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Nannochloropsis gaditana grown outdoors in annular photobioreactors: Operation strategies

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ABSTRACT

Microalgae are a topic of intense research due to their potential applications in bio-based economy. However, sustainable commercial production is still overpriced due to high cultivation costs, harvesting and dewatering processes. In the present study, trials were conducted with the aim to improve daily operation strategies related to microalgae harvesting processes that did not compromise biomass productivity or the biochemical composition of the cultivated microalgae. Two experimental trials were performed in outdoor tubular annular photobioreactors to evaluate the effects of harvesting and medium dilution time (sunrise *vs* sunset) on *Nannochloropsis gaditana* biomass productivity, lipid and fatty acid content. Results showed that harvesting time had no significant effect on cell concentration and biomass productivity. Harvesting and medium dilution time did not affect lipid content. However, lipid content in samples collected at sunset was significantly higher than in samples collected at sunrise for both experimental treatments. The fatty acids profiles were mainly composed by polyunsaturated fatty acids, followed by mono-unsaturated fatty acids and saturated fatty acids. Regardless of medium dilution time, harvesting at sunset indicated that lipidic production (higher polyunsaturated fatty acids and lower saturated fatty acids) was favored without affecting the biomass productivity. The current study showed harvesting in the afternoon is a viable option for large production units that use semi-continuous strategy, without compromising biomass cell and lipid productivity.

1. Introduction

Microalgae are photosynthetic organisms that have during the past three decades been used as a food source for aquaculture [1] and for human food production [2]. More recently, their ability to produce value-added products, such as antioxidants and pigments, triacyclglycerols (TAG) and polyunsaturated fatty acids (PUFA) [3,4] attracted significant interest from researchers. Consequently, microalgae biomass production is perceived as an attractive industrial investment option [5], recognized and pursued by the food, feed, cosmetic and nutraceutical markets [6]. However, the feasibility of outdoor industrial production of algal biomass requires extensive pilot-scale research to determine the optimal strategy to optimize cultivation technology and the selection of an ideal lipid profile for the cultured strain. Such advancements are critical for the economical sustainability of outdoor production of algal biomass and its transition to large scale applications. Previous studies have reported improved practices that can increase biomass and lipid productivity in outdoor culturing facilities [5,7–9], but have also highlighted the challenges in accounting for how varying weather conditions can greatly affect microalgae growth rates [10] and biochemical composition [11–14]. These works indicate that efficient outdoor large-scale cultivation in closed systems is necessarily a balance between production costs, maximum biomass productivity and lipid productivity [15].

Most studies on optimization of microalgae cultivation are based on trial-and-error and/or design heuristics [16,17], focusing on laboratory algae growth systems without accounting for outdoor weather variations [18]. Conventional approaches to improve biomass and lipid productivity of microalgae involve manipulation of nutritional and environmental factors [19]. Few studies address the combined aspects of operating outdoor closed photobioreactors (PBRs) in large scale units and its effects on growth and productivity parameters on microalgal production [5,7,15]. Algae grown in outdoor PBRs are inevitably exposed to variable incident light and water temperature, due to diurnal

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and seasonal differences in irradiation with the potential to result in a circadian adaptation [20]. Examples of processes under control of the circadian clock are chemotaxis, phototaxis, photosynthesis and cell division [21], all likely to have important consequences for the productivity of outdoor PBRs. Several studies reported over 30%-night losses of the biomass fixed during the day in outdoor cultivation regardless of the production technology adopted (open or closed PBRs) [22–24]. Such losses can significantly impact the biomass production capacity of solar-based algae cultures and therefore the suitability of strains for lipid production.

Harvesting of microalgae biomass in the afternoon has been proposed as a measure to mitigate respiration losses and increasing the productivity of in outdoor cultivation systems [25]. Richmond et al. [26] has also reported an increase in net productivity of 15–20% when harvesting was carried out mainly in the late afternoon, rather than in the morning. From a logistical perspective, harvesting microalgae from large industrial units at sunset would present the operational advantage of reducing the number of early morning procedures (*e.g.* harvest and medium dilution) and consequently allowing longer sunlight exposure to the diluted (renewed) cultures. This approach could have direct positive implications on the productivity parameters of microalgae, especially in winter season, when shorter exposure to light periods needs to be maximized to maintain sustainable microalgae growth rates. Additionally, this harvesting strategy could be economically advantageous, since the high cost of harvesting operations is mostly associated with power consumption of water pumps [27]. A shift to a higher power consumption during the night, when its electricity costs are lower in many regions, would result in lower auxiliary production costs.

