Algal Research 39 (2019) 101438

Contents lists available at ScienceDirect

Algal Research

journal homepage: www.elsevier.com/locate/algal

Unconventional alternative biofuels: Quality assessment of biodiesel and its blends from marine diatom *Navicula cincta*

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ARTICLE INFO

Keywords:
Acid-catalyst transesterification
Biodiesel quality
Biofuel
Microalgae
Triacylglycerols

ABSTRACT

The use of microalgae as triacylglycerides (TAG) feedstock for biodiesel is a widely researched field in the development of new technologies. In order to underpin the potential of microalgal biodiesel as a possible substitute in the biofuel industry, more detailed data is therefore required on these aspects. This study assesses the growth, TAG accumulation, kinetics of dissolved nutrients and the quality of biodiesel and its blends from the marine diatom *Navicula cincta* grown in a photobioreactor, using seawater enriched with nutrients and soil extract. Acid-catalyst transesterification was tested for biodiesel production. This species presented during nutrient depletion: 1) a total lipid content of 49.7% of ash-free dry weight (AFDW) with neutral lipid (mainly TAG) as the dominant fraction (ca. 81.5% of total lipids), and 2) favourable fatty acids for biodiesel, such as palmitic acid (28%) and palmitoleic acid (46%). The yield of biodiesel (B100) derived from diatom *N. cincta* (B_{NC}) and analysed by Gas Chromatography was 97.6% of FAME, being the FAME composition similar to the fatty acid profile of *N. cincta* neutral lipids. The fuel properties (including FAME percentage, cetane number, heat of combustion, color and sulfur content) of diatom blends prepared with petrodiesel in a 7% ($B7_{NC}$) and 10% ($B10_{NC}$) vol ratio were evaluated using a PetroSpec (PAC) and compared with commercial soybean blends ($B7_S$ and $B10_S$). B7 and B10 derived from *N. cincta* met the requirements of ASTM D6751 and presented improved cetane numbers (50.2 and 51.6, respectively) with respect to soybean blends. Furthermore, biodiesel derived from the studied species showed the lowest sulfur content (0.0056% w/w). The rapid accumulation of neutral lipids in response to natural nutrient deficiency in *N. cincta* and its capacity of autoflocculate are good selling points for the use of this microalga in the biofuel industry.

1. Introduction

The persistent trend towards depletion of non-renewable petroleum sources and the climate change associated with the combustion of fossil fuels are important issues to be dealt with on a global scale. In the framework of emergent technologies [1], increasing focus is being placed on renewable and sustainable sources of energy such as oleaginous microalgae which synthesize and accumulate substantial amounts of neutral lipids, mainly as triacylglycerols (TAG) [2], making them potential cell factories for the production of biodiesel [3–11].

Biodiesel (B100) is defined as *n*-fuel comprised of mono-alkyl esters of long-chain fatty acids (FAME) derived from vegetable oils or animal fats [12]. Their safe use in diesel engines requires them to meet all the biodiesel standards set by the United States standard (ASTM D 6751) and the European standard (EN14214). In brief, these specifications cover diverse aspects related to biodiesel quality, which can vary depending on the: (1) feedstock nature; (2) production processes; and (3) subsequent handling and storage [13].

Regarding the first aspect, biodiesel quality is largely determined by the fatty acid composition of the feedstock [14]. Therefore, depending on the major fatty acid content, biodiesel from different feedstocks may show variable properties in terms of cetane number, cold flow,

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https://doi.org/10.1016/j.algal.2019.101438
Received 27 September 2018; Received in revised form 11 February 2019; Accepted 11 February 2019

oxidative stability and lubricity, among others [12]. Most biodiesel fuels from both conventional (soybean, rapeseed/canola, sunflower, palm) and alternative oils (jatropha) contain mainly five fatty acids: palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2), and linolenic (C18:3), the amounts varying in their fatty acid profiles [12]. These fatty acids are well represented in most of the green oleaginous microalgae (Chlorophyceae) that have already been studied [2,15–17]. Problems associated with biodiesel derived from vegetable and green microalgae oils are largely related to cold flow and oxidative stability, the severity of the problem varying with the exact amount of these fatty acids. Several approaches have been proposed to improve biodiesel properties, including: 1) antioxidants and additives, 2) different alcohols and 3) alternative feedstocks with different fatty acid profiles [12,18].

Diatoms are very promising microalgae for biofuel production [6,19,20], having an unusual distribution of fatty acids compared to green algae and land plants [21]. The C14, C16 and C20 acids comprise the bulk of diatom fatty acids, while unsaturated C18 acids, particularly linolenic acid, are either absent or present at very low levels [16]. However, although there are more than 200 genera of living diatoms and approximately 100,000 species [22], this issue has not received the same level of attention as other microalgae species.

