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Screening Microalgae Strains for Biodiesel Production: Lipid Productivity and Estimation of Fuel Quality Based on Fatty Acids Profiles as Selective Criteria

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Abstract The viability of algae-based biodiesel industry depends on the selection of adequate strains in regard to profitable yields and oil quality. This work aimed to bioprospecting and screening 12 microalgae strains by applying, as selective criteria, the volumetric lipid productivity and the fatty acid profiles, used for estimating the biodiesel fuel properties. Volumetric lipid productivity varied among strains from 22.61 to 204.91 mg l^{-1} day^{-1}. The highest lipid yields were observed for *Chlorella* (204.91 mg l^{-1} day^{1}) and *Botryococcus* strains (112.43 and 98.00 mg l^{-1} day^{-1} for *Botryococcus braunii* and *Botryococcus terribilis*, respectively). Cluster and principal components analysis analysis applied to fatty acid methyl esters (FAME) profiles discriminated three different microalgae groups according to their potential for biodiesel production. *Kirchneriella lunaris*, *Ankistrodesmus fusiformis*, *Chlamydocapsa bacillus,* and *Ankistrodesmus falcatus* showed the highest levels of polyunsaturated FAME, which incurs in the production of biodiesels with the lowest (42.47–50.52) cetane number (CN), the highest (101.33–136.97) iodine values (IV), and the lowest oxidation stability. The higher levels of saturated FAME in the oils of *Chlamydomonas* sp. and *Scenedesmus obliquus* indicated them as source of biodiesel with higher oxidation stability, higher CN (63.63–64.94), and lower IV (27.34–35.28). The third group, except for the Trebouxyophyceae strains that appeared in isolation, are composed by microalgae that generate biodiesel of intermediate values for CN, IV, and oxidation stability, related to their levels of saturated and monosaturated lipids. Thus, in this research, FAME profiling suggested that the best approach for generating a microalgae-biodiesel of top quality is by mixing the oils of distinct cell cultures.

Keywords Biodiesel quality · Fatty acid profiles · Lipid productivity · Microalgae bioprospection · Microalgae selection for biodiesel production

Abbreviations

ANP	Brazilian National Agency for Petroleum, Natural Gas and Biofuels
ASTM	American Society for Testing and Materials

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CFPP	Cold filter plugging point
CN	Cetane number
DU	Degree of unsaturation
FA	Fatty acids
FAME	Fatty acid methyl esters
GHG	Greenhouse gases
IV	Iodine value
L_c	Total lipids content
LCSF	Long-chain saturated factor
L_p	Volumetric lipid productivity
MJ	Mega Joule
MUFA	Monounsaturated fatty acids
OD	Optical density
OS	Oxidative stability
P_{dwt}	Biomass productivity
PUFA	Polyunsaturated fatty acids
SV	Saponification value
TAG	Triglycerides

Introduction

The current opinion is that biofuels represent a cleaner alternative to fossil fuels, which can attend to most of the energy needs of the transportation sector and, consequently, the importance of such renewables in the current market cannot be underestimated [1]. The success achieved in the production and consumption of ethanol and biodiesel is notorious, but these biofuels are mostly obtained from land-crops feedstocks. Therefore, the expansion of such industry is now of concern due to the requirements of large extension of arable land, increasing the dependency of fertilizers, deforestation, and changes in land use. The resultant of such activities would generate additional impact on the release of CO_2 into the atmosphere in a higher ratio than the biofuels market would offset, thus negating their benefits regarding minimization of greenhouse gas (GHG) emissions [2]. Nevertheless, the use of biofuels has a generic effect of reducing CO_2 impact. The overall life cycle emissions of CO_2 from the use of 100 % biodiesel is 78.45 % lower than those of petroleum diesel, and a blend with 20 % biodiesel fuel can reduce the net CO_2 emissions by 15.66 % [3]. These estimations thus suggest that biofuels are most likely to cause a more immediate impact in the transportation sector than any other sources of renewable energy, such as hydrogen or electricity [4].

Biodiesel is the mono-alkyl ester of linear chain fatty acids (FA) derived from renewable feedstock, such as vegetable oil or animal fats [5]. It offers comparable performance to that observed with conventional diesel, but less harmful in terms of toxic emissions [6]. Another additional advantage is that biodiesel at any blend ratio can be directly used in existing diesel engine without the need of mechanic modifications. Biodiesel is less volatile than petroleum diesel, and it is also safer for transportation (flash point, >150°C) [7]. The global biodiesel market is estimated to reach 166 billion l/year by 2016, with an average annual growth of 42 % [8]. In order to attend to such expected rate of expansion in a sustainable manner, it is necessary to investigate the potential for using non-edible oil sources and non-land-crops-based feedstocks [9–11].

The cultivation of microalgae can be used as a renewable source of biodiesel with the potential to displace petroleum-derived transport fuels at the same time that it will also contribute to GHG savings [12, 13]. Compared with oil crops, microalgae have higher photosynthesis efficiency, faster growth, and can synthesize and accumulate larger quantities of lipids [12–15]. Oil yield per area of microalgae cultures can largely exceed the yield of oilseed crops. Approximately 46 ton of oil ha^{-1} $year^{-1}$ can be produced from diatom algae [15]. Nevertheless, in a more conservative estimation, algal based technologies can produce 20,000 l of oil ha^{-1} $year^{-1}$ [16], about three times more than the oil production from palm. The majority of lipid producing algae species have a lipid-profile suitable for biodiesel in terms of the type and amount produced [17]. Typically, biodiesel from seed oils (rapeseed or soybean) produces 37 MJ kg^{-1}, comparable to the energy density of petroleum diesel (higher heating value of 42.7 MJ kg^{-1}) while biodiesel derived from algae yields 41 MJ kg^{-1} [17, 18]. Therefore, with innovative technological advances, biofuels from algae show the potential to be economically attractive [19–21]. Nevertheless, several issues need to be addressed in order to foment the development of biofuels industry based on microalgae as feedstock. Currently, the processes involved with the algae-to-biofuels routes are considered as comparatively expensive, energy consuming [22], and the resultant productivity does not satisfy a conventional market [9]. These constrains could be overcome by selecting resilient and productive species [19–21], optimizing cultivation conditions [13, 21, 23], developing innovative harvesting systems [12, 13, 23], adopting less energy-intensive processes from algal biomass to biodiesel [22–25], and by using biorefinery strategies [9, 15].

