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ORIGINAL PAPER

Effects of outdoor cultures on the growth and lipid production of *Phaeodactylum tricornutum* using closed photobioreactors

David U. Santos-Ballardo[1] · María Del Carmen Rendón-Unceta[2] · Sergio Rossi[3] · Rosa Vázquez-Gómez[2] · Sergio Hernández-Verdugo[4] · Angel Valdez-Ortiz[5]

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Abstract One of the principal challenges for large scale production of microalgae is the high costs of biomass production. Aiming for minimize this problem, microalgal biodiesel production should focus on outdoors cultures, using available solar light and allowing lower energy cost process. Testing species that proved to be common and easy to culture may be a good approach in this process. The present work reports indoor-outdoor cultures of *Phaeodactylum tricornutum* using different bioreactors types, using cell growth, biochemical composition, and the profiles of the fatty acids produced as the parameters to test the optimization processes. The results show that the use of outdoor cultures is a good choice to obtain *P. tricornutum* biomass with a good potential for biodiesel production. The microalgae produced reached better growth efficiency, major lipid content and showed an increment in the percentage of saturated fatty acids (required on the biodiesel production) respect indoor cultures. These results are important to show the relevance of using outdoor cultures as a way to improve the efficiency and the energetic balance of the biodiesel production with *P. tricornutum* algae.

Keywords Third generation biodiesel · Biomass production · Outdoor cultures · Photobioreactors · *Phaeodactylum tricornutum*

✉ Angel Valdez-Ortiz
angelvaldezortiz@yahoo.com.mx

[1] Maestría en Ciencias Aplicadas, Universidad Politécnica de Sinaloa, Carretera Municipal Libre Mazatlán Higueras Km 3, 82199 Mazatlán, Sin., Mexico

[2] Department of Biology, Faculty of Marine and Environmental Sciences, University of Cadiz, Marine International Campus of Excellence (CEI·MAR), University Campus of Puerto Real, 11510 Puerto Real, Spain

[3] Institute of Environmental Science and Technology (ICTA), Universitat Autònoma de Barcelona (UAB), Building C Campus UAB, 08193 Cerdanyola del Vallès Barcelona, Spain

[4] Facultad de Agronomía, Universidad Autónoma de Sinaloa, Carretera Culiacán-El dorado, Km. 17.5, 25 Culiacán, Sin., Mexico

[5] Programa Regional de Posgrado en Biotecnología, Facultad de Ciencias Químico-Biológicas, Universidad Autónoma de Sinaloa, Av. de las Américas y Josefa Ortiz S/N, 80030 Culiacán, Sin., Mexico

Introduction

Nowadays, biodiesel has generated extensive interest as renewable, carbon neutral, economically viable and high quality fuel (Moheimani 2013). It is well known that some microalgae species are able to produce important amounts of lipids (15–80 % per dry weight), and for this reason have been mentioned as oleaginous microalgae (Chisti 2007). Interestingly, the high lipid productivity of microalgae may reach theoretical biodiesel yields superior than the crops used traditionally (15–300 times) comparing the same areal extension (Kurpan-Nogueira et al. 2015). Nevertheless, the microalgal biodiesel production has not reached an economic/energetic feasibility. One of the biggest challenges for commercial production on a large scale is the overwhelming capital investments, the relatively high costs of biomass production and the hard process of lipid extraction for biodiesel production (Chisti 2007). Within this context, the cultivation system is one of the principal areas of opportunity for optimization of the microalgae biodiesel production process (Santos-Ballardo et al. 2015a). Many efforts are addressed to design economical-efficient models for microalgae biomass cultivation. Due to this, diverse

bioreactors have been developed for growing/handling microalgae cultures on different scales (Fuentes-Grünewald et al. 2011; El-Sheekh et al. 2013). Within these options, the closed systems are considered as the most suitable reactors to grow some microalgae species, allowing the protection of the cultures from external invasive organisms and better-controlled conditions for the growing and harvesting operations (Schoepp et al. 2014). Furthermore, the use of closed bioreactors for microalgae cultures allows reaching higher productivity yields, also the generated biomass shows convenient properties for its further use as biodiesel source; as an inconvenient, the costs for building and operating are elevated when comparing with the open systems (Becerra-Dórame et al. 2010). Despite the high cost disadvantage of the closed devices, some authors proposed these as the only option for successful cultures of certain microalgae species, such as *Phaeodactylum tricornutum*, which is considered as a source of therapeutic compounds and also is a solid candidate for biofuels production (Acién-Fernández et al. 2003; Sánchez-Mirón et al. 2003).