Transesterification reaction and storage processes can also influence biodiesel quality [13]. In particular, several factors such as the type of alcohol and/or catalyst, reaction temperature and time, biodiesel washing and drying, among others, may cause the presence of contaminants such as unreacted TAG, mono and diglycerides, free fatty acids, glycerol, water and sediments, in turn possibly resulting in severe operational problems (e.g., engine deposits, filter clogging or fuel deterioration) [13]. In addition, biodiesel is a biofuel designed as a blendstock for use in blendings with petroleum diesel fuel. Blends are denoted as BXX by ASTM standards, where XX represents the biodiesel fraction (i.e., B10 is 10% biodiesel and 90% petrodiesel). The most common are B5 (up to 5% biodiesel) and B20 (6% to 20% biodiesel). B20 and lower-level blends can be used in current engines without requiring modifications; however, to ensure proper vehicle performance, ASTM D7467 was introduced for fuel blend grades of B6 to B20. Since it can be used directly in existing diesel engines, biodiesel has the immediate potential of being able to reduce demand for petroleum in the transportation sector and thus protect the environment. Biodiesel reduces emissions of hydrocarbons, carbon monoxide and particulates when used alone or with blends that include petroleum diesel. In addition, it has excellent lubrication properties and generally generates low emissions of sulfur, thus meeting the requirements of the United States Environmental Protection Agency (EPA). United State, Brazil, Germany and Argentina are the main leader in biodiesel export in the world; being the soybean oil the dominant feedstock.

Some studies related to biodiesel production from microalgae oils including: biodiesel from the green alga *Chlorella's* oil by acidic transesterification [23,24], biodiesel from the green alga *Neochloris oleoabundans'* oil extracted with warm isopropyl alcohol coupled with acid-catalysed transesterification [25]; biodiesel from *Neochloris oleoabundans'* biomass by supercritical methanol and CO_2 fractionation [26]; biodiesel from the diatom *Chaetoceros gracilis'* biomass by acidic transesterification [9] and biodiesel from the dinoflagellate *Crypthecodinium cohnii's* oil by basic transesterification [11]. Therefore, the aims of this work were to analyse 1) biomass and lipid production of the Argentinian marine diatom *Navicula cincta* strain growing in a photobioreactor (PBR); 2) the quality of lipids by Gas Chromatography (GC); 3) the transesterification process using acid catalyst; 4) the quality of biodiesel by GC and 5) the verification of blend level by Petro-Spec in comparison with commercial soybean blends. It is expected that these results bridge the gap between research and implementation of biodiesel derived from diatoms in the biofuel industry.

2. Methods

2.1. Strain and inoculum production

Navicula cincta was isolated from the inner zone of Bahía Blanca Estuary (38° 45′S, 62° 22′W), South Atlantic Coast (Argentina), and identified by Bielsa et al. [27]. Stock cultures were kept at 15 ± 1 °C and 40 μmol photons $m^{-2} s^{-1}$ of light intensity provided by cool white Philips L-35 fluorescent lamps under a cycle of 12:12 h light:darkness. A radiometer LI-COR model LI-192SB was used to measure the photosynthetically active radiation (PAR). For inoculum preparation, the cells from the stock culture were acclimated in 250 mL flasks at 20 °C and SWES medium (seawater + soil extract + salts) during at least three generations. The medium was composed of aged and filtered (0.45 μm Millipore) seawater from Bahía Blanca Estuary with a salinity of 32, enriched with $NaNO_3$ (800 μM), K_2HPO_4 (70 μM), $MgSiO_4{\cdot}7H_2O$ (80 μM), soil extract and micronutrient solution. The latter solution was made according to f/2 medium [28]. Third generation cells were collected and concentrated by centrifugation (1200*g*, 3 min) and the supernatant was removed. Maintaining the same environmental conditions, the collected cells were transferred to a 5 L flask containing 4 L of SWES medium and supplied with an air stream containing 1% CO_2. After 7 days, this culture was used as inoculum for a photobioreactor.

2.2. Photobioreactor (PBR) and culture conditions

A cylindrical borosilicate photobioreactor (Figmay S.R.L. Córdoba, Argentina) was used for the experiments with a working volume of 25 L. The temperature was kept at 20 ± 2 °C in a temperature-controlled room. Illumination was supplied by cool-white Philips L-35 fluorescent lamps placed around the PBR under a 12:12 light:darkness cycle photoperiod. Light intensity on the surface of the PBR was 60 μmol $m^{-2} s^{-1}$. The culture was supplied with an air stream containing 1% CO_2 every day during 4 h and maintained with continuous stirring at 30 rpm by means of a system of central paddles. The stirring ensured good mixing and prevented cell sedimentation. A culture of *N. cincta* of 16 L in SWES medium was carried out for 16 days by duplicate. Harvest time was determined according to Nile Red kinetics, which characterizes the TAG accumulation status. Temperature (T), pH and dissolved oxygen (DO) were measured daily in situ with a multiparameter digital meter CONSORT C562. Temperature was 20 ± 2 °C, pH was between 8.13 and 8.86 and DO ranged between 0.55 mg L^{-1} and 5.5 mg L^{-1}.