Bioprospection is the basic achievement in production line, which is capable of guaranteeing the success of algae-based biodiesel industry [19]. Therefore, the focus should be the identification of appropriate microalgae strains, which are characterized by high yields, resistance to contamination and tolerance to a wide range of environmental parameters [12, 13, 19]. Native species are supposed to be adapted to local environmental changes and thus resilient and competitive. These are critical characteristics supporting the success of large-scale production. Nevertheless, bioprospection of autochthonous algal species for biodiesel production may be considered as an emergent research activity in many countries [19]. The only two characteristics relevant to biodiesel production

that have been measured in a relatively small number of species are growth rate and total lipid content [26]. The appropriate oil quality, besides its yields, is fundamentally important to the success of algal-based biodiesel industry [8, 26, 27]. Reliable data on this subject are somewhat sparse and not coherent with the increasing worldwide interest in the development of algae biofuels industry [8, 26–28]. There is insufficient quantitative information in the literature about the structural features of the fatty acids composing the microalgae oils, which will determine the biodiesel fuel quality [27, 29, 30]. The present research aimed to address this shortfall by comparing 12 local strains and pointing out the most suitable candidates for biodiesel production. The approach is to compare their volumetric lipid productivities (milligrams per liter per day) and their fatty acids profiles, responsible for the biodiesel properties. The analysis summarizes several physiological factors capable of ascertaining the biodiesel production potential of the isolates. Such an approach can clearly identify the best strains for biofuel production based not only on the volumetric lipid productivity but also on their adequate oil composition.

Methods

Growth Kinetics, Biomass, and Lipid Productivity

A total of 12 strains, identified and maintained by LABIOMAR (IBL-Microalgae Collection), at the Federal University of Bahia, Brazil, were used in this research. Nine strains of Chlorophyceae were tested: *Ankistrodesmus falcatus* (IBL-C113), *Ankistrodesmus fusiformis* (IBL-C111), *Kirchneriella lunaris* (IBL-C118), *Chlamydomonas* sp. (IBL-C108), *Chlamydocapsa bacillus* (IBL-C103), *Coelastrum microporum* (IBL-C119), *Desmodesmus brasiliensis* (IBL-C106), *Scenedesmus obliquus* (IBL-C110), and *Pseudokirchneriella subcapitata* (IBL-C112). Additionally, three species of Trebouxiophyceae were tested: *Chlorella vulgaris* (IBL-C105), *Botryococcus braunii* (IBL-C117), and *Botryococcus terribilis* (IBL-C115). The organisms were collected from a eutrophic lagoon located at Salvador City, Bahia, Brazil (12°93′ 26.38″ S–38°39′09.41″ W and 12°94′56.02″ S–38°21′ 29.40″ W). Trebouxiophyceae and Chlorophyceae strains were maintained respectively in a modified CHU 13 and LC Oligo media, described as nutrient-sufficient media [31, 32]. The choice was to have the results under standard growth conditions to provide comparison between the tested strains, without the risks of variations in the proportion of the resultant lipid classes, due to variation on experimental conditions [33]. The trials were carried out in triplicate using Erlenmeyer flasks containing 600 ml of standardized medium and a 10 % volume of algal inoculums in the exponential growth phase. The flasks were kept under constant temperature and agitation (25±2°C and 90 rpm, respectively); the aeration rate was maintained at 0.50 vvm of atmospheric air enriched with 2 % of CO_2. Cells were incubated at a neutral pH range (6.8± 0.6), and light (140 μE m^{-2} s^{-1}) was provided within a photoperiod of 12:12 h light and dark cycles. Growth was monitored every 48 h, by haemocytometer cell counting and by optical density (OD). OD was determined at 680 nm using a UNICAM® Spectrophotometer model Helios Epsilon. Counting of cells per ml and/or OD_{680} measurements were plotted against time and used to estimate the growth kinetics. Growth kinetic parameters were obtained in triplicates for the 12 distinct strains and data were compared using ANOVA test. The growth curves were adjusted using Boltzman sigmoid model described in Origin software version 7 (Origin Lab Data Analysis and Graphing Software®), which was also tested for the model validity ($p \geq 0.05$). The analyzed parameters were:

1. Specific growth rate (μ), based on the equation $\mu = \ln(N_y/N_x)/(t_y - t_x)$, where N_y and N_x are the numbers of cells (N) at the start (t_x) and the end (t_y) of the logarithmic growth phase [34].
2. Biomass productivity (P_{dwt}), as the dry biomass produced (in grams per liter per day), during the exponential growth phase [30]. For P_{dwt} determination, samples were collected at the end of the exponential phase and cells were harvested by centrifugation for 5 min at 5,000×g at 4°C (Sorvall ultracentrifuge ®, Evolution RC). Supernatant discarded pellets were washed with distilled water, freeze-dried, and the dry weight was determined gravimetrically [35].
3. Total lipids content (L_c), reported as percentage of the total biomass (in % dwt), determined by using the chloroform/methanol approach [36].
4. Volumetric lipid productivity (L_p), calculated according to the equation $L_p = P_{dwt} \times L_c$ and expressed as milligrams per liter per day [29]. The results were compared using ANOVA and multiple-range test, based on GraphPad Software Inc [37].

Fatty Acids Profiles of Microalgae Strains

Fatty acids profile was determined by the capillary column gas chromatographic method applied to the oil methyl esters [38]. The amount of total fatty acids (sum of free and bounded fatty acids) of each microalgae species was obtained by transesterification into the corresponding methyl esters (fatty acid methyl esters (FAME)), through saponification with NaOH in methanol, followed by methylation with BF3 catalyst (12 % in methanol). The FAME were extracted with iso-octane and stored in an inert atmosphere (N_2) in freezer at −18°C. The FAME separation was performed on a gas chromatograph (Varian ® 3800) equipped with a

flame ionization detector and a fused silica capillary column Elite-WAX (30 m×0.32 mm×0.25 mm). The analysis parameters were: injector temperature of 250°C and detector temperature of 280°C. The following thermal program was used: 150°C for 16 min, then increasing 2°C/min up to 180°C, held for 25 min, following an increase of 5°C/min up to 210°C, held for 25 min more. Helium was used as carrier gas at 1.3 ml min^{-1}. Nitrogen gas was used as make up gas (30 ml min^{-1}); flow of hydrogen gas and synthetic air were provided at 30 and at 300 ml min^{-1}, respectively. The injections were performed in duplicate for each extraction in volume of 1 μl. FAME were identified by comparing their retention times with those of authentic standards (189-19, Sigma-Aldrich ®, USA). The quantification of fatty acids, expressed in milligrams per gram of lipids, was performed by adding internal standard (C23:0 Sigma®, USA) and calculating the extracted lipids according to Eq. 1. Reported yields were averaged from three replicate extractions:

$$\text{Concentration}\left(\text{mg g}^{-1}\right) = \left(A_x \times W_{is} \times CF_x\right)/ \left(A_{is} \times W_s \times CF_s\right) \times 1000 \quad (1)$$

A_x Area of methyl ester fatty acid peek in the chromatogram of the sample.
W_{is} Weight (in milligrams) of internal standard added to the sample.
CF_s Conversion factor of fatty acid methyl ester to fatty acid.
A_{is} Area of internal standard methyl ester of fatty acid peek in the chromatogram of the sample.
W_s Sample weight (in milligrams).
CF_x Correction factor response of each fatty acid methyl ester ionization detector, relative to C23:0.