On the other hand, in order to avoid the high energy costs related with microalgal biodiesel production is highly recommended the use of the available natural solar light, through the development of outdoors cultures (Fernández-Sevilla et al. 2004; Sevigné-Itoiz et al. 2012). Is very difficult to report standard values for growth kinetics, cell behavior and biochemical composition of microalgae cultures under outdoor conditions, due to the extreme variations of the climatological conditions around the world; actually, it is normal finding differences between microalgal cultures developed in nearby regions (Becerra-Dórame et al. 2010). Otherwise, for successfully microalgal outdoor cultures is necessary the screening of different species, searching for some specific characteristics such as the resistance to external contamination, tolerance to variations on the culture conditions (irradiance and temperature changes), rapid CO_2 uptake and tolerance to shear force (Griffiths et al. 2012). Various oil-rich microalgae have been suggested as potential sources of renewable energy under outdoor conditions, such as *Arthrospira*, *Botryococcus braunii*, *Chaetoceros*, *Chlorella*, *Dunaliella*, *Haematococcus*, *Isocrysis*, *Karlodinium veneficum*, *Nannochloropsis*, *Porphyridium*, *Scenedesmus* and *Tetraselmis* (Rodolfi et al. 2009; Bazaes et al. 2012; Fuentes-Grünewald et al. 2012; Schoepp et al. 2014). On the other hand, there are some oleaginous microalgae that are not able to grow under outdoor conditions or present limited growth characteristics, because the conditions of light and temperature may be too harsh to survive or being productive (Bazaes et al. 2012). For example, Moheimani (2012) made a long term outdoor cultivation of oleaginous microalgae *Tetraselmis suecica* and *Chlorella* sp. in Australia, but failed to scale up the microalga *Dunaliella tertiolecta* after many attempts under the same conditions. Fuentes-Grünewald et al. (2012) demonstrated that too high or too low temperatures kill the strains in outdoor conditions, but controlling the harvesting times made the cultivation feasible. Due to this, the development of particular studies comparing outdoor and indoor cultures, and the analysis of different materials for the bioreactors, will be essential to make possible a profitable culture of the third generation biodiesel. The present study describes the culture of the microalga *P. tricornutum* in hanging bag photobioreactors and methacrylate containers under outdoor natural temperate climatic conditions in Cadiz, Spain. Its potential as a source of biomass for biodiesel production was analyzed and compared with the biomass obtained under indoor conditions. Also, the properties that frequently are used in biodiesel development, such as biomass yield, cell growth behavior, biochemical composition, and fatty acids (FAs) profile, were compared, in order to obtain a useful protocol for future scale up of the biodiesel and by-products production.

Materials and methods

Indoor photobioreactors

The photobioreactors using under indoor conditions consisted in tubular 10 L methacrylate containers (0.65 m height and 0.125 m radius). The microalgae cultures were exposed to a continuously light illumination (24 h) with an intensity of 6000–6500 lx Illumination provided by fluorescent lamps (Sylvania Gro-Lux F30 W/GRO-T8), generating radiation of 120–130 µmol photons m^{-2} s^{-1}. The culture agitation was developed with filtered air, supplied constantly through a 1 µm filter and using a pump with an average flow (0.5 vvm) (Fuentes-Grünewald et al. 2012).

Outdoor photobioreactors

Two types of photobioreactors were used in the outdoor growing experiments (Fig. 1); (1) 30 L polyethylene hanging bags (PHB) (W × L = 0.20 × 1.0 m). The bags were folded in half, hanged in hooks on the metal structure; a small hole was made in the top of each half to make possible the medium addition, inoculum and aeration lines during the culture (Moheimani 2012). In Fig. 1, (2) the methacrylate tubes are showed; 50 L bubble column photobioreactor (BCP) made by polymethylmethacrylate (PMMA) tubes (1.0 m height and 0.125 m radius) were used for outdoor cultures, the BCP were inclined at a 15° angle with respect to the incident sunlight to maximize the solar luminous radiation (Fuentes-Grünewald et al. 2012). The agitation of the microalgae cultures was achieved by

Fig. 1 Different photobioreactors used for *P. tricornutum* outdoor cultures

continuously injecting pre-filtered atmospheric air, with an average air flow rate of 0.5 vvm (volume per volume per minute) (Fuentes-Grünewald et al. 2012).

Experimental design

The diatom *P. tricornutum* was obtained from the microalgae collection of Marine Culture facilities of Facultad de Ciencias Marinas y Ambientales, Universidad de Cádiz (UCA). The microalga cultures were developed using the growth medium reported by Guillard and Ryther (1962), which is based on natural seawater enriched with nutrients with a net composition per liter of: 75 mg KNO_3, 5.65 mg $NaH_2PO_4{\cdot}2H_2O$, 4360 mg EDTA, 3150 mg $FeCl_3{\cdot}6H_2O$, 0.010 mg $CuSO_4{\cdot}5H_2O$, 0.022 mg $ZnSO_4{\cdot}7H_2O$, 0.010 mg $CoCl_2{\cdot}6H_2O$, 0.180 mg $MnCl_2{\cdot}4H_2O$, 0.006 mg $Na_2MoO_4{\cdot}2H_2O$, 2 g crystalline cyanocobalamin (B_{12}), 0.100 mg thiamine hydrochloride (B_1) and 0.001 mg crystalline biotin., with natural seawater (average pH and salinity between 7.3–7.45 and 34–35 g L^{-1}, respectively). The inoculum used for develop the outdoor cultures was grown under indoor controlled conditions under the settings mentioned before. The outdoor cultures were performed in a prepared installation, next to the saltwater distribution tank from the aquaculture plant in the Marine Culture facilities of Facultad de Ciencias Marinas y Ambientales, UCA, Puerto Real (Latitude: 36°53′N, Longitude: −6°28′W, Altitude: 32 m above sea level), Spain. The experiment started in middle of May and finished in the end of July. Outdoor cultures were run in batch mode; the biomass was harvested when the species reached the stationary phase using a Westfalia separator (KA-05-250) at 12,290 RPM, the biomass was freeze-dried and stored at −20 °C until its use. The following cultures were started immediately the harvesting of the batch. Ambient temperature and solar radiation data were acquired from the State Meteorology Agency (http://www.aemet.es), using the nearest station to the experiments location. The average solar radiation per day was calculated (between 10:00 and 18:00 h) during the outdoor experiments, it was expressed in μE m^{-2} s^{-1} (Fuentes-Grünewald et al. 2012).