The aim of this study was to investigate practices that could contribute to better operational strategies in large scale cultivation units without compromising biomass productivity and biochemical composition of the cultivated microalgae. In the experimental trials we evaluated the effect of two harvesting and medium dilution times on the lipid content, fatty acid profile and biomass productivity of outdoor *Nannochloropsis gaditana* cultures. This robust microalga can cope with varying environmental conditions, such as salinity, nutrient deficiency and light intensity, being cultivated in various types of facilities, both indoor and outdoor [5,8,28–32].

2. Materials and methods

2.1. Facilities

The experiments were performed at Buggypower, a microalgae commercial production unit located in Porto Santo Island (Madeira Archipelago, Portugal) that uses 400 L vertical tubular photobioreactors (PBRs) for the production of biomass of several species, including *Nannochloropsis gaditana*.

Regular operation of the industrial unit applies semi-continuous regime, collecting a variable percentage (5–40%) of the culture every morning, followed by the addition of new medium. Due to the large culture volumes harvested daily, temporary storage of the biomass is normally done prior to centrifugation using a 110 m^3 tank supplied with paddles to mix the microalgae cultures of the different PBRs.

2.2. Microalgal strains and culture medium

The marine species *N. gaditana* (Lubián CCMP 527) was grown in laboratory conditions in 1 L flasks filled with autoclaved 36 ppm seawater and Algal medium ($NaNO_3$, 2 mM; NaH_2PO_4, 0.1 mM; $ZnCl_2$, 1 μM; $MnCl_2$, 1 μM; Na_2MoO_4, 1 μM; $CoCl_3$, 0.1 μM; $CuSO_4$, 0.1 μM; $C_6H_5FeO_7$, 20 μM; EDTA, 26.4 μmol l^{-1} adapted from Fabregas et al. [33]). Temperature was kept at 21 °C, and light was provided in a 12 light: 12 dark cycle at 150 μEm^{-2} s^{-1} measured on the outer surface of the culture flasks (LI-250A light meter). Cultures were continuously bubbled with compressed HEPA-filtered air, supplied at a rate of 1.5 L min^{-1}. To ensure even distribution of nutrients and light exposure, besides air supply, flask cultures were manually stirred three times a day. Laboratory cultures were allowed to grow until nutrient exhaustion and then transferred outdoor into four recirculating 40 L flat panels. The flat panels cultures were allowed to grow autotrophically during a week to achieve the cell concentration needed for the trials (2×10^8 cells mL^{-1}). To avoid contamination, these seed cultures were maintained in UV-treated natural seawater (36 ppm), chemically sterilized with sodium hypochlorite, and further deactivated by strong aeration and filtration over active carbon. Algal medium was added to the seawater to adjust nitrate to a starting concentration of 250 ppm in the seed cultures. Daily samples of 25 mL were manually collected to determine cell number, nitrate concentration and biomass dry weight from the seed cultures (see Section 2.5 Analytical procedures).

2.3. Photobioreactors (PBRs) and operation mode

Six annular PBRs were used to perform the trials in Buggypower facilities, Porto Santo (Portugal). Each PBR had a working volume of 100 L, consisting of two acrylic cylinders of distinct diameters (400 and 300 mm) with the inner cylinder placed inside the outer one and closed at the bottom and at the top tube (Fig. 1). The arrangement of the PBR's was optimized for solar radiation capture, being oriented east-west with the distance between the outer tubes set to 0.30 *m* to minimize shading. Each tube was provided with bottom aeration and 2% CO_2 mixed with air.

The pH of the culture medium was kept at 7.5 by on-demand injection of pure CO_2 into the inlet of the air stream, located at the bottom of the chamber. The air flow rate entering from the bottom of each photobioreactor was 0.1 (v/v/m) in all PBR's. Air outlet was located at the top opening of each annular chamber. The remaining area of the chamber's top was capped to limit entry of air-borne contaminants. The solar radiation received by the facility was measured with a thermoelectric pyranometer (LI-200) connected to a PC control unit.