Estimation of Biodiesel Fuel Properties Based on FAME Profiles

The parameters attesting for the quality of the biodiesel were estimated in relation to the molecular structures of FAME, which may vary according to carbon chain sizes and the amount and/or position of double bonds [39]. These molecular characteristics greatly influence the main parameters of biodiesel quality such as cetane number, iodine value (IV), the cold filter plugging point (CFPP), and the oxidation stability [39, 40].

The cetane number (CN) is indicative of the time delay in the ignition of fuel, for diesel cycle engines. The higher the CN, the shorter is the ignition time. CN increases with the length of the unbranched carbon chain of the FAME components [39]. Thus the hexadecane (cetane) is set to the default value of 100 on the cetane scale, where the minimum value is 15. The higher the carbon chain length of the methyl esters, the higher is the density and viscosity of the biodiesel, characteristics that will decrease with the increasing number of double bonds [40]. Both characteristics are also related to the CFPP [39–41]. Range of required CN for a quality biodiesel is usually 40–50; American standards American Society for Testing and Materials (ASTM) D675 (minimum CN of 47) and European standards EN 14214 (minimum CN of 51) differ from the one in Brazil, where Resolution Brazilian National Agency for Petroleum, Natural Gas and Biofuels (ANP) 07/2008 requires a minimum CN of 45 [42].

The IV refers to the tendency of biodiesel to react with oxygen at near ambient temperature. This characteristic depends on the number and the position of the double bonds in the carbon chains of the alkyl esters. The higher the IV (the mass of iodine, in grams, that is consumed by 100 g of a chemical substance), the higher the possibility of oxidation, deposits formation and deterioration of the biodiesel lubricity. The maximum IV accepted in Europe is 120 g I_2/100 g. The IV for soybean oil, in the range of 120 to 141 is indicative of a higher susceptibility to oxidative attack [43].

The CFPP is usually used for the prediction of the flow performance of biodiesel at low temperatures [39, 41]. ANP standardizes a maximum of 19°C for this parameter [42]. At lower temperatures, the crystallization of the FAME molecules grow and agglomerate, clogging fuel lines and filters. The larger the size of the carbon chains or the higher degree of saturation of FAME molecules composing biodiesel, the higher will be the value of CFPP, and the worse their low temperature properties [41]. However, additives can be used to inhibit the crystals agglomeration. The standards do not mention a low-temperature parameter in their lists of specifications.

The necessity of specialized apparatus, not always available, besides a high amount of oil to directly measure those critical biodiesel properties, lead to the use of predictive models that allow preliminary evaluation of potential feedstocks, if their FA composition is known. In the present work, the estimation of algae-biodiesel properties followed an empirical correlative model ($CN=a+b/x+cy$), whose predictive capacity was previously defined, based on diverse vegetable oils [43]. The two independent variables, x and y, represented the chain length and the degree of unsaturation (DU) of each component ester. They were expressed, respectively, in terms of saponification value (x=SV, in milligrams of potassium hydroxide required to saponify 1 g of oil, which is inversely related to the esters' molecular weight), and the IV (y=IV, directly related to the DU or number of double bonds in the oil). The values of the three constants (a, b, and c) required three independent equations that have been generated based on the SV and IV values from the vegetable oils of palm, peanut and soybean. The equation precision has been

ascertained by a correlation, which indicates that the increase of each unit of IV lowers the CN by 0.225 [43]. Thus, the CN, SV, and IV for each microalgae biodiesel were estimated by using the derived Eqs. 2–4 [44]. The final values for these estimated properties for each microalgae oil-based biodiesel were calculated as the average of the products of these values for each FAME and its percent in the mixture [43, 44].

$$\mathrm{CN} = 46.3 + (5,458/\mathrm{SV}) - (0.225 \times \mathrm{IV}) \quad (2)$$

SV and IV were calculated respectively by using Eqs. 3 and 4, where D is the number of double bonds, M is the FA molecular mass, and N is the percentage of each FA component of the microalgae oil.

$$\mathrm{SV} = \sum(560 \times N)/M \quad (3)$$

$$\mathrm{IV} = \sum(254 \times DN)/M \quad (4)$$

The DU was calculated based on Eq. 5, as the amount of monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA; in weight percent) present in the microalgae oil. Equation 6 has been generated by correlating the value of the CFPP with a factor related to chains saturation and length (long-chain saturated factor (LCSF)). The LCSF value was estimated through Eq. 7, applied to several oils sources, by weighing up values of the longer chains (C16, C18, C20, C22 and C24 are the weight percentages of each of the fatty acids) to reproduce their impact on the fuel cold flow properties [41, 44]. A regression, based on data of these two properties (LCSF and CFPP), defined the cetane response for different levels of saturation, with a good correlation (R^2=0.966) [41].

$$\mathrm{DU} = \mathrm{MUFA} + (2 \times \mathrm{PUFA}) \quad (5)$$

$$\mathrm{CFPP} = (3.1417 \times \mathrm{LCSF}) - 16.477 \quad (6)$$

$$\mathrm{LCSF} = (0.1 \times \mathrm{C16}) + (0.5 \times \mathrm{C18}) + (1 \times \mathrm{C20}) + (1.5 \times \mathrm{C22}) + (2 \times \mathrm{C24}) \quad (7)$$

All these equations have been previously used to estimate the quality of algal biodiesel in comparison to biodiesel from different vegetable oils [44]. In previous research [41, 43, 44], the accuracy of those empirical equations has been tested in reference to vegetable and microalgae oils, proving that the molecular structure of fatty acids directly affects the quality of the produced biodiesel.

