons, especially C15–C30 alkenes/alkanes, exhibiting HHVs of 29–36 MJ/kg which were close to the HHV of crude triglyceride [16,20]. Catalytic processes have also been developed to improve the quality and quantity of *B. braunii*-derived bio-oils. It was found that zeolites decreased the temperature of biomass conversion and yielded high amounts of liquid hydrocarbons by promoting the deoxygenation and reducing the content of *N*-containing compounds [20,21]. Therefore, *B. braunii* can be catalogued as a hydrogen-rich solid fuel that is more analogous to fossil fuel than other terrestrial and algal biomass.

Other abundant and valuable algae species is *Spirulina platensis* (known as *Spirulina*), which is cultivated in a large scale and is extensively used to produce protein-rich alimentary material and bio nutrients [22]. Different thermochemical degradation processes have been applied for the transformation of *Spirulina* into high value-added products. Catalytic and non-catalytic fast pyrolysis led to bio-oils with more amount of nitrogen and less amount of oxygen than lignocellulosic biomass. The catalytic process in the presence of zeolites can be used to increment the oil yields (up to 38%) and to promote *O*- and *N*-reduction as well as aromatic hydrocarbon formation [23,24]. Microwave pyrolysis of *Spirulina* in presence of activated carbon or Fe_3O_4 provided high quantities of bio-oil (31–48% yields) at 500 °C [25]. At the same temperature, the catalytic solar pyrolysis of this alga produced 36% yield of bio-oil using hydrotalcite. Both pyrolytic oils showed high content of aromatic and aliphatic hydrocarbons, which are interesting compounds for biofuels.

This study focuses on the formation of bio-oils with biofuel properties obtained from three different types of algae, *Botryococcus braunii*, *Spirulina platensis*, and *Pithophora* sp., by applying the fast pyrolysis technique under vacuum conditions. As mentioned above, different thermochemical methodologies have been investigated for the degradation of *B. braunii* and *Spirulina*; however, processes at lower pressures were not evaluated. It is well known that pyrolysis carried out in vacuum media has certain advantages compared to other techniques such as very short residence time of the organic vapor in the reactor, which significantly facilitates the formation of bio-oil and enhances the selectivity of certain compounds [4]. Based on these features, we decided to study the behavior of the algal materials using fast pyrolysis under vacuum in order to make a contribution to the knowledge of pyrolysis of different algae using non-conventional conditions. In addition, to the best of our knowledge, the pyrolytic transformation of *Pithophora* sp. has not already been evaluated. Therefore, this study can be a starting point for the use of bio-oils obtained from this alga for some particular purposes. Bio-oil production from the three algae was investigated between 300 and 600 °C applying pressures of 0.1–0.2 Torr. An exhaustive analysis of the components of each oil was conducted to determine the best bio-oil for fuel applications. Also, for pyrolysis of *B. braunii*, it was evaluated as affecting different reaction times and quantities of biomass in the quality and yield of the bio-oil.

2. Experimental section

2.1. Materials

Spirulina platensis was commercially acquired (Sigma-Aldrich) in the form of fine powder and used without further treatment. *Botryococcus braunii* was collected at San Agustín lagoon in Gral. Levalle and *Pithophora* sp. was collected in a garden pond from Córdoba (Córdoba Province, central Argentina). Voucher material of *Botryococcus braunii* and *Pithophora* sp. has been deposited at CORD herbarium (Córdoba, Argentina) and images of fresh collections used in this study are provided in Supplementary Information. The taxonomic identification was based on Bourrelly [26] and Guiry et al. [27] The algal material was held in plastic bags with water until processing in the laboratory. Shortly after harvested, the algae were carefully washed under running water to exclude non-target material. Next, the material was air-dried at room temperature for subsequent analyses. Elemental and biochemical composition of algal biomass was determined, these analyses were performed in duplicate and informed as mean value ± standard error.

Acetone and hexane used in this study were analytical grade and used without further purification.

2.2. Pyrolysis experiments

The fast pyrolysis reactions were carried out using the methodology previously reported [5,6]. Experiments were conducted in a horizontal fixed bed semipreparative reactor heated externally by a tube furnace with a temperature-controller device. The reactor was connected to a high vacuum pump where pressures were in the range of 0.1–0.2 Torr and a flow of oxygen-free dry nitrogen permanently circulated inside the reactor at rates of 0.1 mLs^{-1}. Nitrogen (carrier gas) and vacuum improves the transportation of products from the pyrolysis region to the condensation trap. In this process, short contact times (<0.1 s) can be achieved. Liquid products were trapped at liquid nitrogen temperature (−196 °C) immediately after its escape from the reactor. In a typical experiment, the algal sample (0.20 g) was placed in a sliding ceramic boat which was introduced into the pyrolysis furnace when the temperature, inert gas flow, and vacuum conditions were reached. These conditions were maintained throughout the experiment. The conversion of the starting material took place in a period of 10–30 min. The pyrolysate was collected from the condensation trap with 5 to 20 mL of organic solvent (acetone or hexane) to ensure total bio-oil extraction and was subsequently analyzed by Gas Chromatography-Mass Spectrometry (GC–MS). After evaporation of the organic solvent in a rotary evaporator, the yield of the liquid fraction was determined as follows: the solid and liquid fractions were weighed, and the gas yield was calculated by difference. Each pyrolysis experiment was carried out in duplicate reporting the data as the average of the two replicates with the standard error.