2.4. Experimental procedure

The trials were performed outdoors during winter season and divided into two experimental sets: 1- Harvest time; and 2- Harvest and medium dilution time. First, the tubular PBR's were inoculated with seed cultures of *N. gaditana* from the 40 L flat panel reactors at the end

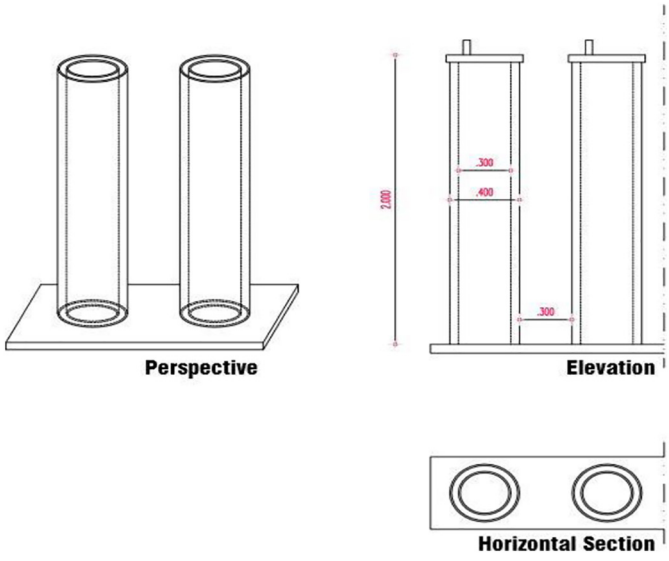

Fig. 1. Schematic representation of the experimental 100 L photobioreactors set outdoor.

of exponential phase grown. These cultures were used as an inoculum at a ratio of 1:5 (v/v) to achieve starting cell concentrations of 4×10^7 cell mL^{-1}.

Once cultures approached late exponential phase ($1.35 \times 10^8 \pm 0.15$ cell mL^{-1}), harvesting and daily dilutions at a renewal rate of 15% of the volume of cultures were carried until the end of both trials. To ensure that cultures were nutrient sufficient during the trials, sub-samples of the harvested biomass were used for nitrate and phosphate determination (see Section 2.5 Analytical procedures) prior to medium dilution. Preliminary tests showed that a nitrate concentration of 150 ppm in the tubular PBRs used in our experiment do not deplete in less than 24 h. The seawater used to dilute the cultures was then prepared by supplementing UV-treated seawater with Algal medium adjusted to keep the cultures with 150 ppm nitrates. Samples of 100 mL of the harvested biomass were also used to assess the algae biomass dry weight (g L^{-1}), cell concentration (10^6 cell mL^{-1}) of the previous day (see Section 2.5 Analytical procedures) and to monitor cultures pH and temperature (°C). Daily ambient temperatures ranged from 17 to 21 °C and irradiance between 2 and 20 MJ m^{-2} day^{-1}.

2.4.1. First experimental set- effects of harvest time

The first experimental set ran for twelve days and aimed at evaluating the effect of harvesting *N. gaditana* cultures at sunset without simultaneous medium dilution. In three of the six replicate PBR's, harvesting was performed daily after sunset (6 p.m.), and medium dilution occurred in the following morning (sunrise), approximately 12 h after harvesting (named HSSDSR). In another set of three PBR's (control set), harvesting and medium dilution was performed daily in the morning at 7 a.m. (named $HDSR_1$). At day one and day twelve of harvesting, additional samples were collected at sunrise and sunset from each biological replicate to determine lipid and fatty acid content (see Section 2.5 Analytical procedures).

2.4.2. Second experimental set- effects of harvest and medium dilution time

On the second experimental set, the effect of harvesting time with simultaneous medium dilution was evaluated for nine days. PBR's were inoculated as in the first experimental set with *N. gaditana* cultures from the flat panels. Three PBR's with N. *gaditana* were harvested at sunset (6 p.m.) followed by medium dilution (named HDSS). On the other three PBR's harvesting and medium dilution occurred at sunrise (named $HDSR_2$) as done in trial 1 for the HDSR (control set). Similarly, to the harvest time experimental set, samples for chemical analysis were collected at the beginning and at the end of the experiment.

2.5. Analytical procedures

Cell concentration, biomass dry weight, lipid and fatty acid content were used as parameters of the culture state in both experimental sets. Daily samples of 100 mL were collected to determine nutrient concentration (2.5.1) and microalgae productivity parameters (2.5.2). For biochemical analysis (2.5.3, 2.5.4), 1 L of each PBR culture was centrifuged, the supernatant was discarded and tubes containing microalgal biomass were stored at −20 °C.

2.5.1. Nutrient determination

Prior to analysis, 10 mL aliquots of the culture samples were filtered through glass fiber filters. The concentration of nitrate and phosphate in the culture media were determined by spectrophotometric determination, according to Clesceri et al. [34].