Maximum absorbance determination

For the microalga *P. tricornutum*, the maximum absorbance was inspected by scanning sample cultures between 550 and 800 nm, using a UV–visible spectrophotometer (Jasco V-630, USA). The maximum absorbance value for each microalga was used to perform the growth curve by optical density (OD) (Santos-Ballardo et al. 2015b).

Cell growth kinetics

To analyze the microalga cell growth daily measurements were performed by quadruplicate. Two analytical methods were used: (1) cell counting by using microscope (Olympus CX40, NY, USA) and a Thoma chamber obtaining the cell density as cell number per milliliter (cells mL^{-1}) (Godoy-Hernández and Vázquez-Flota 2006); and (2) by measuring the absorbance of microalgae suspensions (using the specific wavelength for this specie) with a UV visible spectrophotometer (Jasco V-630, USA) (Mikschofsky et al. 2009). The Pearson correlation coefficient (r) was obtained for cells mL^{-1} and absorbance (abs) data, using the Graphpad Prism 5.0 software.

Cell growth efficiency

Using the growth kinetics, specific growth rate and duplication time were calculated (Godoy-Hernández and Vázquez-Flota 2006):

Specific growth rate:

$$\mu = \frac{\ln X - \ln X_0}{t} \quad (1)$$

Duplication time:

$$dt = \frac{\ln 2}{\mu} \quad (2)$$

wherein: μ, specific growth rate; dt, duplication time; X_0, initial biomass concentration; X, final biomass concentration and t, time (days).

Organic matter content and biochemical analyses

The organic matter (OM) content was calculated using five samples of approximately 10 mg (±0.1 mg) of dried biomass from each bioreactor. Samples were dried (80 °C/48 h), and subsequently combusted at 500 °C for 4 h. The residual inorganic ash was weighed. The OM content was calculated using the difference between the dry weight and remaining ash weight (Rossi et al. 2006a). The biochemical analyses (protein, carbohydrate and lipid contents) were performed spectrophotometrically. Protein analysis was developed using the method reported by Lowry et al. (1951) adapted by Elias-Piera et al. (2013). A sample of 10 mg (±0.01 mg) was weighed in a microbalance and homogenized, grinding in a crystal potter with NaOH (1 N), using albumin as a standard. The carbohydrate content was quantified according to Dubois et al. (1956) as adapted by Elias-Piera et al. (2013). A sample of 10 mg of dried biomass was weighed and homogenized in distilled water, using glucose as a standard. Lipids were analyzed following the method reported by Barnes and Blackstock (1973) adapted by Elias-Piera et al. (2013) 10 mg of dried biomass was homogenized in chloroform–methanol (2:1 v/v), using cholesterol as a standard. These methods have been successfully applied to other microalgae species (Rossi and Fiorillo 2010). Results are presented as μg protein (P), μg carbohydrate (C) and μg lipid (L) per mg OM.

Fatty acid analysis

Fatty acids (FAs) samples were analyzed following the methodology described by Rossi and Fiorillo (2010), Rossi et al. (2006b), and Viladrich et al. (2015). Five samples of approximately 10 mg (±0.1 mg) of dried biomass from the different experiments were dissolved in dichlorolmethane:methanol (3:1); the FAs were analyzed with a splitless injection technique using gas chromatography analysis performed with an instrument (Agilent Technologies 7820A GC) equipped with a DB–5 ms Agilent column (60 m length, 0.25 mm internal diameter and 0.25 lm phase thickness). The oven temperature was programmed with an increment from 50 to 180 °C at 10 °C min^{-1} and from 180 to 320 °C at 4 °C min^{-1}. Temperatures of 300 and 320 °C, for the injector and detector were used, respectively. Methyl esters of FAs (FAMEs) were identified by comparing retention times with those of standard FAs (37 FAME compounds, Supelco^{TM} Mix C^4–C^{24}; trophic markers). FAs were quantified by integrating the areas under peaks in the gas chromatography traces (CHROMQUEST 4.1 software), with calibrations derived from standard FAs. The results are presented as μg FA mg organic matter $(OM)^{-1}$, % of saturated fatty acids (SFAs), mono-unsaturated fatty acids (MUFAs), poly-unsaturated fatty acids (PUFAs) and % of each marker.

Statistical design

Statistical analyses were conducted to analyze the potential variances in biochemical content (carbohydrate, protein, lipid) and in the total FAs concentration and profile (SFA's, MUFA's, PUFA's), between indoor and outdoor cultures, as well as using different reactors (PHB and BCP). A one-way analysis of variance (ANOVA) was performed and for statistical significance, a post hoc Tukey's test was performed, indicating differences between different cultures experiments for each biomass property.