2.3. Characterization techniques

Elemental analysis of the raw material was performed using a CHNS Elemental Analyzer 2400 Serie II Perkin Elmer. The weight percentage

of oxygen was determined by difference considering the ash content. The protein, lipids, ash, moisture and carbohydrates contents were determined by the Association of Official Agricultural Chemists (AOAC) and Food and Agriculture Organization (FAO) official methods of analysis. Bio-liquids obtained in the fast pyrolysis experiments of all algal biomass were analyzed in a Shimadzu GC–MS-QP 5050 spectrometer, using the conditions already described in previous work [5,6]. For the chromatogram analysis, an approximation that the peak area of an individual compound was directly proportional to the concentration of such compound was applied. Thus, the peak area percentage of a compound was used to compare the change of its relative amount in the bio-oil at the different conditions. The identification of chromatographic peaks was achieved according to NIST MS library (match >85%).

Bio-liquids elemental analysis of *Botryoccocus braunii* alga was also determined. With these results it was possible to determine the higher heating value (HHV), an important parameter that represents the enthalpy of the complete combustion of all the carbon content on a fuel onto CO_2. This value allows to have an approximation of the amount of heat that a liquid can liberate if he is going to be used for fuel proposes. HHV was calculated according to the Dulong's equation: Eq. 1

$$Q = 0.3383C + 1.442\ (H–O/8) \quad (1)$$

where Q is in MJ/kg unit and C, H and O are the content of carbon, hydrogen and oxygen [28].

In the case of the alga *Botryococcus braunii* and the pyrolytic liquid obtained at 500 °C, HHVs were determined using a Berthelot calorimetric pump modified by Mohler and Broker, filled with oxygen (purity 99.99%) under a pressure of 7.85 bar.

3. Results

3.1. Characterization of algal feedstock

Table 1 reports the typical elemental and biochemical composition of the three studied algae: *Botryococcus braunii*, *Spirulina platensis*, and *Pithophora* sp. It is important to highlight that there is no elemental analysis of *Pithophora* sp. previously reported. In general, algae are richer in hydrogen and nitrogen when compared to biomass of lignocellulosic origin, whereas carbon content can be within the same range. In this case, clear differences between the algal materials can be seen. *Botryococcus braunii* contains the largest amount of C and H while *Spirulina* and *Pithophora* sp. contain larger amounts of N. The composition of *B. braunii* was similar to the one reported by other authors [24]. For Spirulina, a lower H content was found in comparison to literature where values are in the range of 4–7 wt% [23,24]. According to these results, it is evident that the composition of the alga depends on the cultivation system and growing conditions of this microorganism among other factors. Mineral content of algae is generally higher than plants and different metals are usually bonded to carbonate ions in the algae structure [29]. It is known that ash content varies in different species, geographical sites, and seasons; for instance, in the samples here evaluated, the ash contents fell in the approximate range of 2–27 wt%. *Botryococcus braunii* was lower in ash content than the other species and even lower than that found for lignocellulosic and terrestrial biomass [6,7]. On the contrary, *Pithophora* sp. has a high content of ashes, similar to the one reported by Sukumuran and Thevanathan for *Pithophora oedogonia* [30]. These values are consistent with the fact that macroalgae usually contains a high ash content compared to microalgae [31].

Active metabolic derivatives are the main algae components with a biochemical arrangement highly influenced by environmental and nutritional conditions. The algae degradation products are typically grouped as protein, lipid, and carbohydrate species. In particular, the lipid content can vary significantly from species to species. *Botryococcus braunii* has a high lipid content, which is evidenced in the high content of C; by contrast, *Spirulina* and *Pithophora* sp. have very small amount of lipids. The content of proteins is notable in *Spirulina* while *Pithophora* sp. has significant quantities of proteins and carbohydrates. In this case, *Spirulina* was found to have a higher amount of carbohydrate and a lower protein content than expected [24], which could be related to the nutrient-limited culture medium where it was produced and which would favor a higher reserve compounds production such as carbohydrates [32,33]..

Table 1.
Elemental and biochemical composition of algal biomass (DW basis).

Properties	*Botryococcus braunii*	*Spirulina*	*Pithophora* sp.
Moisture content (w/w%)	1.7	7.3	7.0
Ash (w/w%)	1.6	18.58	27.25
Protein (w/w%)	12.8	51.3	38.5
Lipids (w/w%)	58.9	3.01	5.31
Carbohydrates (w/w%)	26.8	27.1	28.9
% C	75.7 ± 0.3	36 ± 3	34 ± 2
% H	10 ± 1	1.7 ± 0.2	0.2 ± 0.1
% N	1.81 ± 0.01	7.2 ± 0.5	4.8 ± 0.3
% O[a]	11 ± 1	37 ± 3	35 ± 2

Elemental analysis is shown as mean ± standard error (n = 2).
[a] Calculated by difference, error equal to the sum of errors of C, H and N.