2.5.2. Microalgae productivity

The cell concentration was determined by cell counting under an optical microscope (Axio Scope A.1) using a Neubauer chamber (20×) and the biomass dry weight determination was performed according to Zhu & Lee [35]. Briefly, 75 mL of samples were taken from each replicate, filtered and washed with 100 mL of a 0.5 M NH_4HCO_2 solution through a pre-dried and pre-weighted Whatman GF/C filters of 0.7 μm pore size (Millipore Ireland Ltd.). Then, the samples were dried for 24 h at 95 °C, cooled down in a vacuum desiccator for 2 h and weighted again.

The daily biomass productivity (BP; g L^{-1} day^{-1}) during the culture harvesting periods was calculated from the equation:

$$BP = [(V_{PBR} * (\%harvest/100) * dw]/day/V_{PBR}$$

where, V_{PBR} is the PBR volume (L); % harvest is the daily harvest (15%) and dw is the biomass dry weight (g L^{-1}).

The cellular lipid productivity (LP; mg L^{-1} day^{-1}) was calculated as the product of BP and the lipid content in the dry biomass (w/w), according to Dickinson et al. [36]:

$$LP = BP * lipid/biomasss\ (w/w)$$

Prior to the beginning of semi-continuous regimen and at the end of the experiments, harvested biomass was washed with distilled water, centrifuged at 4000 rpm for 10 min (HLMac CT Series, rotor T6AP; 4170 maximum RCF) and freeze-dried (Labconco Freezone freeze dry 4.5 L) until further chemical analysis.

2.5.3. Lipid extraction

The lipid extraction was performed according to modified Bligh and Dyer [37]. Briefly, to dried algal biomass was added 3 mL of a methanol: chloroform mixture (2:1, v/v) followed by 400 μL of a saturated solution of KCl and 2 mL of chloroform. After homogenization, 2 mL of distilled water were added, and the mixture was left stirring for 15 min. Then, the sample was let to set, and the organic phase was removed and dried in Na_2SO_4 filters. At the end, solvent was evaporated in a Büchi rotavapor R-200, in order to proceed to lipids quantification. Lipid content was quantified gravimetrically. Lipids contents are presented relatively to dry biomass weight (% dw) as average of at least two replicates.

2.5.4. Fatty acid analysis

Lipid extracts were analysed for their fatty acid composition as fatty acid methyl esters (FAMEs) as described in Nogueira et al. [38]. Briefly, the fatty acids were converted to FAMEs by adding a mixture of ethyl acetate-methanol (1:19; v/v) to lipid extract aliquots that were after placed at 80 °C for 1 h. FAMEs were analysed by gas chromatography (Agilent HP 6890) equipped with a mass selective detector (Agilent 5973) and a fused silica capillary column Supelcowax™ 10 (30 m × 0.25 mm inner diameter, 0.25 μm film thickness) from Supelco. The chromatographic conditions were: initial temperature, 40 °C for 5 min; temperature gradient, 2 °C min^{-1}; final temperature, 250 °C for 5 min; injector temperature, 260 °C; transfer-line temperature, 260 °C; split ratio, 1:100. Helium was used as the carrier gas with a flow of 1.0 mL min^{-1}.

The FAMEs were identified through comparison of retention times and mass spectra obtained by spectra library Wiley-NIST and/or using two standard samples: Bacterial acid methyl esters CP mix; and Supelco 37 component FAME's mix, from Supelco. To quantify the fatty acids of the sample it was used heneicosanoic acid (C21:0) as an internal standard. The results were expressed in percentage of total fatty acids, being the quantification made according to the response factor determined for each fatty acid present in the standards, in comparison with internal standard.

2.6. Statistical analyses

Repeated measure One-way ANOVA was used to test for significant differences between treatments at 5% confidence level on the dependent variables measured daily for 12 days, namely: cell density, biomass dry weight and productivity. One-way Anova was conducted to detect significant differences in chemical parameters that were only measured at the end of the 12 days trials, namely lipids and FAME's.

Table 1
N. gaditana grown outdoors in annular tubular PBR's, in semi-continuous system (15% dilution): cell concentration, dry weight and biomass productivity.