Results

Ambient conditions during the microalgae cultures

The initial experiment under outdoor environment was conducted using the PHB during mid-May. The first attempt permitted to examine the environmental conditions and the feasibility of grown the microalgae under the present outdoor cultures. Due to the difficulties of grown microalgae using this plastic bag bioreactor type, the use of BCP was started in parallel, searching a more robust methodology for microalgae cultivation. High variations in solar radiation and temperature were observed through the outdoor cultures during the period of study (Fig. 2a). The average time of daily sunlight during the experiment was around 12 h. The average temperature recorded during the experiment was 22.15 °C, the minimum (14.6 °C) was reached during end-May corresponding to an unusual storm and the maximum (35.5 °C) was reached during mid-July. The maximum night-day temperature amplitude during the experiment period was close to 11 °C and was recorded three times (end-May, mid-June and mid-July), killing the cultures twice (red arrows, Fig. 2b). The lowest values of irradiance on the outdoor cultures was reported at the end of May (1915 μE m^{-2} s^{-1}), while the highest values was recorded at the mid of June, 3278 μE m^{-2} s^{-1} (Fig. 2a).

Absorbance pattern of *P. tricornutum*

The light absorbance pattern for *P. tricornutum* is shown in Fig. 3. The wavelength was scanned from 550 to 800 nm, the maximum absorbance was observed at 677 nm. The maximum absorbance was measured at different cellular growth stages, showing the same trend in all experiments. The cell growth kinetics based in cell absorbance was developed using this maximum wavelength obtained.

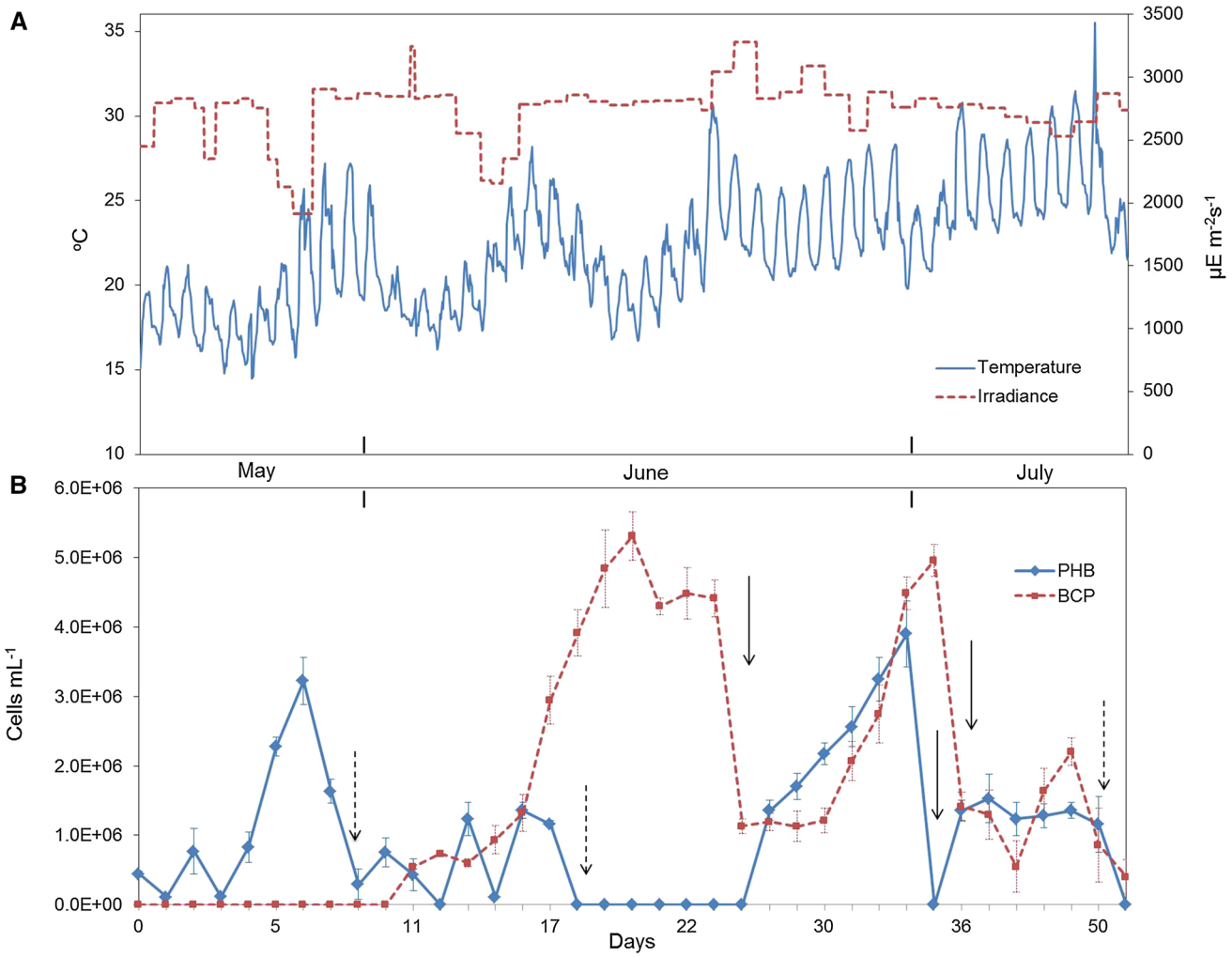

Fig. 2 **a** Daily average of temperature (*solid line*) and solar radiation (*dashed line*) during the experimental outdoor cultures; (**b**) growth kinetics under outdoor conditions of *P. tricornutum* (*dashed arrows* indicate death events, *solid arrows* indicate harvest time). Cells mL^{-1} = cells per milliliter; °C = centigrade; µE m^{-2} s^{-1} = micro Einsteins per m^2 per s; *PHB* polyethylene hanging bags, *BCP* bubble column photobioreactor