3.2. Fast pyrolysis experiments

Fast pyrolysis reactions of algal materials were carried out at different temperatures: 300, 400, 500, and 600 °C for 20 min. Yields of liquid, gaseous and solid products were determined (Table 2). All bio-oils were dark brown with an acrid or smoky odor [34]; however, the polarity of these liquids was very different depending on the starting alga. It was necessary to use acetone to recover bio-oils from *Spirulina* and *Pithophora* sp. while hexane was used to recover the *B. braunii*-derived oil from the trap. In general, the water-soluble phase was in the range of 3–5% of the total liquid fraction.

For reactions of *Botryococcus braunii*, the temperature increment from 300 to 500 °C enhanced the yield of pyrolytic oil, up to 65% at 500 °C. At this temperature, the formation of oil decreased and gaseous products were promoted. When pyrolysis temperature is too high (>500 °C), a greater degradation of biomass occurs due to the secondary cracking of pyrolysis vapors, generating a larger yield of gas phase. The results indicate that the methodology here applied provides better yields of bio-oils compared with pyrolysis experiments found in literature, where bio-oils obtained from *Botryococcus braunii* do not exceed 52% yield [17,20].

When analyzing the pyrolysis of *Spirulina* and *Pithophora* sp., it was found that the liquid fractions reached their maximum yield at 600 °C giving 45% and 28%, respectively. In both cases, biochar was the main product at 300 °C while the gas fraction was predominant from 400 °C.

Table 2.
Fast pyrolysis of microalgae at different temperatures[a].

Microalgae	T (°C)	Yield (%)		
		Solid	Liquid	Gas
Botryococcus braunii	300	35 ± 1	37 ± 1	29 ± 2
	400	6 ± 1	54 ± 1	41 ± 1
	500	2 ± 1	65 ± 4	38 ± 5
	600	1 ± 0	52 ± 2	48 ± 2
Spirulina	300	49 ± 2	18 ± 2	34 ± 0
	400	29 ± 4	37 ± 1	35 ± 3
	500	20 ± 1	40 ± 2	41 ± 3
	600	15 ± 1	45 ± 4	44 ± 3
Pithophora sp.	300	55 ± 1	12 ± 2	33 ± 1
	400	37 ± 2	18 ± 2	46 ± 0
	500	26 ± 2	21 ± 3	54 ± 2
	600	15 ± 1	28 ± 1	59 ± 2

Mean ± standard error, n = 2.
[a] Conditions for pyrolysis experiments: 0.2 g of biomass, 20 min, 50–100 m Torr, 0.2 mL/s of N_2 as carrier gas.

Considering the microalgae pyrolytic oil yields described in literature for non-catalytic pyrolysis of *Spirulina*, the values here obtained are a little better than the ones informed in those studies (25–35% yield between 500 and 550 °C) [23,35]. In the case of studies concerning catalytic systems, yields of oils reached as much as 56% using different clays in a semi-batch reactor at 500 °C [36]. For *Pithophora* sp., the lower bio-oil yield could be attributed to the high ash content, which may act as catalysis promoting secondary cracking with the concomitant production of gaseous products [37].

When comparing the transformation of three microalgal materials between 300 and 600 °C, the highest yield of bioliquid was achieved in the pyrolysis of *Botryococcus braunii*. The high oil recovery is probably due to the low ash content of *Botryococcus braunii*, which means that it has higher volatile matter [38].

The composition of bio-oil was carefully analyzed by GC–MS, where the peak area percentage of the detected compounds is related to the response factor of the mass spectrometer detector, making it difficult to achieve an accurate quantification of products. For this reason, the peak area of each individual compound was considered to be directly proportional to the concentration of such compound in the oil. The peak area percentage of some compounds was used for comparison purposes to assess its variability within the pyrolysis temperature and only those main compounds with an area percentage higher than 0.5% were considered in this study. Between 25 and 65 peaks were identified in the chromatograms, which indicates the high complexity of bio-oil. The results of this analysis are shown in Table 3.

Concerning the composition of all bio-oils, the compounds were classified into the following categories: alkanes, alkenes, nitrogen-containing compounds, oxygenates, aromatic compounds, and anhydrosugars.

Fast pyrolysis of *Botryococcus braunii* resulted in high amounts of long-chain alkenes.