	Cell concentration ($\times 10^6$ cell mL^{-1})	Dry weight (g L^{-1})	Productivity (g L^{-1} day^{-1})
$HDSR_1$	126.36 ± 31.15	0.52 ± 0.08	78.04 ± 14.20
HSSDSR	144.94 ± 30.04	0.55 ± 0.06	81.70 ± 9.80
$HDSR_2$	121.17 ± 35.28	0.69 ± 0.06	105.33 ± 8.40
HDSS	111.63 ± 32.41	0.64 ± 0.08	94.26 ± 9.72

Data are given as means ± SD (n = 3). $HDSR_1$ and $HDSR_2$ = harvesting and dilution at sunrise; HSSDSR = harvesting at sunset followed by dilution later at sunrise; HDSS = harvesting and medium dilution at sunset.

Data in figures and tables are reported as mean ± SD. All variables were checked for normality, homogeneity of variance, using the Kolmogorov-Smirnoff and the Levene tests, respectively. Sphericity of repeated measure ANOVA was checked with Mauchly's test. Statistical analyses were performed using SPSS. v 23 for Windows.

3. Results and discussion

3.1. Effects on biomass, cell density and productivity

The present study was focused on evaluating the effect of harvesting microalgae biomass late in the afternoon in order to expedite morning processes in outdoor microalgae industrial facilities. Experiments were conducted outdoors during winter season and the effects of harvesting and medium dilution at sunset were evaluated on *N. gaditana* cell and lipid productivity.

The first experimental set aimed at evaluating the effect of harvesting the microalgae at sunset, as this procedure can reduce the morning procedures in large industrial plants using semi-continuous regimes. Interestingly, harvesting at sunset did not result in a significant reduction in biomass, cell density or productivity (Table 1). Still, medium dilution time in the HSSDSR treatment was performed almost 12 h after harvesting, which may contribute to the building of foam at the top of the reactor. This phenomenon is commonly observed in microalgal cultures [39] due to the presence of specific molecules excreted by the microalgae and is amplified in confined geometries where small bubbles are generated [40]. The accumulation of foam should be minimized to avoid biomass losses by entrainment and the development of bacteria. On the second experimental set, the advantages of further minimizing procedure times in the morning and avoiding possible foam formation at the top of PBR's were evaluated, as harvesting and medium dilution were both performed at sunset. Similar to the first experimental trial, our results indicate that the employment of a complete semi-continuous system at sunset did not significantly affect cell density, dry weight or biomass productivity (g L^{-1} day^{-1}) ($p > 0.05$) (Table 1).

Biomass productivity from the first experimental set was on average 78 mg L^{-1} day^{-1} for the morning harvesting treatment (HDSR) and 82 mg L^{-1} day^{-1} for cultures harvested at sunset (HSSDSR), which is comparable to other outdoor *N. gaditana* cultures in different PBR's designs ("green wall panels" [41]; plastic tubular PBR's [42]); raceway ponds [8]. Nonetheless, biomass productivity values were low compared to other works [5,28,43,44], representing only 16–30% of the maximum productivity found in the above-mentioned studies. This may be a result of several factors that influence productivity and are related with the design of the PBR's; the bottleneck effect [45]; inefficient mixing [46]; temperature; and or irradiation levels [15]. In fact, differences in the irradiation levels could also contribute to explain the higher biomass productivity found in the second experimental set. Though both experimental sets were conducted consecutively in the same time of the year, the mean irradiance in the second experimental set was more than the double of the first experimental set (13.4 MJ m^2 $day^{-}1$ and 5.6 MJ m^2 day^{-1}, respectively).

3.2. Effects on lipid and fatty acid content

Lipid content (TL) of the two experimental sets (Fig. 2) were comparable to those obtained by other studies working with *Nannochloropsis oculata* grown outdoors, during winter season such as by Olofsson et al. [15]. At the end of the first experimental set, TL of microalgae (% dw) was similar for both harvesting times when lipid samples were collected in the morning (9.69 ± 0.46% for $HDSR_1$ and 10.10 ± 0.41% for HSSDSR). When samples of the same experimental set were collected at sunset, there was a slight, but significant increase ($p < 0.05$) in lipids of both harvesting times (11.49 ± 0.89% for $HDSR_1$ and 12.11 ± 1.19% for HSSDSR). Differences in lipid content of the same cultures collected at different times of the day suggest that harvesting at sunset would be favorable for lipid purposes, without