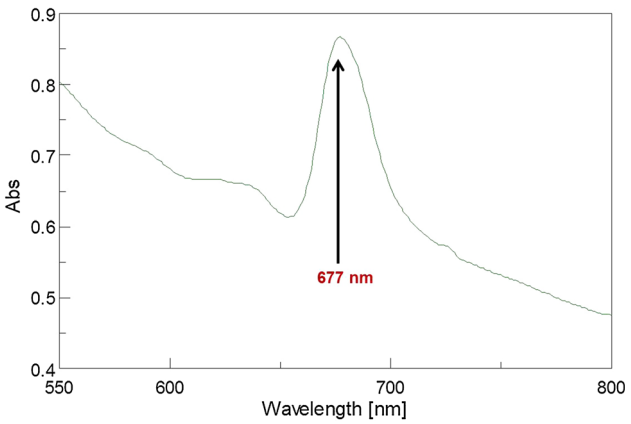

Fig. 3 Light absorbance pattern of *P.tricornutum* scanned from 550 to 800 nm. *Abs* absorbance, *nm* nanometer

Growth performance of *Phaeodactylum tricornutum* under indoor cultures

Figure 4 shows the *P. tricornutum* cell growth kinetics under indoor conditions for both measuring methodologies (cell mL^{-1} and abs) for 21 days. The cultures showed a 3 days lag phase, followed by an exponential growth approximately of 11 days. The stationary phase was reached around the 15th day of culture. After this day, the culture entered to the senescence phase with a decrease in the cell density. The parameters obtained under indoor cultures for cell growth performance such as specific growth rate (µ) and duplication time (*dt*) are shown in Table 1. The maximum density and absorbance obtained in the growth kinetics was reached at the stationary phase.

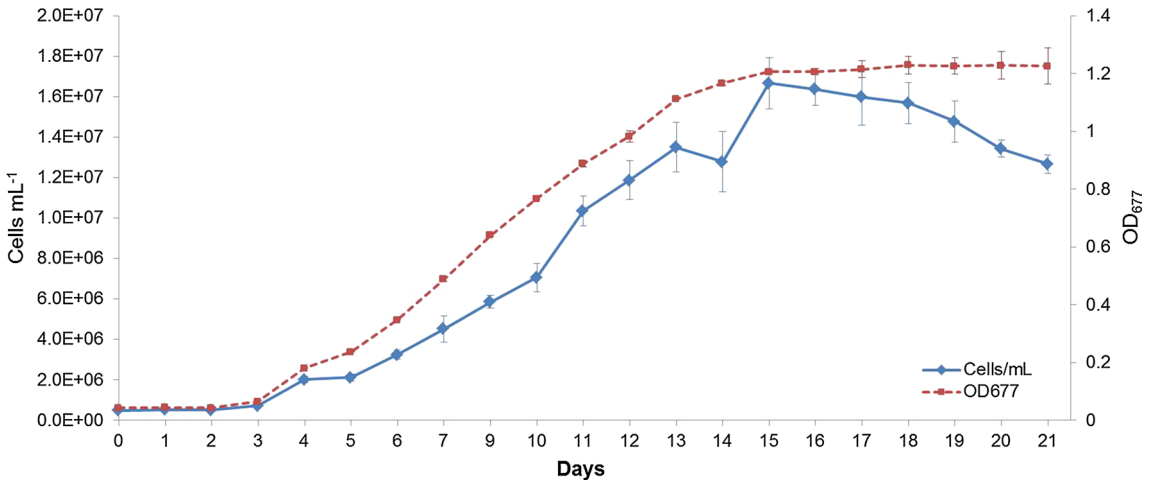

Fig. 4 Growth curve at indoor conditions of *P. tricornutum*. Cells mL^{-1} = cells per milliliter; OD_{677} = absorbance at optical density to 677 nm

Table 1 Growth performance of *Phaeodactylum tricornutum cultures*

	(max) (cells mL^{-1})	Maximum abs[a]	Days for (max) (d)	μ^b (d^{-1})	dt^b (d)
Outdoor PHB	$3.90 \times 10^6 \pm 4.74 \times 10^5$	0.3835 ± 0.0042	7	0.2775 ± 0.0052	2.4978 ± 0.0475
Outdoor BCP	$5.13 \times 10^6 \pm 3.51 \times 10^5$	0.5074 ± 0.0023	10	0.4058 ± 0.0222	1.7104 ± 0.0936
Indoor BCP	$16.66 \times 10^6 \pm 1.26 \times 10^6$	1.2291 ± 0.0031	15	0.2262 ± 0.0008	3.065 ± 0.1080

abs absorbance, *d* days, *dt* duplication time, (*máx*) maximum density, *mL* mililiter, μ specific growth rate

[a] Maximum absorbance at the corresponding wavelength = 677 nm

[b] Average from the growth kinetics developed using absorbance and cell counting

Pearson coefficient of 0.9648 was obtained between the distinct biomass determinations techniques, indicating that the measured cell counting (cells mL^{-1}) has a high positive correlation with the absorbance (abs) from *P. tricornutum* cultures.