2.3. Analytical procedures

The cell density (N° cell mL^{-1}) was determined daily by counting duplicate samples in a Sedgwick-Rafter chamber. The specific growth rate (μ) of the cells was calculated during the period of exponential growth by a least squares fit to a straight line of logarithmically transformed data, which were expressed as day^{-1} [29]. Dry weight (DW) was determined on days 0, 6, 14 and 16 by filtering 30 mL culture samples by duplicate through a pre-weighed filter (Whatman GF/F). The filters were then washed with 10 mL distilled water and dried at 60 °C for 24 h to a constant weight. For the ash-free dry weight (AFDW) measurement, triplicate samples of pellets were resuspended and washed with 10 mL distilled water by centrifugation up to negative chlorination reaction. The samples were dried at 100 °C–105 °C, cooled down in a vacuum desiccator and weighed to obtain the biomass dry weight. These oven samples were then ashed in a furnace at 450 °C for 8 h, cooled in a vacuum desiccator, and weighed to obtain the AFDW.

For the chlorophyll-*a* content determination, 6 mL samples were filtered daily through GF/F filter (Whatman ®). The filters were treated with 99.9% (v/v) methanol three times and kept overnight in the dark at −18 °C [30]. Chlorophyll concentration was determined spectrophotometrically with a spectrophotometer Shimatzu UV–Visible 1603

at different wavelengths (665 and 750 nm).The chlorophyll content in 1 mL was calculated according to Talling and Driver [31]: Chl-*a* ($\mu g\,mL^{-1}$) [Chl-*a*] = 13.9 ($A_{665} - A_{750}$) × v/V, where A_{665} and A_{750} correspond to the absorbance of methanol extracted supernatant at 665 nm and 750 nm wavelength, respectively, with a 1 cm pathway cuvette; v: methanol volume; and V: sample volume. Detection via Nile Red (NR-FI) fluorescence was measured every two days according to Bielsa et al. [27] to evaluate TAG accumulation kinetics.

For harvesting, mixing was stopped on day 16 in order to autoflocculate the suspended cells. After 4 h, the cell-free supernatant was removed by siphoning and flocculated cells were collected. The harvested pellet was washed with distilled water, centrifuged (10 min at 3600*g*) in an IEC Model 2 K centrifuge with 4 L-capacity, lyophilized and kept at −80 °C. Total lipid extraction was performed according to a modified Folch method [17]. Briefly, freeze-dried biomass samples diluted in chloroform:methanol 2:1 (v:v), in a ratio solvent:biomass 125:1 were vortexed thoroughly during 30 s, ultrasonicated for 30 min at ambient temperature and centrifuged (3000*g*, 5 min) three times. The supernatants collected were washed (three times) in a separatory funnel with 20% NaCl 0.9% (w/w). The lower phase containing essentially all the lipids was recovered and the solvent was evaporated in a rotary evaporator under vacuum; the traces of solvent were removed under a weak nitrogen stream. Total lipids were partially separated to analyse the lipid fractions and fatty acid profile according to Bielsa et al. [27]. In brief, lipid fractionation into neutral lipids (NL) and polar lipids (PL) was performed using a silica cartridge Sep-Pack (SP) of 1000 mg (J.T. Baker Inc., Phillipsburg, N. J.). The fatty acid profile was determined by methyl ester derivation and gas chromatography (GC) analysis, using a HP Agilent 4890D gas chromatograph (Hewlett Packard Company, USA), equipped with a flame ionization detector (FID) at a temperature of 260 °C, a split/splitless injector (175 °C), and a capillary column SP–2560 (100 m length, 0.25 mm i.d., and 0.2 μm film thickness; Supelco Inc., Bellefonte, PA) according to Popovich et al. [17]. The relative percentages determined by GC for each fatty acid methyl ester sample are the means of quadruplicate runs.