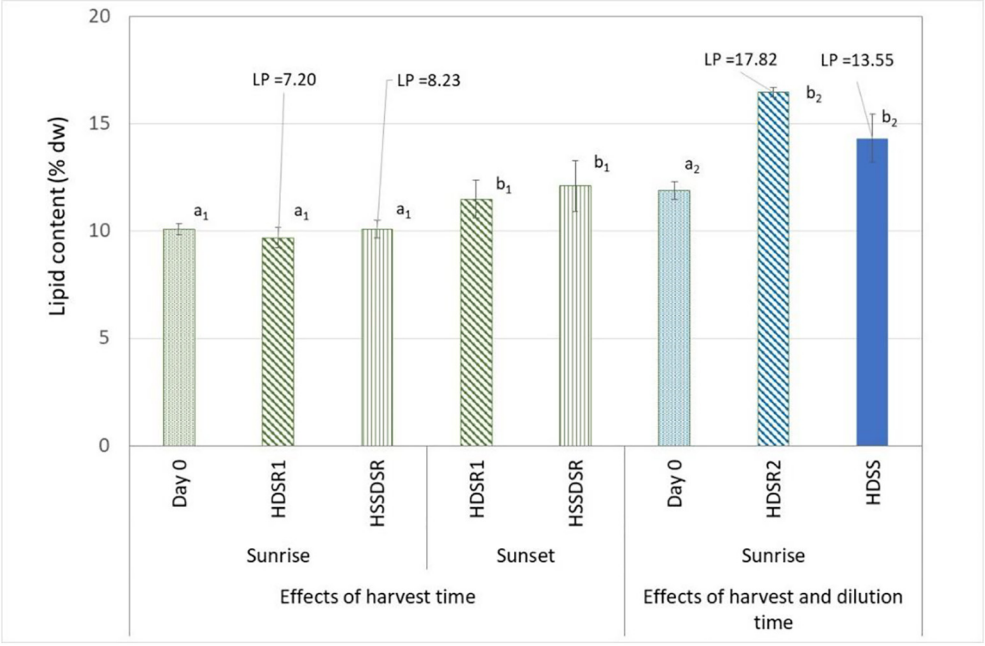

Fig. 2. Lipid content (% dw) prior to the beginning of the semi-continuous systems and at the end of the two experimental sets. In the harvest time experimental set, samples for lipid determination were collected at sunrise and at sunset in both treatments. In the harvest and medium dilution experimental set, lipid samples were collected at sunrise. Error bars represent standard deviation (n = 2). Different subscripts indicate significant differences ($p < 0.05$) in lipid content within each experimental set (1 or 2). $HDSR_1$ and $HDSR_2$: harvesting and dilution at sunrise; HSSDSR: harvesting at sunset followed by dilution later at sunrise; HDSS: harvesting and medium dilution at sunset. LP: Lipid productivity (mg L^{-1} day^{-1}).

affecting cell productivity in terms of number or biomass dry weight. Increased lipid and fatty acid content of samples collected at sunset has also been observed by other authors [47,48] and can be related to the variability in lipid content and fatty acids composition of microalgae during its growth cycle [49,50] in which daily light and temperature play a central role [8]. In accordance to the results of the present study, Tambouric et al. [51] demonstrated that *N. oculata* grown outdoors were more active beyond midday under elevated afternoon and early-evening temperatures. Further, the same authors suggested that high temperatures at the end of the light period (sunset) lead to a positive hysteresis in the oxygen evolution rate, resulting in higher relative photosynthetic activity towards the end of the day.

In the second experimental set, lipid content was also not affected by the harvesting and medium dilution time as both treatments presented similar lipid content (16.48 ± 0.23% for $HDSR_2$ and 14.32 ± 1.12% for HDSS), but in accordance with biomass productivity results, higher lipid content and productivity were observed when compared with the first experimental set. Overall, lower cell density with higher dry weight resulted in higher lipid productivity, with the highest lipid content and lipid productivity being observed in the harvesting and medium dilution at sunrise treatment ($HDSR_2$). Irradiance is a very useful tool to modify the biochemical composition of the marine microalga *N. gaditana* under semi-continuous culture [52,53]. As stated previously, cultures of the second experimental set experienced higher light availabilities than the first experimental set (13.4 MJ m^2 day^{-}1 and 5.6 MJ m^2 day^{-1}, respectively). Further, although the two experiments were performed consecutively it is normal that besides light intensity, short term changes in day length and photoperiod occur, which in accordance to Olofsson et al. [15] may contribute to explain the higher TL levels found in the second experiment.

The fatty acids composition of marine *N. gaditana* after applying the proposed experimental conditions, depicted in Table 2, confirm the potential use of *N. gaditana* as a natural resource of high commercial value PUFAs [54,55].