Outdoor cultures of *Phaeodactylum tricornutum*

PHB and BCP for the *P. tricornutum* outdoor cell growth kinetics are shown Fig. 2b. The PHB cultures showed no lag phase in the outdoor environment passing to exponential growth immediately after the inoculation, whilst the BCP showed a lag phase between 4 and 5 days (d), after that, the cells entered to the exponential phase. The stationary stage was achieved in the range of 6–7 days for PHB and between 8 and 9 days for BCP, which allows the collecting of microalgae biomass (black arrows, Fig. 2b).

The total elapsed time of the experiment the *P. tricornutum* in outdoor conditions was almost 52 days in batch mode. The μ and *dt* recorded for both types of bioreactors during the outdoors cultures are shown in Table 1. The maximum density and absorbance observed for the outdoor cultures was reached between the end of the exponential phase and the start of the stationary phase for both bioreactors used.

Biochemical composition and fatty acids content

The Fig. 5 shows the carbohydrate, protein and lipids contents for *Phaeodactylum tricornutum* cultures. The contents of carbohydrate and protein did not show significant variations between the culture conditions (one-way ANOVA; $P < 0.05$, Fig. 5); the carbohydrate contents for outdoor cultures were 75.8 ± 11.5 and 85.6 ± 11.2 μgC mg^{-1} OM for PHB and BCP, respectively; though indoor BCP showed 87.6 ± 11.3 μgC mg^{-1} OM. On the other hand, outdoor PHB and BCP presented a protein content of 229 ± 14.5 and 250.7 ± 29.7 μgP mg^{-1} OM, respectively; meanwhile indoor BCP showed 249.6 ± 29.9 μgP mg^{-1} OM. The lipids showed significant differences among different cultures of *P. tricornutum* (one-way ANOVA; $P < 0.05$, Fig. 5c), with the lowest value obtained in outdoor PHB (113.7 ± 12 μgL mg^{-1} OM) and the highest value in outdoor BCP (347.5 ± 32.4 μgL mg^{-1} OM). On the other hand, based on the FA profile obtained from *P. tricornutum* lipids (Table 2), the microalga contains more than 20

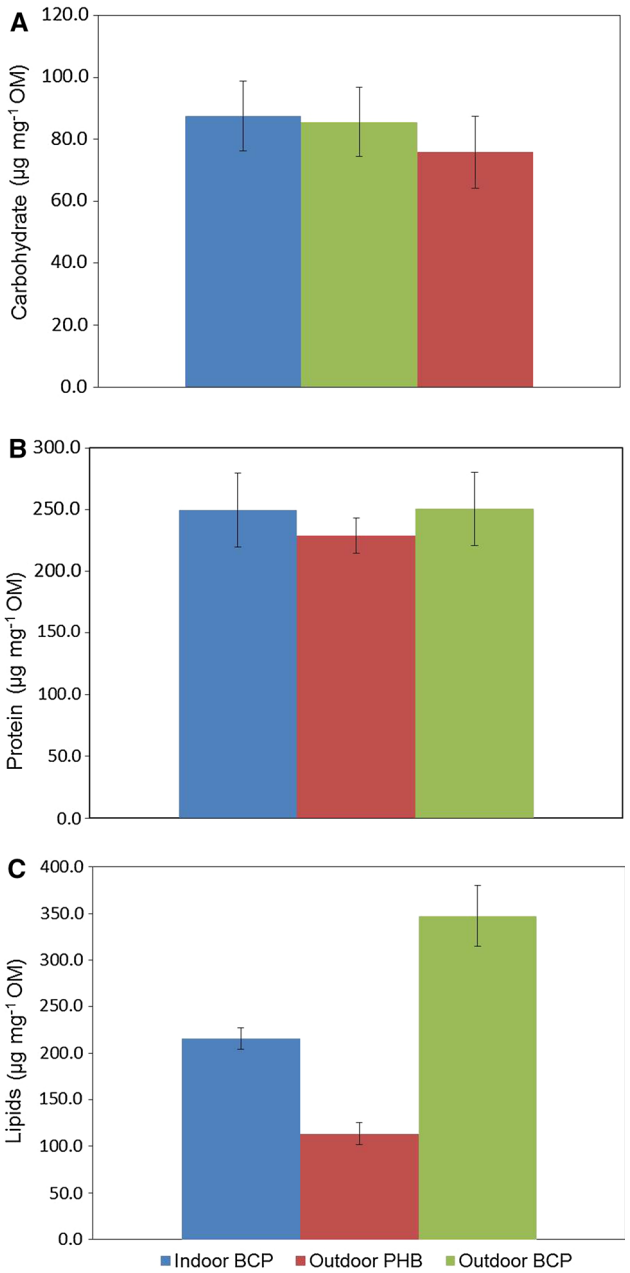

Fig. 5 Biochemical analyses. **a** Carbohydrate, **b** protein and **c** lipid content in the *P. tricornutum* biomass cultivated at different conditions. µg mg^{-1} *OM* micrograms per milligrams of organic matter, *PHB* polyethylene hanging bags, *BCP* bubble column photobioreactor

different FAs. The most abundant FAs observed (more than 5 % respect the total FAs) were the eicosapentaenoic acid (C20:5n3), palmoleic acid (16:1n7), palmitic acid (C16:0) and myristic acid (C14:0), which constituted ranges from 47.27 to 58.38 %, 20.84–22.75 %, 6.14–12.93 % and 5.9–7.37 % of the total FAs present, respectively. The rest of the FAs represented nearly 12 % of the total FAs.