(C12–C20 range) in the pyrolysate at all temperatures (Fig. 1a). At all studied temperatures, the main component identified was 1,19-eicosadiene, a long-chain diene already reported in solvent extracts of *Botryococcus braunii* and in the pyrolysis of other types of biomass [39,40]. Phytadienes with varying unsaturation sites, which are primary chlorophyll-derived products, were also detected [18]. In addition, oxygenated compounds were formed with an important contribution of long-chain alcohols (C10–C30 range) and few mono-unsaturated carboxylic acids (C20, C22) or acid derivatives. Aliphatic compounds such as long-chain alkanes were produced in low quantities at all evaluated conditions. Moreover, an extremely small number of aromatics were detected in the pyrolysis at 600 °C. These results seem quite different than the ones reported by Murata et al. [20] where, apart from aliphatic hydrocarbons, oxygenated compounds and mono-aromatic were formed in significant amounts from pyrolysis (without vacuum) of *Botryococcus braunii* between 450 and 600 °C. However, the fact that the selectivity of oxygenates slightly increases with the rise in the reaction temperature was also observed by these authors.

Liquids recovered from *Spirulina* displayed a large quantity of *N*-containing compounds in the form of amines, amides, heterocyclic derivatives, and nitriles (Fig. 1b). *N*,*N*-diisopropylallylamine was one of the main contributors to *N*-containing products, reaching 37% of the total area at 400 °C and its formation diminished greatly at a higher temperature probably due to deamination, cracking, and cyclization reactions, which also resulted in NH_3 and CH_4 release. Amides were mainly long-chain (C6–C18, 2–9% of the total area) and are likely formed by the reaction of fatty acids existing on algae and the ammonium produced during pyrolysis [41]. Heterocyclic compounds as piperazine, pyridine and pyrrole derivatives were formed in a significant amount at all temperatures (17–34%) and its formation is thought to occur by deamination or dehydrogenation of amino acids [41]. The yield of nitriles was in the range of 1–7% and increased with the temperature rise. The formation of nitriles between 600 and 700 °C can be attributed to the dehydration of amides that are originally found in proteins of the algae, or are formed in the pyrolytic process [24]. Aromatics, in this case phenol derivatives, reached 14% at 600 °C while oxygenates and anhydrosugars did not exceed 10% of the total area. Regarding the elevated nitrogen and oxygen content in *Spirulina*-derived bio-oil, the direct applicability of this oil for bio-fuel industry would not be possible without an appropriate upgrading. It is known that several acid catalysts can be used for this purpose [21]. Alkenes (C19–C44 range), aromatics (phenols), and anhydrosugars were the products observed in small quantities at almost all temperatures. Phenol and methyl-phenols were detected as the main aromatic compounds while levoglucosan (LG) and 1,4:3,6-dianhydro-α-D-glucopyranose (DGP) constituted the fraction of anhydrosugars.

In the case of *Pithophora* sp., the composition of the pyrolisate was highly dependent on the temperature of the process. Between 300 and 400 °C, *N*-containing compounds were predominant and 4-amino-4-methyl-2-pentanone was the main component. At 500 °C, an abrupt change was observed in the *Pithophora* sp. bio-oil composition: The *N*-compounds rapidly decrease and O-compounds take place as the main products. These results may be explained by the fact that between 300 and 400 °C the decomposition of proteins and carbohydrates occurs, while above 400 °C lipids decomposition is favored [8,42]. At 500 °C, the formation of oxygenated compounds was favored and the analysis of the bio-liquid showed that 50% of the total area corresponded to 2,2-dimethyl-1,3-dioxolane-4-methanol or solketal. This ketal is a protected form of glycerol that results from lipid degradation and can be prepared by mixing the triol and acetone in an acidic medium [43]. Taking into account that acetone was used to collect the oil from the condensation trap and this oil displayed an acid pH (2.5) [4,44], it could be considered that the amount of solketal would be representative of the amount of glycerol obtained in the *Pithophora* sp. pyrolysis.

The production of aromatic derivatives increased with the temperature rise with maximum yield of 28% at 600 °C. Xylenes, phenol, and

Table 3.
Composition of bio-oils from microalgae at different temperatures[a].

Microalgae	T (°C)	Alkanes	Alkenes	Nitrogenates	Oxygenates	Aromatics	Anhydrosugars
Botryococcus braunii	300	2.20 ± 0.04	85.51 ± 0.03	–	12.29 ± 0.05	–	–
	400	4.98 ± 0.08	72.55 ± 0.05	0.78 ± 0.01	21.7 ± 0.1	–	–
	500	11.8 ± 0.1	64.57 ± 0.07	0.87 ± 0.01	23.60 ± 0.08	–	–
	600	1.40 ± 0.04	76.1 ± 0.1	0.53 ± 0.01	22.5 ± 0.1	0.06 ± 0.01	–
Spirulina	300	6.10 ± 0.01	–	43.01 ± 0.05	13.14 ± 0.02	10.10 ± 0.02	2
	400	4.23 ± 0.03	–	72.22 ± 0.07	3.02 ± 0.01	11. 39 ± 0.02	2
	500	5.15 ± 0.02	–	67.41 ± 0.07	12.44 ± 0.02	2.36 ± 0.01	3
	600	3.08 ± 0.03	–	60.18 ± 0.05	9.29 ± 0.01	20.18 ± 0.03	1
Pithophora sp.	300	19.8 ± 0.04	–	29.47 ± 0.06	19.13 ± 0.02	–	12.14 ± 0.02
	400	8.07 ± 0.03	3.99 ± 0.01	39.07 ± 0.05	15.84 ± 0.04	15.22 ± 0.02	7.24 ± 0.02
	500	9.84 ± 0.02	–	5.48 ± 0.02	55.20 ± 0.05	–	11.14 ± 0.02
	600	10.1 ± 0.07	1.7 ± 0.01	28.29 ± 0.06	31.4 ± 0.1	14.65 ± 0.02	3.2 ± 0.02

Data shown as mean ± standard error, n = 2.
[a] Values expressed as area % and determined by GC–MS.