2.4. Biodiesel production

2.4.1. Acid catalysis reaction

Pure methanol (reagent grade), concentrated sulfuric acid (98% reagent grade) and *Navicula cincta* oil were used in a 100 mL jacketed reactor equipped with a magnetic stir bar and a reflux condenser to avoid methanol evaporation. Methanolysis of microalgae oil was conducted employing a 6:1 M ratio of methanol:oil for 6 h at 60 °C and 600 rpm with 10% w/w sulfuric acid/oil as catalyst according to standard parameters [32]. The mixture was then cooled to ambient temperature overnight without agitation (12 h). The fatty acid methyl esters (FAME) separation was carried out according to a modified method [23]. In brief, the separation into two layers was performed in Falcon tubes with screw-caps by addition of petroleum ether, shaking and decanting each time (2–3 times) until the extract was clear. The upper layer containing FAME was collected in a separatory funnel and washed with 20% of distilled water to remove most of the excess methanol and catalyst. Finally, the biodiesel was separated, taken to dryness on a rotary evaporator under vacuum and placed under a weak nitrogen stream to eliminate residual volatile components.

2.5. Biodiesel characterization

2.5.1. Gas chromatography analysis of biodiesel samples

Fatty esters, monoglycerides and diglycerides in biodiesel samples were analysed by gas chromatography in a GC Agilent – 7820A. The GC was assembled with FID and a capillary column (J&W Scientific, model HP-5 ms, 30 m length, 0.25 mm inner diameter, and 0.25 μm film thickness). The analytical method was based on BS EN 14105:2003 to analyse fatty acid methyl esters [33]. The method was slightly modified to analyse FAME, monoglycerides and diglycerides according to analytical methods reported by Dieffenbacher and Pocklington [34] for acylglycerides, using n-tetradecane as internal standard. The injector and detector temperatures were 290 °C and 316.8 °C, respectively. The oven temperature program was initially set at 70 °C for 1 min and included a ramp of 15 °C/min to 180 °C, a ramp of 7 °C/min to 230 °C, and a ramp of 10 °C/min to 310 °C, where the temperature was maintained for 10 min before completing the analysis.

The sample injected onto the chromatograph consisted of 2 μL of a solution prepared with 0.2 mL of the sample (10 mg mL^{-1} in pyridine), 0.1 mL methyl heptadecanoate (10 mg mL^{-1}), 0.1 mL of tetradecane (10 mg mL^{-1}), and 0.1 mL of silylating agent (MSTFA, Sigma Aldrich Argentina). Calibrated curves were prepared with pure standards of methyl heptadecanoate 99.9% (Sigma Aldrich, Argentina), monopalmitin 99.9% (Sigma Aldrich, Argentina), and dipalmitin 99.9% (Sigma Aldrich, Argentina). Samples were analysed in triplicate. The standard deviations obtained in replicated injections were up to 1.5% (w/w) in total FAME concentration, 1.0% (w/w) in MAG composition and 0.6% (w/w) in DAG composition.

2.5.2. Quality parameters of biodiesel/diesel blends

A soybean biodiesel (B_S) and a pure diesel (D) supplied by PETROBRAS Laboratory were employed together with *N. cincta* biodiesel (B_{NC}) produced by acid catalysis to prepare four petrodiesel/biodiesel blends. Each biodiesel was mixed with pure diesel to obtain blends of 7% and 10% (w/w). B7 and B10 were used in this study because the Argentinian market for transport fuels established the addition of 7% (w/w) of biodiesel in 2010 and the biodiesel blend has increased to 10% (w/w) since 2016. PetroSpec fuel TD PPA (PAC), a near- and mid-infrared spectroscopic analyser, was provided by PETROBRAS Laboratory (Refinery Ricardo D. Eliçabe, Bahía Blanca, Argentina) in order to determine: fatty acid methyl esters (FAME), cetane number (CN), heat of combustion (HOC) and color. This instrument allows the determination of the above mentioned parameters with repeatability and reproducibility. Each unit is calibrated with a diverse matrix of > 600 fuels, which was developed worldwide together with the main fuel producers (Shell and Ethyl Corp) to guarantee a reliable equivalent for each analysis in accordance with ASTM methods. In addition, the instrument provides high sampling rates; the test results are obtained in 3 min using only 10 mL of sample and without previous sample preparation. The TD PPA uses unique infrared analysis which combines both near- and mid-infrared information to evaluate multiple analytes and physical properties simultaneously.

2.6. Statistical analysis

The differences in FAME content, CN and HOC in both biodiesel blends (B_{NC} and B_S) were assessed with Student's *t*-test, being statistically different at a significance level of 5% and n = 4.