The fatty acid profiles of microalgae are an important indicator of downstream process requirements, particularly with respect to unsaturated fatty acid content [7]. In all our experimental conditions, fatty acids profiles were highest in polyunsaturated fatty acids (PUFAs), followed by monounsaturated fatty acids (MUFAs) and lowest in saturated fatty acids (SFAs). Though the proportion of PUFAs of each strain may be the most diverse [15], it is assumed that PUFAs increase in winter season, due to lower light intensity [15,56] and lower temperatures [54]. Similar PUFAs contents were found by Olofsson et al. [15] in *N. oculata* grown outdoors in winter season at analogous temperature and radiations levels. Moreover, results found in the present study could also be explained by the fact that fatty acids samples were collected in the morning. SFAs and MUFAs, along with carbohydrates, act as storage material [48,57] decreasing during the night, whereas the relative proportion of PUFA, namely EPA (C20:5 - eicosapentanoic acid), mainly associated with galactolypids, increases in the dark period. In future experiments, it would be interesting to collect samples for fatty acids determination both in the morning and in the afternoon to further investigate if the fatty acids composition decreases significantly during the night following Chini Zitelli et al. [57].

Still, our findings highlight the tendency of *N. gaditana* cultures to produce higher PUFAs and lower SFAs, when harvesting of biomass occurred at sunset, regardless of the medium dilution time. Thus, if PUFAs are intended, the proposed harvesting timetable of the current study would be beneficial.

Furthermore, the fatty acid profiles showed that predominance was consistent in all the experimental conditions, with palmitic acid (C16:0) accounting for 12.9 to 20.5% of the SFAs; palmitoleic acid (C16:1) for 21–38% of the MUFAs and EPA for 45–85% of total PUFAs. Palmitic and palmitoleic acid are major fatty acids in semi-continuous [58], batch [59] and continuous cultures [60,61] and are assumed to be the major fatty acids of microalgae strains of Eustigmatophyte [62]. EPA, a functional fatty acid with great benefits to human health [63], accounted for 18 to 38% of the total fatty acids detected (Table 2). In comparison with previous studies *N. gaditana* grown in our experimental conditions presented higher amounts of EPA, regardless of the semi-continuous timetable [5,43]. However, when normalizing EPA to total lipid content (Fig. 3), similar values to those reported by Olofsson et al. [15] were found. Normalized EPA content ranged between 5.5% in the $HDSR_2$ treatment and 18% in the samples collected at sunset and medium renewed 12 h later (HSSDSR).

3.3. Implications for feasibility of outdoor cultivation

The majority of microalgal production occurring in outdoor cultivation still faces biotechnological and economical challenges [4]. In order for these algal production systems to increase their competitiveness and economic viability, different strategies and routines would need to be applied [4]. The operation strategy applied in the current work aimed at increasing the exposition time of the cultures to sunlight, particularly in winter season, with the potential advantage of reducing harvesting costs without compromising biological and biochemical parameters of the cultures. Our results indicate that harvesting and renewing the medium in late afternoon is feasible and present no significant disadvantages in term of biomass production and lipid content. In fact, lipid content of algae harvested in the afternoon was significantly higher in our trials, suggesting that this procedure could

Table 2
Fatty acid (% relatively to the total of the fatty acids detected) of *N. gaditana* grown in outdoor tubular annular photobioreactors at the end of semi-continuous systems.

	$HDSR_1$			HSSDSR			$HDSR_2$			HDSS		
C14:0	2.64	±	0.05^a	2.39	±	0.05^b	2.49	±	0.14^a	2.03	±	0.11^a
C16:0	16.82	±	0.10^a	12.90	±	0.01^b	20.51	±	0.01^a	17.89	±	0.20^b
C18:0	0.23	±	0.01^a	0.41	±	0.01^b	0.78	±	0.01^a	0.53	±	0.01^b
C16:1	37.99	±	0.16^a	30.39	±	0.01^b	27.12	±	0.60^a	20.51	±	0.02^b
C18:1	1.76	±	0.01^a	1.59	±	0.01^b	7.26	±	0.12^a	4.52	±	0.09^b
C18:2	0.94	±	0.08^a	1.43	±	0.02^b	2.39	±	0.10^a	2.94	±	0.06^b
C18:3	0.36	±	0.03^a	0.37	±	0.02^a	6.90	±	0.01^a	7.39	±	0.02^b
C20:4	4.77	±	0.14^a	7.41	±	0.01^b	2.25	±	0.06^a	3.33	±	0.05^b
C20:5	33.89	±	0.08^a	41.56	±	0.08^b	18.76	±	0.08^a	30.48	±	0.10^b
Total – SFAs	20.04	±	0.17^a	16.19	±	0.04^b	24.59	±	0.19^a	21.20	±	0.30^b
Total –MUFAs	39.74	±	0.16^a	31.99	±	0.04^b	34.41	±	0.48^a	25.03	±	0.06^b
Total – PUFAs	40.22	±	0.31^a	51.83	±	0.04^b	41.00	±	0.28^a	53.77	±	0.24^b