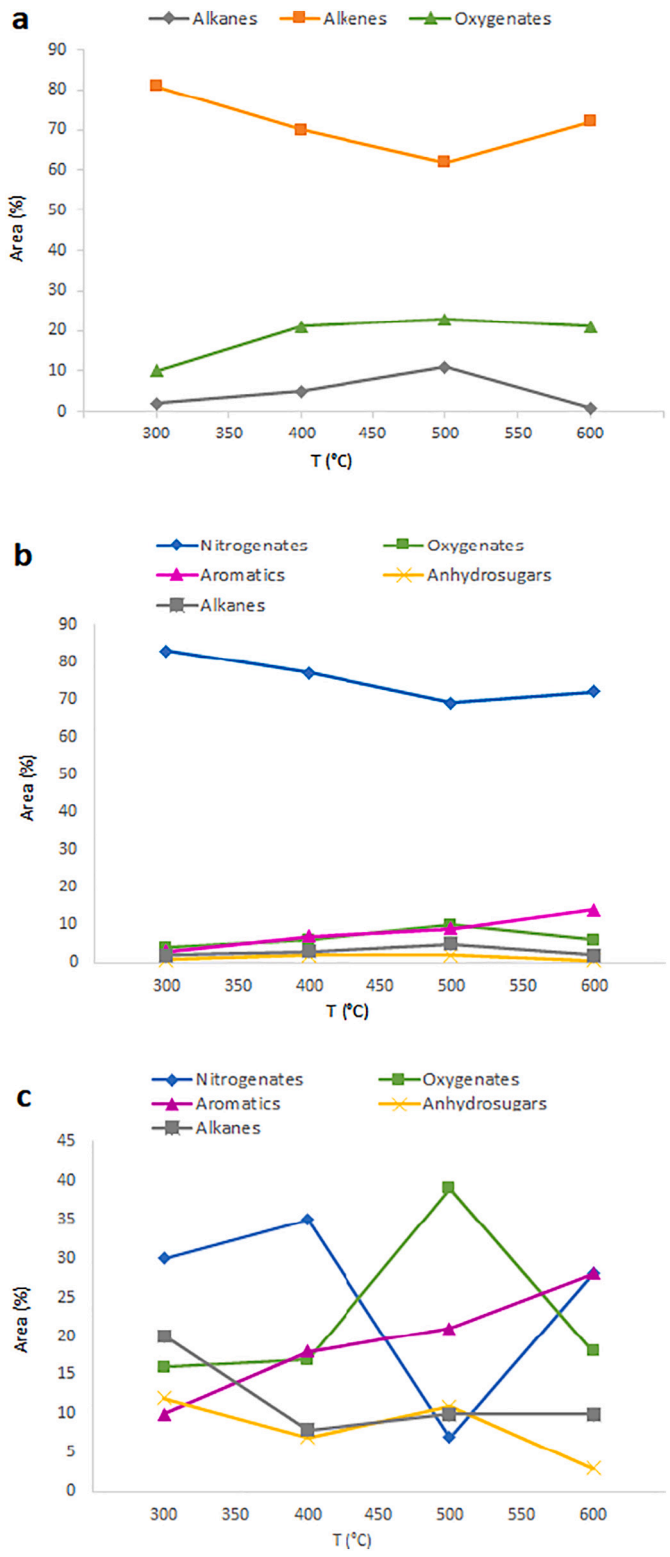

Fig. 1. Composition of bio-oils: a) from pyrolysis of *Botryococcus braunii*, b) from pyrolysis of *Spirulina platensis* and c) from pyrolysis of *Pithophora* sp. Values correspond to the mean ± standard error (n = 2).

methyl-phenols were the main components observed at almost all temperatures. The presence of alkanes (C14–C18 range) decreased from 300 °C and sugars (LG, DGP) were minor products.

Typically, the van Krevelen diagram has been used to evaluate the evolution and origin of coal and oil samples. In such plots, trends along lines are indicative of structural relationships among groups of compounds, which arise from several typical reactions (dehydration, decarboxylation, oxidation, among others) that affect both the H and O indexes [45]. The O index in the van Krevelen plot is suitable for assessing the upgrading of bio-oils or the production of fuels with a focus on increasing the calorific value of the materials [46].