3. Results and discussion

3.1. Biomass and lipid production of Navicula cincta in a PBR

Fig. 1a shows the growth curve and dry weight values of *Navicula cincta* growing in a PBR. The species presented an exponential growth-phase until day 8, a stationary-phase until day 13 and a declining-phase until day 16. The maximum specific growth rate (μ) was 0.47 ± 0.03 d^{-1} and the maximum biomass production in dry weight was 318.5 mg L^{-1}. These values are comparable with the marine diatom *Cylindrotheca closterium* growing in a PBR under the same light intensity condition [35]. In addition, the robustness of *N. cincta* compared with other benthic marine diatoms cultured at 600 μmol m^{-2} s^{-1} was evident [36]. This feature is advantageous for sustainable use of the species as biofuel resource, especially since larger scale cultures may have a shadow effect due to biomass accumulation. Another interesting

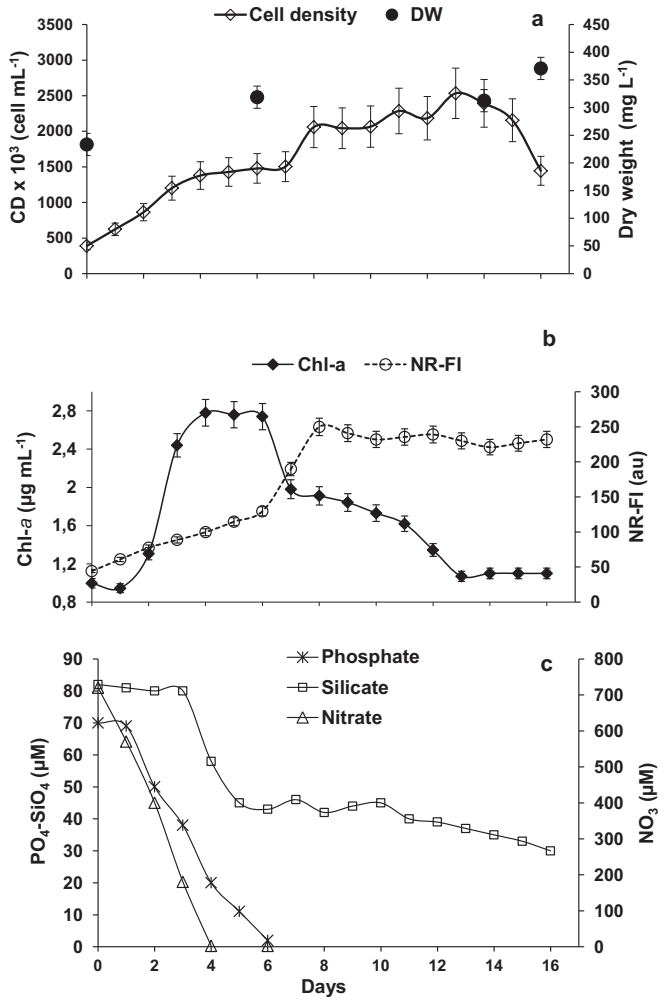

Fig. 1. *Navicula cincta* growing in a PBR. Cell density kinetics and dry weight variation (a), chlorophyll-*a* content and TAG kinetics (b), and nutrient dissolved kinetics (c). Phosphate (PO_4); Nitrate (NO_3) and Silicate (SiO_4). Bars indicate standard deviations.

aspect of this species is its capacity to autoflocculate when air is suspended prior to harvesting. For example, gravity sedimentation together with siphoning off the supernatant is widely accepted as a viable harvesting method in wastewater treatment processes [37].

Fig. 1b depicts chlorophyll-*a* content (Chl-*a*) and the triacylglycerol (TAG) kinetics (NR-FI values). The Chl-*a* values showed a trend similar to the species' growth curve, exhibiting an exponential increase until day 4; then a stationary phase until day 6; and finally, a declining phase until the end of the culture. The NR-FI signals peaked up on day 8, indicating a TAG accumulation-phase until the end of the culture (Fig. 1b). In correspondence with this trend, total lipid (TL) content ranged from 20 (± 1.7) % of ash- free dry weight (AFDW) on day 6 to 49.7 (± 2.2) % AFDW on day-16 (Fig. 1b). Neutral lipids on day 16 were the main fraction with a value of 81.5 (± 0.26) % of TL (40.5% AFDW), while polar lipids represented 18.5 (± 0.26) % of TL (9.2% AFDW). These values indicate a high accumulation of neutral lipids with respect to polar lipids with culture ageing (i.e. when growth stopped and chlorophyll decreased), being TAG determination the clearest sign of the amount of suitable biodiesel feedstock [2]. Lipid productivity is another key criterion for evaluating the potential of microalgae for biodiesel production [38].Thus, the total lipid and neutral lipid productivities of *N. cincta* during the lipid accumulation phase were 23 $mg\,L^{-1}\,d^{-1}$ and 18.7 $mg\,L^{-1}\,d^{-1}$, respectively. It is important to note that total lipid productivity was three time higher than that of this species grown in batch culture [27] and higher than that of benthic diatoms such as *Navicula* sp. [39] and *Nitzschia* LBK-017 [40].