Statistical Analysis

For a more accurate selection of microalgae species for biodiesel production, the observed data were first organized in a meaningful way through a cluster analysis representing similarities in algae fatty acid composition. A Euclidian distance matrix based on FA percentages for all the algae strains was used in an unweighted pair group average algorithm to construct dendrograms. This procedure was done in R PACKAGE VEGAN 2.1–3 [45]. This same analysis was done to represent the algae similarities based on FA saturation and unsaturation levels, in relation to the lipid productivity and the estimated properties (CN, IV, CFPP, and OS) of biodiesel generated from algal oil.

The influence of each fatty acid in the formed groups was synthesized by a principal components analysis (PCA). Samples envelopes were constructed based in the cluster analysis results and superimposed in the PCA ordination diagram. This procedure was done in CANOCO 4.5® [46, 47]. The PCA analysis was used to reduce dimensionality and to identify which components were more closely related to each microalgae group in terms of the strains able to generate oils and biodiesels with similar characteristics.

Results and Discussion

Growth Kinetics, Biomass, and Lipid Productivity

Growth rate and oil content (in % dwt) have been the two most studied parameters in the search for the success of large-scale cultivation of microalgae for biofuels production [30]. However, fast growth only rarely correlates with high lipid productivity. Lower growth rates and/or small cell size contribute to lower the biomass productivity, even when the lipid content is high [33]. Therefore, biomass yields may be considered as an adequate criterion for biodiesel production only when associated with lipid productivity (L_p) [30]. Generally, high specific growth rate depends on cell proliferation and it does not reflect the microalgae specific capacity for producing and storing lipids. The production of lipids is commonly observed during the stationary phase, when the cells have most of their biosynthetic capacities redirected to the production of triacylglycerols [14] or hydrocarbon lipids [48, 49]. For this reason, the lipid volumetric productivity and the qualitative lipid composition should be considered as the most appropriate parameters to facilitate decision making on species selection for biodiesel [26].

The obtained data on biomass productivity (Table 1) are in agreement with previous findings in the literature for some of the studied strains [30, 33]. It should be highlighted that lipids bioprospection of some of the local studied strains

Table 1 Growth kinetics, lipid content, and lipid productivity of microalgae strains

Local strains	Specific growth rate $\mu=\ln(N_y/N_x)/(t_y-t_x)$ (day^{-1})	Biomass productivity P_{dwt} (g l^{-1} day^{-1})	Lipid content (L_c; % dwt)	Volumetric lipid productivity $P_{dwt}\times L_c\times 10^3$ (mg l^{-1} day^{-1})
Ankistrodesmus falcatus	0.57	0.34	16.49±0.44	56.07±1.75
Ankistrodesmus fusiformis	0.39	0.24	20.66±2.07	49.58±5.74
Kirchneriella lunaris	0.25	0.14	17.30±1.12	24.22±1.81
Chlamydomonas sp.	0.30	0.24	15.07±0.95	36.17±2.61
Chlamydocapsa bacillus	0.75	0.32	13.52±0.65	43.26±2.40
Coelastrum microporum	0.13	0.11	20.55±0.99	22.61±1.26
Desmodesmus brasiliensis	0.28	0.13	17.99±0.42	23.39±0.63
Scenedesmus obliquus	0.21	0.16	16.73±1.37	26.77±2.53
Pseudokirchneriella subcapitata	0.27	0.08	28.43±5.40	22.74±4.97
Chlorella vulgaris	0.53	0.73	28.07±4.31	204.91±6.37
Botryococcus braunii	0.14	0.25	44.97±4.00	112.43±11.52
Botryococcus terribilis	0.13	0.20	49.00±1.48	98.00±3.42

N_y number of cells at the end of the log phase, N_x number of cells at the beginning of the log phase, (t_y-t_x) time of the log phase

has not yet been referred elsewhere previously. However, the local strain *C. vulgaris*, showed a higher P_{dwt} (0.73 g l^{-1} day^{-1}) than previously reported (0.25 to 0.31 gl^{-1} day^{-1}) [50]. For other six strains (Table 1), biomass productivity varied between 0.20 and 0.34 gl^{-1} day^{-1}. Nevertheless, the top biomass producers in the present study did not correspond to the top lipid producers, what is in agreement with the fact that biomass productivity did not correlate (r^2=0.018) with lipid content (in % dwt). Two Chlorophyceae species (*C. bacillus* and *A. falcatus*) and one Trebouxiophyceae (*C. vulgaris*) showed the highest specific growth rates (above 0.50 day^{-1}) and biomass productivities (above 0.30 g l^{-1} day^{-1}), while their lipid contents have not comparatively been the most conspicuous (Table 1). On the other hand, the two *Botryococcus* strains showed the highest lipid contents, while presenting the lowest specific growth rates, even though with biomass productivities above 0.20 gl^{-1} day^{-1}. Similar performance has also been observed with *B. braunii* grown in photobioreactors [31] under nitrogen-sufficient condition.

Literature data on volumetric lipid productivity for most of the species focused in the present research are relatively scarce. However, recently, this characteristic was averaged for 55 microalgae species, as 50 mg l^{-1} day^{-1} [30], even though some of them showed outstanding values (i.e., *A. falcatus,* 109 mg l^{-1} day^{-1}); in the present study, this species showed a similar lipid productivity (56.07 mg l^{-1} day^{-1}). Trebouxiophyceae strains presented the highest volumetric lipid productivities (corresponding to daily productions of 204.91, 112.43, and 98.00 mg l^{-1} of oil, respectively for *C. vulgaris*, *B. braunii*, and *B. terribilis*), which differed significantly (p<0.05) from all the other native strains in this study (Fig. 1). Species from the class Trebouxiophyceae have been extensively referenced in the literature as promising for biofuel production. It is believed that this group is capable of supporting high oil production systems [48–53]. In spite of this, some species from this taxonomic group may not be the best choice for biodiesel production, as argued in this paper.