The stoichiometric ranges of elemental H/C and O/C ratios of the molecular formulas are used to establish the boundaries of the classification space for the components found in natural organic matter. The van Krevelen space for this study was classified into the following regions: lipids (H/C = 1.5–2.0, O/C = 0–0.3); proteins (H/C = 1.5–2.2, O/C = 0.3–0.67); carbohydrates (H/C = 1.5–2.4, O/C = 0.67–1.2); aromatic hydrocarbons (H/C = 0.7–1.5, O/C = 0–0.1); and polycyclic aromatic hydrocarbons (H/C = 0.2–0.7, O/C = 0–0.67).

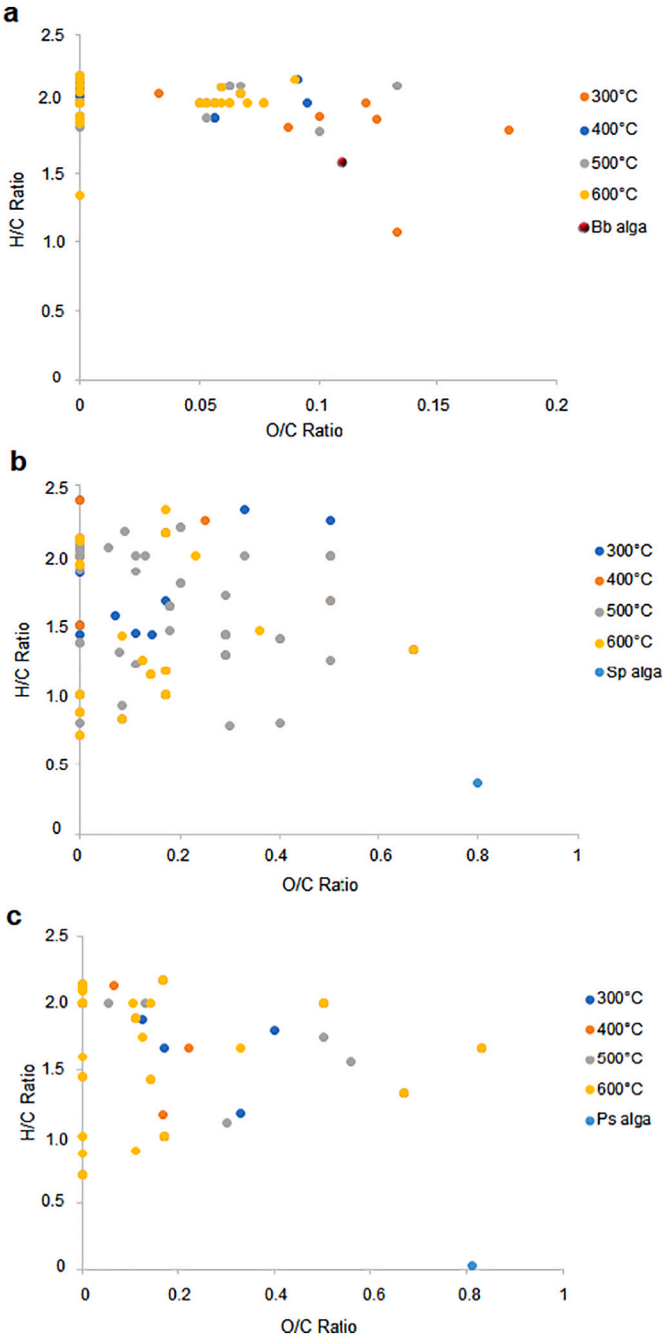

Fig. 2. Van Krevelen plots: a) bio-oils from *Botryococcus braunii*, b) bio-oils from *Spirulina platensis* and c) bio-oils from *Pithophora* sp.

The H/C and O/C evolutions presented in Fig. 2 show the increment of products from de-oxygenation reactions when temperature increased. This trend was more remarkable in the thermal transformation of *Botryococcus braunii* where the O/C ratio of products detected at 600 °C did not exceed 0.1. The diagram shows a proximity of the molecules detected in *Botryococcus braunii* bio-oil, mainly from degradation of lipids in the starting algae. For its part, compounds in *Spirulina* and *Pithophora* sp. oils were more dispersed along the two-axis due to their lipid, protein and carbohydrate origin. *Spirulina* oil contained an important number of products derived from protein degradation (Fig. 2b, grey points at O/C ratio higher than 0.2), especially at 500 °C, while products derived from carbohydrates (O/C ratio higher than 0.7) were lower for all species.

When analyzing the region that comprises aromatic hydrocarbons in the diagrams, only *Spirulina* and *Pithophora* sp.-derived oils obtained from 500 °C covered this area with the presence of few compounds.

Regarding the starting biomass and pyrolytic bio-oils, H/C atomic ratio increased between 31 and 93 times for *Pithophora* sp. and between 2 and 7 times for *Spirulina* while O/C was reduced, which corroborates the loss of oxygen content in the original alga. This behavior was less noticeable in *B. braunii*-derived oils most probably because the starting alga displayed a high H/C ratio.