In terms of nutrient kinetics (Fig. 1c), nitrate and phosphate were rapidly absorbed at the initial stage of cell growth, decreasing below the limiting values indicated by Sarthou [41] ($P < 8.9\,\mu M$; $N < 10.2\,\mu M$), while silicate concentrations never reached limiting values ($Si < 22\,\mu M$). It is evident that N and P stress caused a natural scenario to trigger TAG accumulation to the detriment of cell division. From the point of view of biofuels-from-algae technology development, the microalgal strains of interest are those that produce 20% or more of oil relative to their biomass in dry weight [42]. In general, only oleaginous microalgae produce these oil values by using chemical or physical stimuli [43]. It is remarkable that the species studied reached an oil content of ca. 50% AFDW without applying stress conditions, except those naturally created by nitrate and phosphate limitation. Moreover, in outdoor scaled-up microalgae cultivation systems (such as raceway ponds), nutritional stress has been recognized as a strategy to produce oils from marine diatoms for biodiesel production [20,44].

3.2. Fatty acid profile of *Navicula cincta* oil

The differences in fatty acid profiles of raw materials serve as parameters to estimate biodiesel properties [14]. Twenty fatty acids were identified in both the neutral and polar lipid fractions of *N. cincta* grown in a PBR (Table 1). In the neutral lipid fraction, monounsaturated fatty acids (MUFA) and saturated fatty acids (SFA) were the principal classes, accounting for 51% and 31.6%, respectively, while polyunsaturated fatty acids (PUFA) presented the lowest percentage of 15.9%. The dominant SFA, MUFA and PUFA were: palmitic (C16:0) acid (ca. 28%), palmitoleic (C16:1 n-7) acid (ca. 46%) and eicosapentaenoic (20:5n-3) acid (ca.11%), respectively. Thus, according to the neutral lipid FAME profile of *N. cincta*, the cetane number (CN), heat of combustion (HOC, MJ/kg), and iodine value (IV, g I_2/100 g) of

Table 1
Fatty acid profile (percentage of total fatty acids, %) in neutral and polar lipid fractions. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids and PUFA, polyunsaturated fatty acids. The values presented are means of four replicates (n = 4). Standard deviations are indicated in brackets.

Fatty acids	Lipid fractions	
	Neutral	Polar
Saturated fatty acids		
C12:0	0.33 (0.00)	0.99 (0.02)
C14:0	2.00 (0.01)	3.08 (0.05)
C15:0	0.61 (0.01)	0.78 (0.02)
C16:0	28.93 (0.17)	39.51 (0.12)
C17:0	0.15 (0.00)	0.23 (0.01)
C18:0	2.16 (0.01)	1.35 (0.02)
C20:0	0.04 (0.00)	0.23 (0.00)
Monounsaturated fatty acids		
C15:1n-5	0.11 (0.00)	0.08 (0.00)
C16:1n-7	46.32 (0.15)	30.76 (0.45)
C17:1n-7	2.64 (0.02)	4.77 (0.08)
C18:1n-9 (c and t)	2.16 (0.01)	5.18 (0.04)
C24:1n-9	0.13 (0.00)	0.15 (0.02)
Polyunsaturated fatty acids		
C18:2n-6t	0.28 (0.01)	0.35 (0.01)
C18:2n-6c	0.76 (0.01)	1.49 (0.15)
C18:3n-6	0.44 (0.00)	0.35 (0.01)
C18:3n-3	0.13 (0.00)	0.13 (0.00)
C20:2n-6	0.56 (0.02)	0.74 (0.03)
C20:4n-6	1.43 (0.02)	1.14 (0.04)
C20:5n-3	10.82 (0.19)	6.28 (0.07)
C22:6n-3	1.57 (0.04)	2.40 (0.08)
ΣSFA	32.66 (0.18)	46.18 (0.14)
ΣMUFA	51.36 (0.12)	40.94 (0.34)
ΣPUFA	15.98 (0.25)	12.89 (0.22)

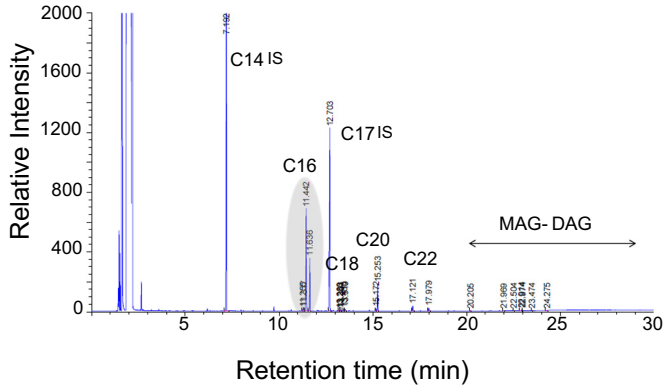

Fig. 2. GC analysis of biodiesel samples from *Navicula cincta* oil produced by acid-catalyst transesterification. Internal Standards as tetradecane (C14 IS) and methyl heptadecanoate (C17 IS).