Table 2 Fatty acid profile (%) from *Phaeodactylum tricornutum cultures*

	Outdoor PHB	Outdoor BCP	Indoor BCP
C14:0	5.90 ± 0.77	6.08 ± 1.60	7.37 ± 0.84
C15:0	0.17 ± 0.10	0.28 ± 0.07	0.23 ± 0.005
C16:1(n-7)	21.32 ± 3.36	20.84 ± 1.51	22.75 ± 0.56
C16:0	11.71 ± 1.10	12.93 ± 4.04	6.14 ± 0.32
C18:3(n-6)	0.86 ± 0.083	0.94 ± 0.10	0.39 ± 0.02
C18:2(n-6)	2.42 ± 0.28	2.44 ± 0.37	0.64 ± 0.01
C18:1(n-9)	1.84 ± 0.42	2.33 ± 0.62	0.92 ± 0.06
C18:1(n-7)	0.29 ± 0.51	0.65 ± 0.23	0.38 ± 0.044
C18:0	0.22 ± 0.17	0.46 ± 0.13	0.14 ± 0.03
C20:5(n-3)	49.91 ± 4.79	47.27 ± 7.61	58.38 ± 0.42
C20:4(n-6)	0.41 ± 0.03	0.69 ± 0.045	0.46 ± 0.09
C20:3(n-6)	1.18 ± 0.21	1.73 ± 0.51	0.49 ± 0.016
C20:2(n-6)	0.015 ± 0.002	0.06 ± 0.018	0.05 ± 0.006
C20:0	0.05 ± 0.03	0.06 ± 0.01	0.02 ± 0.007
C21:0	0.12 ± 0.07	0.20 ± 0.03	0.14 ± 0.01
C22:6(n-3)	1.86 ± 0.21	1.29 ± 0.27	0.63 ± 0.011
C22:1(n-9)	0.029 ± 0.009	0.12 ± 0.05	ND
C22:0	0.16 ± 0.009	0.18 ± 0.03	ND
C24:1(n-9)	0.087 ± 0.03	0.15 ± 0.04	0.10 ± 0.008
C24:0	1.15 ± 0.18	0.89 ± 0.15	0.16 ± 0.007
SFAs	19.48	21.08	14.2
MUFAs	23.56	24.09	24.15
PUFAs	56.65	54.42	61.44

Data are the means of three measurements with standard deviations

PHB polyethylene hanging bags, *BCP* bubble column photobioreactor, MUFAs = Mono-unsaturated fatty acids, *PUFAs* poly-unsaturated fatty acids, *SFAs* saturated fatty acids, *ND* non detected

Discussion

Feasibility of growing *P. tricornutum* under outdoor conditions

The present study confirms that *P. tricornutum* outdoor cultures can be maintained under the summer conditions of a warm temperate area. There are previous reports using outdoor cultures of some microalgae species at different world latitudes, some authors developed different culture systems such flask bioreactors, bag columns and outdoor raceways (Becerra-Dórame et al. 2010; Bazaes et al. 2012; Moheimani 2013; Schoepp et al. 2014). Other studies of outdoor cultures from different microalgae species in the Mediterranean area were measured over shorts time periods (Rodolfi et al. 2009) and/or avoiding the summer (Sánchez-Mirón et al. 2003; Fuentes-Grünewald et al. 2012). The present study was over a period of time considered as medium (2 months) and covering one period of

time (summer) which is usually avoided for other studies at similar latitudes.

Microalgae maximum absorbance

The maximum absorbance observed at 677 nm, is similar to former reports, where some authors measured microalgae growth using different wavelengths ranges: 684 nm (Ribeiro-Rodrigues et al. 2011) and from 677 to 688 nm (Santos-Ballardo et al. 2015b). The difference on the maximum absorbance between microalgae species could be explained due to different pigments, such as chlorophyll a, c and carotenoids present in the cells (Santos-Ballardo et al. 2015b).

Microalgae behavior under indoor cultures

The results presented in Table 1 for indoor cultures of *P. tricornutum*, are very similar to previous works, which reports values of μ: 0.2–1.44 d^{-1}, *dt*: 3.46–0.48 d (C. Fuentes-Grünewald et al. 2012) and μ: 0.29 d^{-1}, *dt*: 2.36 d (Santos-Ballardo et al. 2015b); that allows the use of the microalgae cultured under this conditions as inoculum for the outdoor cultures, also permitted the comparative for cell growth and biochemical composition on further experiments. Regarding the correlation of the biomass determination techniques, the obtained results are close to previous reports. High positive Pearson coefficients have been found between the number of cell per milliliter and spectrophotometric absorbance of compounds presents in microalgae cells, such as chlorophyll and some carotenoids (Lee et al. 2013). On the other hand, Santos Ballardo et al. (2015b) reported the correlation between the cell counting and spectrophotometric absorbance of *P. tricornutum*, reaching a high positive Pearson coefficient of 0.9827.

Phaeodactylum tricornutum growth under outdoor cultures

The *P. tricornutum* strain showed successful growth in the BCP made by PMMA instead the PHB, this could be explained due to the material used in BCP, the PMMA, which is a typical material used for manufacture of photobioreactors due to its higher refractive index, besides is considered an efficient material for investigations of light wavelength effects on cell growth (Kunjapur and Eldridge 2010).