By examining the results obtained in the pyrolysis of microalgae and considering the yield and composition of bio-oils, the oil obtained from *Botryococcus braunii* at 500 °C was selected as the most promising material for biofuel applications and it was further characterized (Table 4). Following the elemental analysis, the content of H, C, N and S in this bio-oil was found to have a 1.0–1.5-fold increase compared to the starting material. By contrast, the O content decreased 1.6-fold in the *Botryococcus braunii*-derived oil. Table 3 compares typical properties of fossil oil [47] and bio-oils derived from fast pyrolysis of wood [1,8] and microalgae [10]. According to the literature, *Botryococcus braunii* oil contains more C than other biomass-derived liquids, although less than fossil oils. The alga oil contained a much lower amount of oxygen in their chemical composition than liquids from lignocellulosic feedstock but was still high compared to fossil oils. *Botryococcus braunii*-derived oil also displayed a considerable quantity of nitrogenates compared with other liquids. Considering that the combustion of bio-oils containing sulfur and that nitrogen generates NO_X and SO_X, responsible for acid rain and smog [48], an upgrading stage of *Botryococcus braunii* oils should be carried out before their use as fuel or precursor of fuels.

The HHV of the starting raw material and pyrolytic oils were evaluated. The results showed that the liquids from the reactions at 300 and 500 °C had a better performance, the liquid obtained at 500 °C being the best. On the contrary, the oil obtained at 600 °C showed the lowest HHV, even less than the starting material. As it can be seen, bio-oil from *Botryococcus braunii* exhibited better HHV than bio-oils produced from wood biomass [1] and from other microalgae [10], and it was within the range of fossil fuels [46]. In general, the main reason for low HHV in algal bio-oils is the oxygen content, for instance, the high oxygen content results in a low energy density [49], which means that bio-oil must be treated to reduce the oxygen and improve the bio-oil quality.

Considering that the aim of this work was to study new sources of renewable bio-fuels from microalgae and the results obtained from fast pyrolysis reactions of *Botryococcus braunii*, we tried to optimize some technical parameters of the process. Accordingly, Fig. 3 shows the effect of the reaction time on the product distribution for pyrolysis of *Botryococcus braunii* at 500 °C. After 10 min of the experiment, the yield of liquid fraction was 57%. After 20 min, the highest production of liquids was accomplished (65%) and at the longer reaction time, the yield decreased up to 50%. This would indicate that the total conversion of the material takes place at around 20 min of reaction, thus extending that time would favor the formation of gaseous products from the condensed oil.

In addition, it was important to analyze the effect of changing the biomass quantity on the yield of bio-oil keeping 20 min of reaction time (Fig. 4). From the initial amount (0.2 g), employed in all previous experiments, three increments were evaluated: 1, 3, and 5 g. As the amount of biomass to be pyrolyzed increased, the liquid fraction was favored,

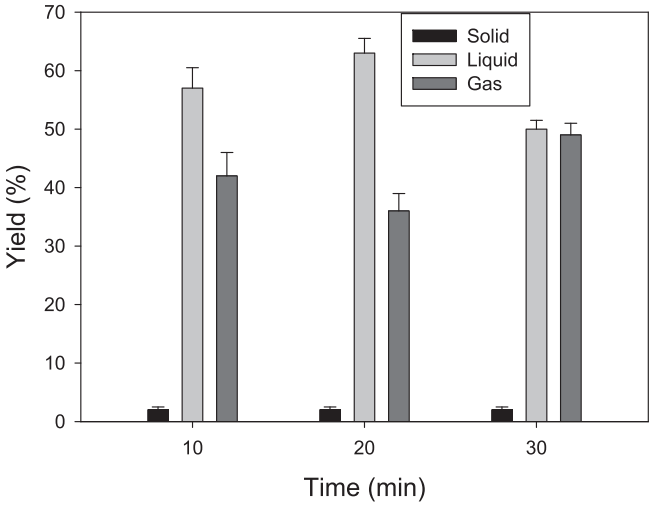

Fig. 3. Product yields for pyrolysis of *Botryococcus braunii* at 500 °C evaluating different reaction times. Values correspond to mean ± standard error (n = 2).

Table 4.
Elemental analysis and HHV values for raw *Botryococcus braunii*, *Botryococcus braunii*-derived bio-oils and for other oils.