Fatty Acids Profiling

Microalgae FA profiles are shown on Table 2. Palmitic acid (C16:0) was the predominant fatty acid in most of the algal lipid extracts. The highest percentage was obtained with *S. obliquus* and *Chlamydomonas* sp. Exceptions were

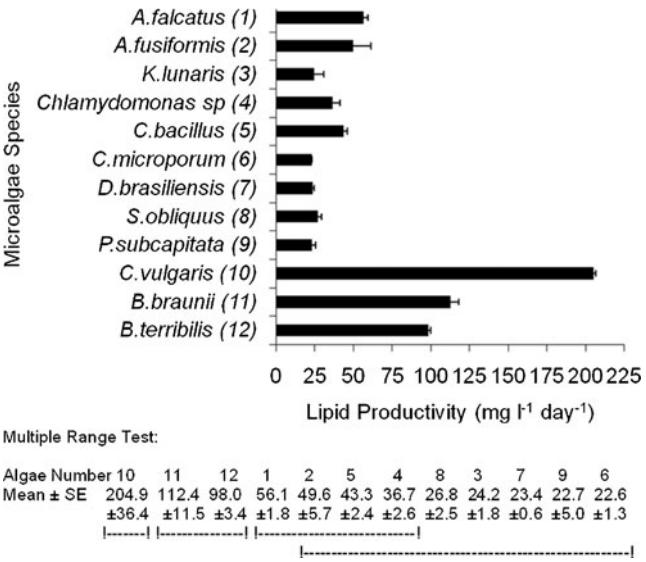

Fig. 1 Multiple range test comparison of lipid productivity (mean± SE) applied to distinct microalgae strains collected in Bahia, Brazil. Values united by *dashed lines* are not significantly different ($p\leq0.05$)

Table 2 Fatty acids profiles (%) in microalgae oils

Fatty acids	Names	*Ankistrodesmus falcatus*	*Ankistrodesmus fusiformis*	*Kirchneriella lunaris*	*Chlamydomonas* sp.	*Chlamydocapsa bacillus*	*Coelastrum microporum*	*Desmodesmus brasiliensis*	*Scenedesmus obliquus*	*Pseudokirchneriella subcapitata*	*Chlorella vulgaris*	*Botryococcus braunii*	*Botryococcus terribilis*
C4	Butyric	1.52	0.74	n.d.	3.93	n.d.	0.60	0.43	1.25	1.65	n.d.	n.d.	n.d.
C6	Caproic	0.91	0.94	1.21	1.79	0.63	0.69	0.61	2.23	n.d.	n.d.	n.d.	n.d.
C8	Caprylic	n.d.	0.09	n.d.	0.18	0.24	n.d.	n.d.	0.68	n.d.	n.d.	n.d.	n.d.
C10	Capric	0.48	0.51	n.d.	0.77	0.50	0.52	0.41	1.91	n.d.	n.d.	n.d.	n.d.
C11	Undecanoic	n.d.	n.d.	n.d.	0.11	0.80	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C12	Lauric	n.d.	n.d.	n.d.	0.14	0.60	n.d.	n.d.	n.d.	n.d.	0.10	n.d.	0.62
C13	Tridecanoic	n.d.	0.14	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C14	Myristic	1.07	2.02	1.53	1.61	1.44	0.80	0.66	1.06	1.01	0.63	0.73	n.d.
C15:1	Pentadecenoic-*cis*	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.44	n.d.	n.d.
C16	Palmitic	30.23	26.95	25.28	50.77	24.51	25.66	27.61	52.07	28.00	40.31	7.17	35.22
C16:1	Palmitoleic	0.47	0.25	n.d.	0.28	0.84	1.00	n.d.	n.d.	n.d.	3.16	n.d.	n.d.
C17	Margaric/ Heptadecanoic	0.48	0.20	n.d.	1.81	1.31	n.d.	n.d.	n.d.	n.d.	0.51	n.d.	n.d.
C17:1	Heptadecenoic	1.52	1.31	1.89	n.d.	3.19	0.73	1.37	n.d.	0.42	0.82	1.26	n.d.
C18	Steararic	2.72	2.10	2.01	11.54	2.96	2.91	3.34	7.48	2.85	8.01	1.59	3.12
C18:1c	Oleic	24.12	18.80	18.50	7.82	7.01	44.24	42.42	21.46	46.15	29.29	76.29	39.74
C18:1t	Elaidic/ Octadecenoic	0.67	0.81	2.06	5.95	11.12	1.07	n.d.	n.d.	0.38	0.60	0.93	n.d.
C18:2w6	Linoleic	2.00	12.23	4.50	3.93	13.33	8.58	12.03	4.60	7.49	8.54	5.16	5.02
C18:3w6	Linolenic	0.37	0.22	n.d.	0.82	4.49	11.12	0.97	2.83	0.60	n.d.	n.d.	n.d.
C18:3w3	α-linolenic	26.49	26.28	39.66	1.94	20.96	n.d.	8.46	n.d.	9.27	1.57	5.34	7.22
C20:1	Eicosenoic	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.95	n.d.	n.d.
C20:1w9	Gadoleic	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.81
NI		6.84	5.81	3.35	6.66	6.90	3.08	1.69	4.43	2.18	4.01	1.53	5.25

NI not identified, *n.d.* not detected

observed with *K. lunaris*, which showed the highest percentage of α-linolenic, and with *C. microporum*, *D. brasiliensis*, *P. subcapitata*, and both *Botryococcus* strains, which showed high percentages of oleic acid (a maximum of 76.29 % for *B. braunii*). Isomers of the monoenoic acids C16 and C18 were detected in low quantities except for *K lunaris*, *A falcatus* and *C bacillus*, where α-linolenic was found above 20 % (Table 2). For some of the studied strains, the results are in agreement with previous findings [8]. For *B. braunii*, however, distinct FA ratios have been reported [54], when compared with the ones obtained in the present work. Differences in the overall ratios can be explained by the diversity of strains and cultivation conditions. Nonetheless, the results of the present study also show that *Botryococcus* strains are mostly represented by saturated or MUFA, with chain lengths from C12 to C32. Fatty acids with C21 to C32 (not reported in the present research) were observed in very low quantities (0.4 to 2.4 %). As opposed to fuels composed mostly by polyunsaturated FAME, the high proportion of saturated and MUFA in microalgae oils of *Botryococcus* strains [54], may incur in less problems with fuel polymerization during combustion [57].

The FA concentrations (Table 3) varied from 26.53 to 270.56 mg g^{-1} of total lipid for *S. obliquus* and *C. vulgaris*, respectively. The observed averaged value for the total lipid extracted from all the studied strains, was 140.42 mg g^{-1}. The total lipids ratios (as percent of dry biomass) and the percentages of saturated, MUFA and PUFA in the dry biomass are shown in Table 3. Comparatively, *Chlamydomonas* sp. and *S. obliquus* produced values above 70 % of saturated FA and the lowest percentages of PUFA (6.76 and 7.46 %, respectively). The highest percentages of PUFA were observed for *K. lunaris*, *C. bacillus*, and *A fusiformis* (44.83, 40.74, and 40.24, respectively). The remaining studied strains showed higher concentrations of saturated (*A. falcatus*, *C. microporum*, *C. vulgaris*, and *B. terribilis*) or MUFA (*D. brasiliensis*, *P. subcapitata*, and *B. braunii*). If saturated and monoenoic FA are considered in combination, all Trebouxyophyceae species show very similar values for these lipids contents (around 89 %). The highest values for the sum of saturated and MUFA, however, were observed for *Chlamydomonas* sp. (93.24 %) and *S. obliquus* (92.54 %).