$HDSR_1$ and $HDSR_2$: harvesting and dilution at sunrise; HSSDSR: harvesting at sunset and dilution later at sunrise; HDSS: harvesting and dilution at sunset. SFAs: saturated fatty acids; MUFAs: monounsaturated fatty acids; PUFAs: polyunsaturated fatty acids. Data are given as means ± SD (n = 3). Different superscripts in the same row indicate significant differences ($p < 0.05$) for the same fatty acid within each experimental set.

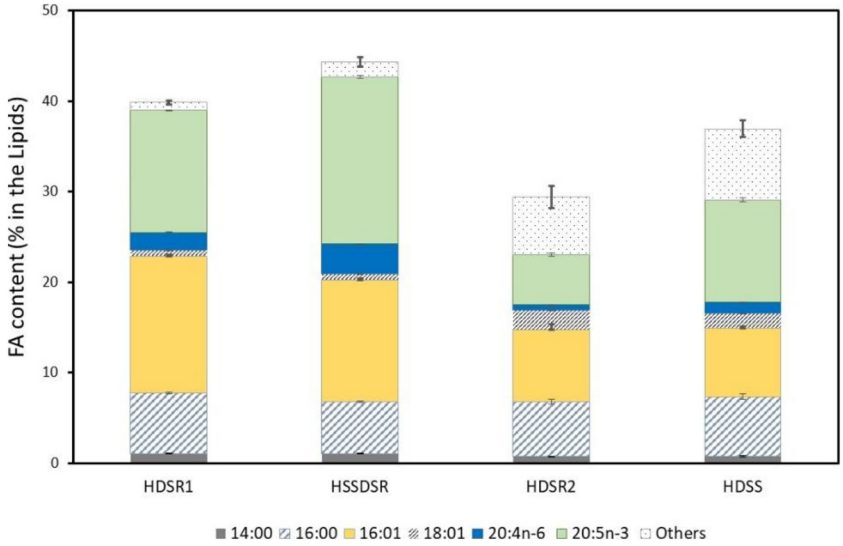

Fig. 3. Effects of harvesting and dilution time on the main fatty acids (FA, % in the lipids) profile of *N. gaditana*. Data are given as means ± SD ($n = 3$). $HDSR_1$ and $HDSR_2$: harvesting and dilution at sunrise; HSSDSR: harvesting at sunset and dilution later at sunrise; HDSS: harvesting and dilution at sunset.

actually benefit microalgal lipid production.

Still, the value semi-continuous at sunset production represents an effective alternative to sunrise processes, should continue to be assessed in future studies. Particularly, the value of adjusting semi-continuous operational routines like harvesting frequency and percentage should be evaluated. Moreover, research on nutrient supply strategies, such as induced stress by N-deficiency [28]; two-stage cultivation [64] and nutrient resupply at sunset [65] should also be combined with sunset harvesting and medium dilution procedures, as these procedures are known to trigger the accumulation of lipids in this microalgae species without loss of productivity.

4. Conclusions

This work tested possible negative effects to biomass production and lipid content of an alternative operation strategy in semi-continuous systems that could be biologically and economically advantageous for industrial microalgae production plants. Biomass productivity of *N. gaditana* was not affected by harvesting and medium dilution at sunset. Actually, higher percentage of PUFAs and fatty acids content were found in cultures subjected to semi-continuous system at sunset. Thus, our results indicated that harvesting at sunset will benefit productivity parameters of lipid and PUFA production, especially in winter season, when a reduced number of natural light hours result in the need to maximize microalgae exposure to light. Economically, these findings may open opportunities to reduce production costs related with the use of harvest and water and centrifugation pumps in regions where special night energy rates are available.

CRediT authorship contribution statement

Natacha Nogueira: Conceptualization, Investigation, Formal analysis, Writing - original draft. **Francisco J.A. Nascimento**: Investigation, Writing - review & editing. **César Cunha**: Writing - review & editing. **Nereida Cordeiro**: Writing - original draft, Writing - review & editing, Supervision.

Declaration of competing interest

The authors declare no conflict of interest financially and personally.

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