On the other hand, the results obtained in the present investigation for growth parameters are close to previous reports for *P. tricornutum* outdoor cultures, even presenting better cell growth performance than the reported for Acién-Fernández et al. (2003) which reached μ: 0.048–0.192 d^{-1} and *dt*: 14.44–3.61 d, using an helical reactor. On the other hand, Hall et al. (2003) reported μ: 0.072–1.632 d^{-1}, *dt*: 9.62–0.42 d, they reached a shorter duplication time, using variations of the superficial gas velocity in an helical tubular reactor. The difference observed for the growth characteristics between indoor and outdoor cultures could be explained because the growing behavior could differ even with the same microalgae species. Besides, the microalgal cell growth is affected by different factors, including the culture conditions (nutrients, temperature, light irradiance, carbon sources, among others), the reactor characteristics and also the biological demands for the microalgae strains (Bazaes et al. 2012; Griffiths et al. 2012).

Effects of culture conditions on proximal composition and FAs profile of *P. tricornutum*

The changes observed in the biochemical composition of the microalgae cells under different culture conditions could be explained because the environmental factors that affect the growth rate and cell yield in cultures, affects the lipid content and biochemical composition of microalgae (Fuentes-Grünewald et al. 2011; Bazaes et al. 2012). Furthermore, Kaixian and Borowitzka (1993) reported the environmental effects (nitrogen limitation, temperature and irradiance) on cultures of *P. tricornutum,* also they determinate that the carbohydrate and protein contents are negatively correlated with irradiance; this suggests that the changes in these parameters are more related to the variations in nitrogen limitation. The structural roles and use of fast energy of the *P. tricornutum* cells is not affected by the culturés conditions. This could explain the differences observed in lipid content within diverse cultures and the similar contents of proteins and carbohydrates.

The FAs profiles reported in the present work are similar to the reported for continuous outdoor cultures of *P. tricornutum* using a helical reactor (Sánchez-Mirón et al. 2003), where obtained a similar FAs profile and also, the eicosapentaenoic acid (C20:5n3) was the major FA present in the biomass. Even if the FAs profile of *P. tricornutum* grown outdoor conditions was not drastically altered by the inducted stress, they showed slightly variations in the cell concentration compared to the indoor cultures. Specifically there was a increase of the PUFAs: C18:2(n-6), C20:3(n-6), C22:6(n-3), C22:1(n-9); and in the percentages of the SFAs, particularly C15:0, C16:0, C18:0, C20:0, C22:0, C24:0. In general, MUFAs showed no variations between different cultures, ranged percentages from 23.56 to 24.15 %; on the other hand, PUFAs (which consisted mainly in eicosapentaenoic acid) showed a slightly decrease from 61.44 to 54.42 %, meanwhile SAFs showed a small increase from 14.2 to 21.08 %, comparing outdoor and indoor cultures. The differences observed in these

experiments could be explained due to the lipid composition within the same species can vary in response to growth conditions and other related factors (Fuentes-Grünewald et al. 2012; Kurpan-Nogueira et al. 2015).

Indoor vs outdoor cultures general performance

The biomass concentration of the microalga *P. tricornutum* was highest under indoor conditions; however total lipid content and accumulation per cell were greater in outdoor conditions. These results are similar to previous reports on the same microalga and even in other diatoms at different world locations (Acién-Fernández et al. 2003; Sánchez-Mirón et al. 2003; Rodolfi et al. 2009; Becerra-Dórame et al. 2010; Fuentes-Grünewald et al. 2011; Bazaes et al. 2012). This could be explained because the outdoor conditions imposed stress conditions to the microalgae, and responding to this the cells stimulated the production of reserves of energy to avoid starvation and non-controlled temperature shifts (Fuentes-Grünewald et al. 2011). On the other hand, one of the principal recommendations for the feedstocks destined for biodiesel production is the presence of high amounts of SFAs, in order to avoid the potential autoxidation of the unsaturated fatty compounds (caused by the presence of double bonds which are susceptible to oxidation) (Knothe 2005), although we observed a majoritarian presence of the eicosapentaenoic acid (C20:5n3), it is important to highlight that under outdoor cultures *P.tricornutum* showed an increase on the general content of the SAFs. Some authors reports that microalgae under stress conditions (irradiance, temperature, and nutrients changes, among others) showed variations within the oil composition, for example, the lipids used for growth and forming of cell membrane stops its production, and in its place the lipids necessary for energy reservoir are stored (Fuentes-Grünewald et al. 2011; Kurpan-Nogueira et al. 2015).

Conclusions

Different strategies for microalgae outdoor cultures were studied for analyzing their potential as feedstock for biodiesel production. The present study showed that the conditions reported of daylight and temperature in the experimental location, allowed to obtain microalgal cultures using different outdoor bioreactors. Besides, based on the results on cell growth behavior, lipid content and FAs profiles, the outdoor cultures using methacrylate BCP is the best option for obtain biodiesel feedstock from *P. tricornutum*, compared with the use of outdoor BHP and the BCP under indoor cultures. This study assessed the importance of the use of outdoor cultures as a way to enhance the efficiency and the energetic balance of the microalgal biodiesel production from *P. tricornutum.* Furthermore, the authors recommend the development of life cycle analysis (LCA) for calculate the real enhancement obtained on the energetic balance with the use of outdoor cultures.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

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