Estimation of Biodiesels' Fuel Properties Related to FAME Profiles of Microalgae Oils

Several types of lipids, such as phospholipids, glycolipids, mono-, di-, and triglycerides, among others [55, 56], are produced by microalgae and their ratios depend on each species and the growing conditions applied (14, 23, 29, 30, 33, and 50). Free fatty acids are generally only about 1–2 % of the lipids in microalgae [29]. Most of the fatty acids are bounded to glycerol molecules forming the acylglycerols. Among these, only triglycerides are easily converted into biodiesel by the transesterification method [39]. Thus, for pure ASTM grade biodiesel, it is the FA composition rather than the lipid content that must be considered for potential biodiesel production [57, 58].

The most important properties of the biodiesel potentially produced from the obtained microalgae oils were empirically estimated in this research (Table 4). This estimation allowed a comprehensive assessment of biodiesel quality according to the properties specifications. The estimated CN for microalgal biodiesels varied between the 12 microalgae strains from

Table 3 Total lipids, total fatty acids, and percentages of saturated, monounsaturated, and polyunsaturated FA in microalgae dry biomass

Microalgae species	Total lipids		Saturated	Monounsaturated	Polyunsaturated
	Total fatty acids (mg/g lipid)	Total lipids in biomass (% dwt)	Fatty acids in biomass (% dwt)	Fatty acids in biomass (% dwt)	Fatty acids in biomass (% dwt)
Ankistrodesmus falcatus	106.36	16.49	41.39	28.41	30.20
Ankistrodesmus fusiformis	131.96	20.66	37.33	22.43	40.24
Kirchneriella lunaris	176.07	17.30	32.06	23.11	44.83
Chlamydomonas sp	93.19	15.07	78.61	14.63	6.76
Chlamydocapsa bacillus	141.79	13.52	35.68	23.58	40.74
Coelastrum microporum	239.05	20.55	45.87	38.03	16.10
Desmodesmus brasiliensis	205.65	17.99	34.54	44.08	21.38
Scenedesmus obliquus	26.53	16.73	70.83	21.71	7.46
Pseudokirchneriella subcapitata	128.90	28.43	35.39	47.36	17.25
Chlorella vulgaris	270.56	28.07	52.15	37.51	10.33
Botryococcus braunii	130.97	44.97	9.85	79.61	10.54
Botryococcus terribilis	34.00	49.00	43.15	44.29	12.56

42.47 to 64.94, with an average value of 54.34. The ANP Resolution [42] specifies a minimum of 45 for CN. In the present study, except for *K. lunaris* (CN=42.47), all the strains showed CN values in a similar range (between 40 and 65) as most of the biodiesels originated from vegetable oils, such as 49 for sunflower, 52.9 for rapeseed, 50.9 for soybean [57], and 61.0 for palm oil [41]. Nevertheless, different oil extraction methods and transesterification approaches may cause some variation in the resultant biodiesels viscosity and, consequently, in the CN values [58–60]. The SV estimated for biodiesels from all the strains in this research, except for *Chlamydomonas* sp. (whose SV was 220.17) varied within the same range observed for vegetable oils (196–202 for palm oil, 189–195 for soybean oil, and 188–194 for sunflower oil) [43]. This parameter, however, is highly variable because it is also directly associated to the technology used for biodiesel production.

The IV is a parameter not included in ASTM or Brazilian standards, even though it represents the DU, involving the weighted sum of the masses of MUFA and PUFA, important for the biodiesel oxidative stability. High unsaturation levels may result in polymerization of glycerides and formation of deposits [44]. In comparison to biodiesel from vegetable oils [43] most of the estimated IV for the biodiesels from the microalgae strains (Table 4) were lower than for soybean oil (120–141) and sunflower oil (110–143). For some microalgae such as *Chlamydomonas* sp., *S. obliquus*, and *C. vulgaris*, the IV (Table 4) were lower than, or similar to palm oil (48–56), indicative of a lower susceptibility to oxidative attack.

Saturated FA have higher melting points than unsaturated FA compounds. When most saturated molecules of FA esters are present in oils, crystallization may occur at temperatures within the normal engine operation range [44], what gives biodiesel poor CFPP properties. Biodiesel rich in stearic and palmitic acid methyl esters have a tendency to present a poor CFPP (equivalent to a higher temperature of plugging point), because when a liquid biodiesel is cooled, these FAME are the first to precipitate [61]. In the present research, the levels of Stearic acid (Table 2) were generally very low (below 3.34 %), except for *Chlamydomonas* sp., *C. vulgaris* and *S. obliquus* (11.54, 8.10, and 7.48 %, respectively). These low values of stearic acid may have contributed for the lower temperatures of CFPP for the majority of the studied strains. The CFPP values estimated for biodiesel from the strains focused in the present work ranged from −0.55 (*D. brasiliensis*) to −12.23 (*Chlamydomonas* sp.). According to previous data, the CFPP obtained for different microalgae oils varied from −12.3 to 20.8°C [44]. Peanut has the highest CFPP value (19°C) among the vegetable oils [62].

Selection of Microalgae for Biodiesel Production

When algae species were compared based on FA saturation and unsaturation levels, three groups were identified by cluster analysis. In the PCA plotting (Fig. 2), the two axis explained 100 % of total variation in algae fatty acid composition. The first axis showed the effect of variations on MUFA, mostly associated with *D. brasiliensis* and *P. subcapitata* and to a less extent, *C microporum* and *B. terribilis.* The left side of diagram was associated with the increase in saturated-FA and involved *Chlamydomonas* sp., *S. obliquus*, and *C. vulgaris.* The second axis was related with the increase in composition of PUFA. The species *K. lunaris, C. bacillus*, *A. fusiformis*, and, to a less extent, *A. falcatus*, were associated with this pattern. The bottom part of diagram was associated with the decrease of PUFA (Fig. 2). The microalgae *B. braunii* appeared isolated in the dendrogram highlighting its different composition of fatty acid types, in particular, the highest percentage (76.29 %) of oleic acid. Among the studied strains, *Botryococcus* have characteristically long chain lipids,