biodiesel derived from this feedstock and calculated according to Hoekman et al. [45] would present the following values: 54.5, 40.7 (MJ/kg) and 106.1 (g I_2/100 g), respectively, in all cases meeting the biodiesel standard specifications (ASTM D6751-08 and EN 14214). In addition, Knothe [12] demonstrated that fatty-acid profiles enriched in palmitoleic acid may impart overall favourable properties to a biodiesel fuel, giving especially improved cold-flow properties. This fatty acid occurs in small amounts in land plant oils; however, it is noteworthy that *N. cincta* presented dominance of methyl palmitoleate (melting point of −34 °C), which in combination with low levels of high-melting saturated species of fatty acids, such as C18:0, C20:0 and C22:0, confers improved fuel properties with emphasis on cold flow. In particular, the cold filter plugging point (CFPP) was relatively low (−3.87 °C).

3.3. Biodiesel production from Navicula cincta oil

3.3.1. GC analyses of biodiesel samples

Fig. 2 shows GC chromatogram of the biodiesel samples obtained via acid catalyst transesterification. GC analysis of the samples showed a high concentration of palmitic and palmitoleic acid methyl esters and a small quantity of monoglycerides (MAG). In particular, Table 2 shows main fatty acid methyl esters analysed in biodiesel samples obtained by acid-catalysed transesterification of microalga oil. Total FAME account for 97.6% (w/w) of the sample, exceeding the minimum FAME content of 96.5% mass (EN 14214) necessary to characterize an adequate transesterification reaction. The main fatty acid esters determined in the reaction products were consistent with the fatty acid profile of the neutral lipids of the original oil (Table 1). On the other hand, MAG were detected as impurities in the biodiesel sample at very low level

Table 2
GC analysis of biodiesel samples obtained by acid-catalyst transesterification. Retention time of the different components and concentration of FAME and monoglycerides (MAG). The values presented are means of four replicates (n = 4). Standard deviations are indicated in brackets.

Components	Retention time (min)	% esters (w/w)
C14:1	11.254	1.7 (0.1)
C14:0	11.292	0.7 (0.1)
C16:1/2	11.315	51.4 (0.6)
C16:0	11.446	23.1 (0.4)
C18:1/2/3	13.23/13.44	1.9 (0.4)
C18:0	13.519	2.2 (0.1)
C20:1/2	15.171	< 0.2
C20:5	15.253	14.2 (1.0)
C22:6	17.122	2.4 (0.2)
MAG	20.21/24.28	< 1.5 (1.0)
Total FAME		97.6

Table 3
FAME, cetane number (CN), heat of combustion (HOC) and color of biodiesel/petrodiesel blends. B_{NC}: *Navicula cincta* biodiesel; B_s: soybean biodiesel; D: petrodiesel. The values presented are means of four replicates (n = 4). Standard deviations are indicated in brackets. Ns = not significant ($p > 0.05$); * = significant ($0.01 > p < 0.05$); ** = very significant ($p < 0.01$).

Blending (biodiesel/diesel)	7% (w/w)		10% (w/w)	
	B_{NC}/D	B_S/D	B_{NC}/D	B_S/D
FAME % (w/w)	6.96 (0.02)	7.04^{ns} (0.05)	9.99 (0.01)	10.04* (0.02)
CN	50.20 (0.8)	48.20* (0.2)	51.60 (0.5)	49.80** (0.2)
HOC (MJ/kg)	44.34 (0.004)	46.341** (0.004)	44.92 (0.004)	45.14** (0.004)
COLOR	2.70 (0.1)	2.30** (0.0)	3.1 (0.1)	3.1^{ns} (0.1)

(< 1.5%), while the diglycerides (DAG) did not were reported because their levels were lower than the standard deviation calculated for DAG [0.6% (w/w)]. Pigments, phospholipids and other biomass residues present in biodiesel samples were not detected in GC-FID analyses [46].

3.3.2. Blending

The addition of biodiesel to petrodiesel produces several changes in parameters, such as cetane number (CN), color, heat of combustion (HOC), sulfur content, volatility, oxidative stability, lubricity, foaming, particles (total contamination), and turbidity [47]. Biodiesel derived from *N. cincta* by acid catalysis was blended with diesel and compared with commercial soybean blends. Table 3 shows the results of FAME, CN, HOC and color determinations of blends at 7% and 10% using PetroSpec TD PPA (PAC). As can be observed, the quality of biodiesel/diesel blends derived from *N. cincta* was in good agreement with ASTM specifications, making it potentially suitable for diesel fuel.