	Raw *B. braunii*	Bio-oil from *B. braunii* at the studied temperatures				Bio-oils from wood biomass[b]	Bio-oils from other micro algae[c]	Fossil oils[d]
		300 °C	400 °C	500 °C	600 °C			
% C	75.7 ± 0.3	81 ± 3	73 ± 4	74 ± 4	66 ± 1	54–58	51.4–76.5	84.9–87.4
% H	10 ± 1	9.0 ± 0.5	8.9 ± 0.9	12.4 ± 0.1	10.1 ± 0.8	5.5–7.0	6.8–11.8	12.1–14.8
% N	1.81 ± 0.01	2.4 ± 0.8	5 ± 1	5 ± 3	6.9 ± 0.6	0–0.2	7.1–16.3	traces
% S	0.4 ± 0.1	0.6 ± 0.2	0.5 ± 0.2	0.50 ± 0.01	0.7 ± 0.2	–[e]	<0,1–0.4	0–1.4
% O[a]	11 ± 1	7 ± 5	15 ± 6	5 ± 7	17 ± 3	35–40	6.8–33-3	0–0.04
HHV (MJ/kg)	40[f] 40.4[g]	42[f]	37[f]	46[f] 45.1[g]	33[f]	19–22	27–37	42–46

Results expressed as mean ± standard error, (n = 2).
[a] Determined by difference.
[b] The data were taken from ref. [1].
[c] The data were taken from ref. [10].
[d] The data were taken from ref. [46].
[e] Not specified.
[f] Values estimated by Dunlog's equation.
[g] Experimental value.

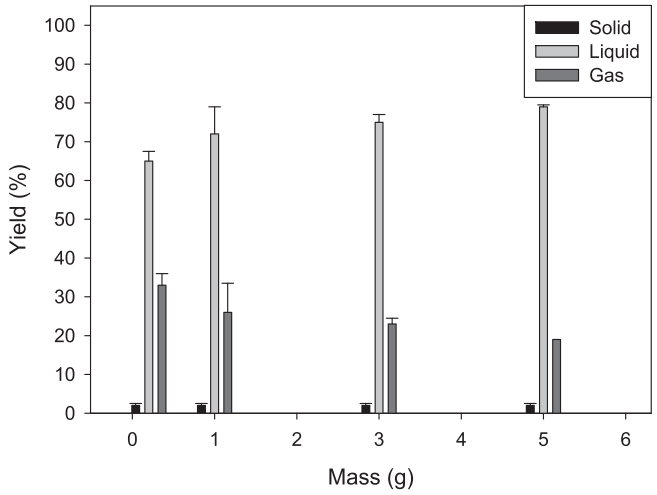

Fig. 4. Product yields for *Botryococcus braunii* pyrolysis at 500 °C changing biomass quantity. Values correspond to mean ± standard error (n = 2).

reaching performance values slightly below 80%. This effect was also observed in pyrolysis reactors with a continuous biomass feeding system [50,51]. With the increase in the initial amount of biomass, there is a greater volume of steam phase, facilitating better condensation of products and minimizing the loss of volatiles. It was evident that the pyrolysis system here studied is more efficient when using higher amounts of processed biomass.

It is important to note that both biomass quantity variation and reaction time variation had no noticeable impact on liquid composition and few differences could be observed (see Supplementary Information). These results suggest that pyrolysis of *Botryococcus braunii* could be scaled into processes with short reaction times in order to generate a bio-oil enriched with hydrocarbons that can be used as a precursor of renewable fuels.

4. Conclusions

The aim of the present study was to evaluate *Botryococcus braunii*, *Spirulina platensis*, and *Pithophora* sp. microalgae as possible feedstocks for bio-fuel production through fast pyrolysis methodology under vacuum. We found that *Spirulina*-derived bio-oil obtained with good yields (up to 45% at 600 °C) was mostly composed by nitrogen-containing compounds, particularly amines and heterocyclic compounds. These features indicated that oils from pyrolysis of *Spirulina* could not be applied directly in fuel industry; therefore, a conversion of nitrogenates to aromatics or other derivatives would be indispensable before its use.

In the case of *Pithophora* sp., the yield of liquid fraction was the lowest of the three studied materials and the oil composition was extremely dependent on the temperature. Between 300 and 400 °C, nitrogen derivatives were predominant while oxygenated compounds were significant at higher temperatures. While the *Pithophora* sp.-derived oil would not be a suited candidate for biofuel precursor, the significant formation of glycerol, which was trapped as solketal in the crude, could provide an added value to this organic liquid. These studies indicate that this alga, which had not been studied up to this point, might be a source of important chemicals.

The pyrolysis of *Botryococcus braunii* displayed an interesting way to obtain high yields of bio-oils (up to 65% at 500 °C) enriched with long-chain alkenes and alkanes. The HHV of this liquid product was 45.05 MJ/kg, which was similar to fossil oils and greater than the HHV of oils from lignocellulosic sources or another algal biomass. Moreover, the scaling of the process from 0.2 to 5 g of microalgae improved the liquid yields without altering its chemical composition. In view of the results, the application of pyrolysis at low pressures for the treatment of *B. braunii* promotes the formation of an oil with excellent properties as a precursor to biofuels. In this sense, a deoxygenation step is necessary to further improve the quality of this oil.

Statement of informed consent, human/animal rights

No conflicts, informed consent, or human or animal rights are applicable to this study.

Declaration of authors contributions

R.V.P. performed the laboratory work, data analysis, and drafted the manuscript. I.C.D. and C.U. contributed to the selection and provision of algal materials. E.L.M. conceived the main project, contributed to the design, reviewed and edited the article and provided the financial support for the research project.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.algal.2021.102206.

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