Table 4 Estimated properties of biodiesel from microalgae oils

Microalgae strains	Cetane number	Saponification value	Iodine value	Degree of unsaturation (wt.%)	Long-chain saturation Factor (wt.%)	Cold filter plugging point (°C)
Ankistrodesmus falcatus	50.52	201.97	101.33	88.81	1.69	−10.43
Ankistrodesmus fusiformis	48.00	199.85	113.81	102.91	1.78	−10.14
Kirchneriella lunaris	42.47	202.21	136.97	112.77	1.94	−9.62
Chlamydomonas sp	64.94	220.17	27.34	28.15	1.11	−12.23
Chlamydocapsa bacillus	48.38	197.50	113.95	105.06	1.77	−10.17
Coelastrum microporum	52.95	205.63	88.42	70.23	1.98	−9.52
Desmodesmus brasiliensis	53.28	205.46	87.05	86.84	1.97	−0.55
Scenedesmus obliquus	63.63	216.04	35.38	36.63	1.23	−11.87
Pseudokirchneriella subcapitata	53.94	207.68	82.83	81.86	1.95	−9.60
Chlorella vulgaris	61.83	199.37	52.63	58.17	1.57	−10.81
Botryococcus braunii	52.67	197.36	94.60	100.69	2.47	−7.96
Botryococcus terribilis	59.50	193.22	66.90	69.41	1.74	−10.26

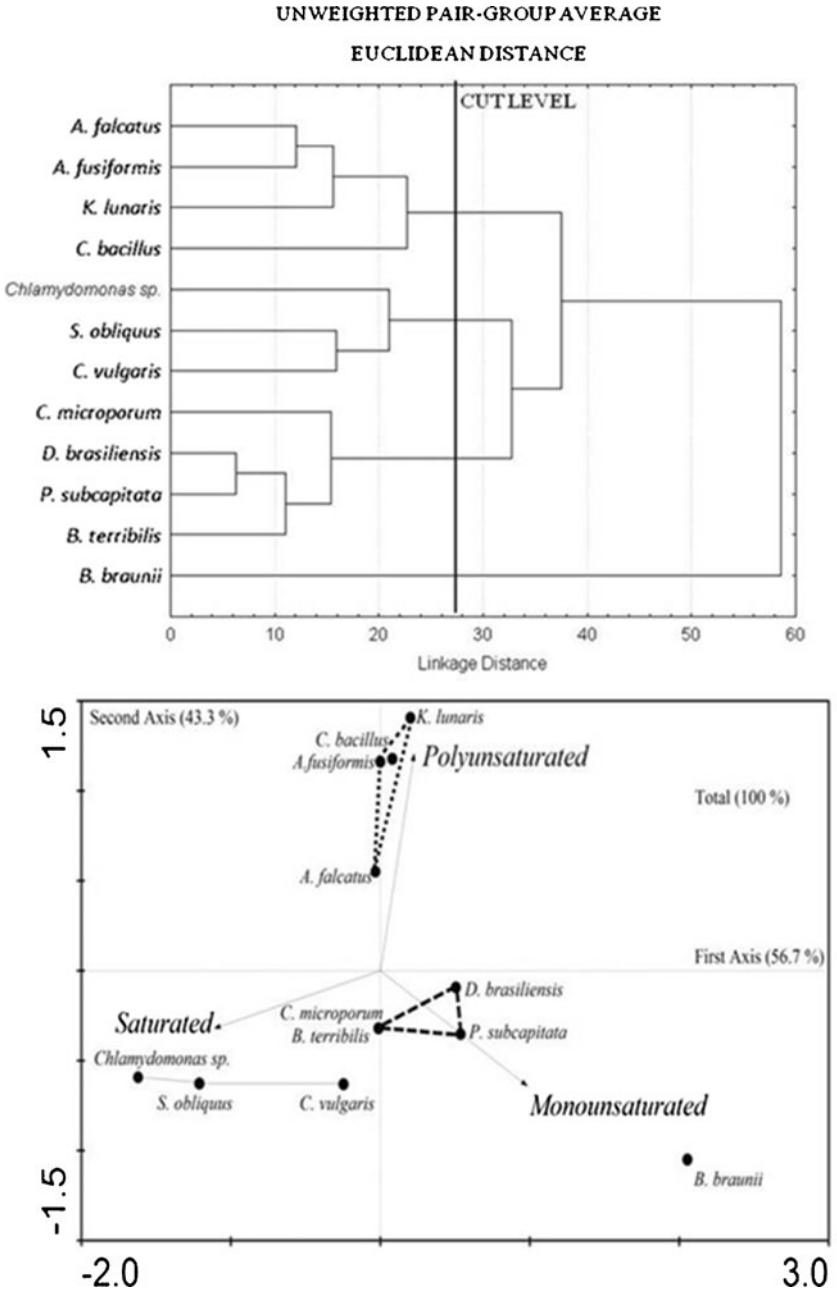

Fig. 2 Cluster Analysis (up) and PCA results (down) comparing the variation of microalgae saturated and unsaturated fatty acids profiles. The first and the second axis of the PCA explain, respectively, 56.7 and 43.3 % of the observed variations

which included chains longer than C30 [48, 49, 54] while most oils currently used for biodiesel production are mainly composed of C16 and C18 [63]. These long chains would generate a biodiesel with higher density and viscosity that would contribute to increase the CN and the CFPP, influencing negatively the motor combustion process.

The statistical approach applied to microalgae strains based on FAME profiles in relation to the estimated biodiesel characteristics (CN, SV, DU, IV, LCSF, and CFPP) and the volumetric lipid productivity (Fig. 3) is indicative that the degree of saturation/unsaturation represents an important discriminatory factor. The two PCA axis explained 80.2 % of the total variation. The first axis, explaining 56.8 %, was related to CN and SV increase and associated with *Chlamydomonas* sp. and *S. obliquus*, richer in saturated fatty acids. These species will generate biodiesel of higher CN, lower IV and higher oxidation stability. In the left side of the diagram, *K. lunaris*, *A. fusiformis*, *C. bacillus*, and *A. falcatus*, characterized by generating the highest percent of polyunsaturated FAME (higher DU), compose a group able to generate biodiesel more prone to oxidation, with the lowest CN, and the highest IV. The second axis explained 23.4 % of total variation and was related with increases of CFPP and LCSF values. The strains *C. microporum*, *D. brasiliensis*, and *P. subcapitata* were associated with this pattern. These species, having oils with higher percentages