Total fatty acid esters analysed by PetroSpec in the biodiesel sample (B100) from *N. cincta* oil accounted for 98.6% (w/w). The two methods (GC and PetroSpec) did not differ significantly ($p > 0.05$) in terms of the total FAMEs recovered. In addition, B7 and B10 derived from *N. cincta* showed higher CN than B7 and B10 derived from soybean. This property improves fuel combustion, reduces white smoke on startup and particulate matter (PM) emissions. Other important parameter for the quality of a fuel is its heat of combustion (HOC, MJ/kg). HOC is a measure of the amount of heat energy released when a given fuel is burned. Thus, the more thermal energy is released, the faster the temperature of the heated fuel increases. Diesel has a HOC of 45 MJ/kg, while that of biodiesel is about 12% lower, so that more is required to achieve a yield equal to that of diesel, explaining the higher consumer demand for biodiesel [48]. B7 and B10 derived from *N. cincta* biodiesel presented HOC values of 44.3 MJ/kg to 44.9 MJ/kg, respectively. Although these values were lower than B7 and B10 derived from soybean biodiesel, they were similar to the diesel values.

On the other hand, a major characteristic of biodiesel is its ability to reduce total particulate emissions from diesel engines, contributing to environmental protection [49]. In particular, Table 4 shows that the

Table 4
Sulfur oxide content from soybean's and *N. cincta*'s biodiesel and petrodiesel. B_{NC}: *Navicula cincta* biodiesel; B_s: soybean biodiesel; D: petrodiesel. The values presented are means of four replicates (n = 4). Standard deviations are indicated in brackets. Ns = not significant ($p > 0.05$); * = significant ($0.01 > p < 0.05$); ** = very significant ($p < 0.01$).

Fuel	B_{NC}	B_S	D	ASTM standard D 6751*a*
Sulfur (% w/w)	0.0056** (0.0004)	0.0085** (0.0005)	7 (0.17)	0.0015 max (S15)

exhaust emissions of sulfur oxide of B100 from soybean and *N. cincta*'s biodiesels were essentially eliminated compared to diesel one. Moreover, for the purpose of characterizing the effect of biodiesel fuel on pollutant emissions as defined by the EPA, other specifications should also be taken into account, including total nitrogen oxides (NOx), carbon monoxide and total hydrocarbon. Specifically, biodiesel derived from the diatom *Chaetoceros gracilis* was found to have a very low value of NOx output, even lower than petroleum diesel's [9].

4. Conclusions

This study focuses on the various aspects to be taken into account when selecting a microalgal species to produce biodiesel. The high TAG content and the biodiesel quality obtained from *N. cincta* oils make this species a good candidate for use in new biofuel technologies, meriting further efforts to ensure the profitable production of *N. cincta* biomass on a large-scale in marine waters. The biodiesel obtained from *N. cincta* oil by conventional acid transesterification consists mainly of methyl palmitoleate (51.4%), which confers value-added properties on biodiesel fuel. In particular, our research leads to improvements in the cetane number and sulfur content with respect to soybean biodiesel/diesel blends. These combined features facilitate controlled combustion, thus ensuring engine endurance, and mitigate sulfur emissions without diminishing the cold flow properties. Biodiesel derived from *N. cincta* may be tested as a blendstock for use primarily as a value-added blending component with diesel fuel. However, in order to avoid the use of an acid catalyst on an industrial scale, non-catalytic supercritical alcohol-transesterification should be tested as an alternative chemical route to carry out the transesterification of microalgae oils. Recent findings in this field [26] provide encouraging data for future research with oils produced by bioenergetic cultures of *N. cincta* sustained with seawater. Additionally, *N. cincta* has shown remarkable capacity in terms of the co-production of valuable exopolysaccharides [27] in parallel with TAG production, the sale of which can be offset against the cost of biodiesel production from this strain. The combined features reported here render the biodiesel obtained from *N. cincta* and its blends an attractive alternative for diversifying the biofuel portfolio.

Acknowledgements

Funds have been provided by the Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina (PIP 112-2015 01-00510), the Agencia Nacional de Promoción Científica y Tecnológica (BID PICT 2015-0800) and the Universidad Nacional del Sur (PGI 24/B246) to PIL. We would like to thank Petrobras for their assistance in blend analysis. CAP and MP are Research Members of Comisión de Investigaciones Científicas de la Provincia de Buenos Aires (CIC), PH, LM and PIL are Research Members of CONICET.

Statement of informed consent, human/animal rights

No conflicts, informed consent, human or animal rights applicable.

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