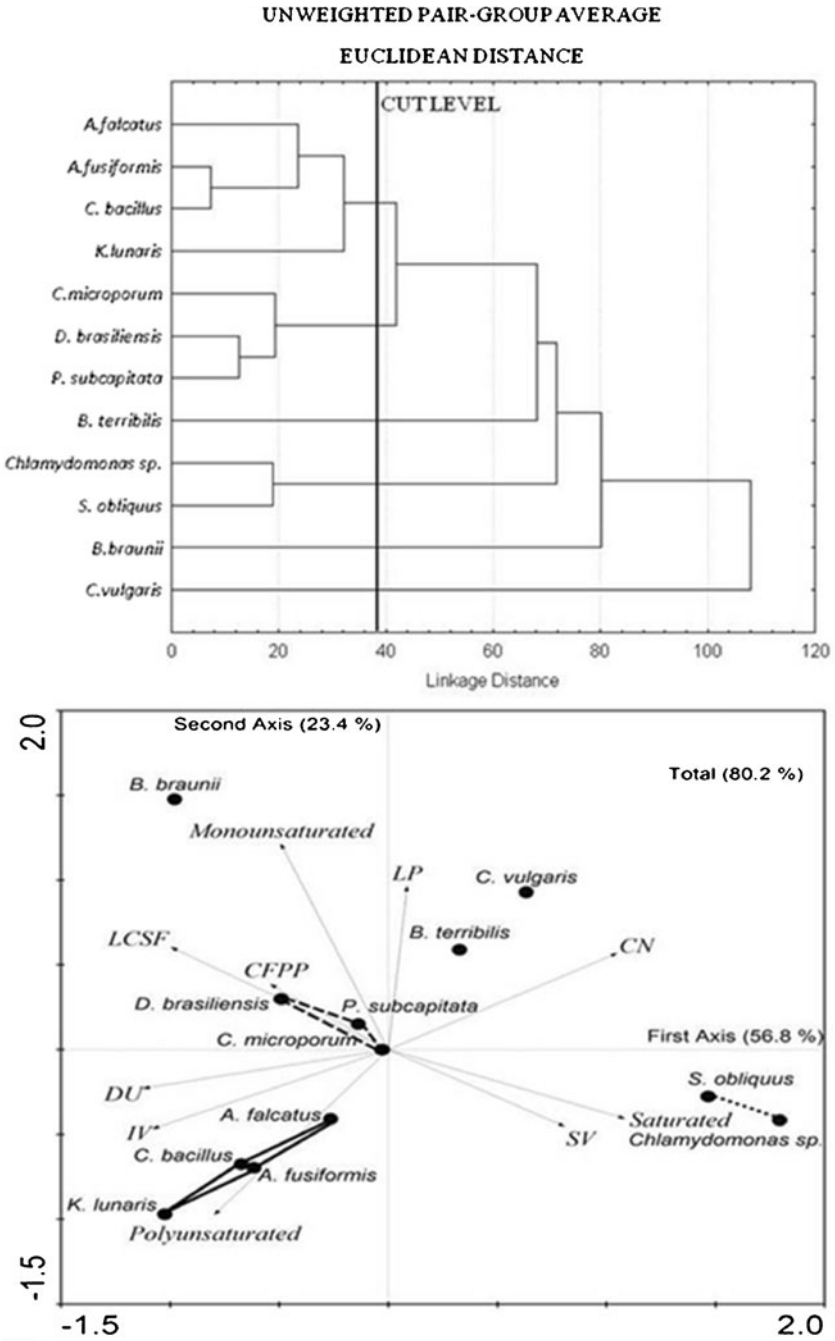

Fig. 3 Cluster Analysis (up) and PCA results (down) comparing the variation of microalgae lipid productivities (LP) in relation to estimated biodiesel characteristics (CN, cetane number; SV, saponification value; DU, degree of unsaturation; IV, iodine value; LCSF, long-chain saturated factor; CFPP, cold filter plugging point). The first and the second axis of the PCA explain, respectively, 56.8 and 23.4 % of the observed variations

of MUFA, generate biodiesel of intermediate CN (52.95 to 53.94), IV (82.83 to 88.42), and also, intermediate susceptibility to oxidation. PCA analysis (Fig. 3) discriminated, in isolation, the Trebouxyophyceae species (*B. braunii*, *B. terribilis*, and *C. vulgaris*), as a result of their high lipid yields when compared with the other studied strains. *C. vulgaris* was found to be very productive, but not comparatively the best among the studied species as feedstock for biodiesel, as previously suggested [44]. In the present study, qualitative analysis of FAME showed this species has a predominance of saturated (52.15 %) and MUFA (37.51 %), tending to generate a biodiesel with a good oxidative stability (Table 4) but not the best in terms of ignition quality and lubricity (CN of 61.83, and IV of 52.63 g I_2/100 g). As previously recommended [28], a good quality biodiesel should be composed of a 5:4:1 mass ratio of C16:1, C18:1, and C14:0, with a low oxidative potential while retaining good cold flow characteristics and high CN. These characteristics were not found in any of the studied microalgae oils. Thus, the desirable quality of biodiesel may be achieved by selecting an appropriate mixture of oils from different organisms.

Conclusions

In this study, 12 microalgae strains were compared according to their biomass and lipid productivities. The top biomass producers did not correspond to the top lipid producers. The total lipids ratios in dry biomass varied between 13.52 % (*C. bacillus*) to 49.00 % (*B. terribilis*).

The range of lipid productivity was from 22.61 to 204.91 mg l^{-1} day^{-1}. The highest lipid yields were observed for Trebouxyophyceae species, *C. vulgaris* (204.91 mg l^{-1} day^{-1}) and *Botryococcus* strains (112.43 and 98.00 mg l^{-1} day^{-1} for *B. braunii* and *B. terribilis*, respectively), which differed significantly ($p<0.05$) from all the other autochthonous strains tested in this study. Nevertheless, the FA-profiling showed that the studied *Botryococcus* strains were not the most appropriate for producing a pure ASTM- or ANP-grade biodiesel.

As the physical-chemical properties of biodiesel are determined by the molecular structures of the constituents FAME, this research suggests that the adequate fatty-acids composition of microalgae oil and the volumetric lipid productivity must be priority criteria for strains selection, to make viable the algae-based biodiesel industry.

According to their FA profiles, several microalgae show the potential to produce biodiesel within most of the biodiesel standards. However, none of the investigated species would naturally produce a lipid capable of fulfilling all the requirements for a biodiesel of the top quality grade. On the other hand, as most of them contained one or more of the main characteristics describing such top quality, it is suggested that a good quality biodiesel may be achieved using a mixture of the distinct lipid extracts, obtained from different species.

Taking into consideration the dependence of biodiesel fuel properties on the structure of fatty acid alkyl esters and the scarce information on the qualitative composition of microalgae oil, this research provides an important contribution for further bioprospection related to microalgae for biodiesel production.

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