Influence of irradiance on the growth and biochemical composition of *Nitzschia aff. pellucida*

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Abstract

In this paper the influence of irradiance and the operation modes on biomass production and biochemical composition, especially the fatty acid profile, on the diatom *Nitzschia aff. pellucida* was studied. Experiments were carried out under laboratory conditions, but simulating those outdoors, at irradiances ranging from 100 to 1000 μmol photons $m^{-2}\,s^{-1}$, both in batch and semi-continuous mode. The data show that the growth rate increased with irradiance, following a hyperbolic relationship with the average irradiance (E_{av}). However, at irradiances higher than 450 μmol photons $m^{-2}\,s^{-1}$, photo-inhibition was observed, with F_v/F_m values notably decreasing at irradiances above 1000 μmol photons $m^{-2}\,s^{-1}$. To take account of this phenomenon, a growth model is proposed that considers the saturation constant modification (E_k) as a function of available irradiance. The data indicate that *N. aff. pellucida* is better adapted to low irradiances, below 300 μmol photons $m^{-2}\,s^{-1}$. Regarding the biochemical composition, the highest content of lipids (~20%) and carbohydrates (~40%) was achieved at high irradiances (≥ 450 μmol photons $m^{-2}\,s^{-1}$), while the highest protein content (>8%) was found at low irradiances (≤ 200 μmol photons $m^{-2}\,s^{-1}$). Elevated amounts of saturated fatty acids (SFA) (>60%) were measured at high irradiances, while the highest content of unsaturated fatty acids (USFA) (>35%) was found at low irradiances. Eicosapentaenoic acid (C20:5 n-3) (EPA) and arachidonic acid (C20:4 n-6) (ARA) were only detected at 200 μmol $m^{-2}\,s^{-1}$ but not at higher irradiances. The results confirm the effects of irradiance and the cultivation mode on the growth and biochemical composition of *N. aff. pellucida*. The model is an important tool when using this valuable strain to produce compounds of biological interest.

Keywords *Nitzschia aff. pellucida* · Irradiance · Growth model · Saturation constant · Biochemical composition · Fatty acids

Introduction

Microalgae are considered as a promising source of compounds of biological interest with diverse applications. They are known to contain a wide variety of bioactive compounds, many of which have commercial applications such as pharmaceuticals, cosmetics, nutraceuticals, human food, and animal feed (Kelman et al. 2012). However, some groups of microalgae deserve greater worldwide attention due to their current applications. Bacillariophyceae, or diatoms, are a microalgae group that represents a major phytoplanktonic class present in the oceans, contributing to global photosynthesis and 20% of global carbon fixation as well as producing large amounts of biomass (Kroth et al. 2008; Pierella Karlusich et al. 2020). They constitute an abundant source of important biological compounds such as lipids, carotenoids, sterols, isoprenoids (Stonik and Stonik 2015), fatty acids

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(Zulu et al. 2018), carbohydrates (Kroth et al. 2008), proteins (Alzahrani et al. 2018), antibacterial substances (Wang et al. 2018), and others.

A comparison of the literature reporting on *Spirulina* and *Chlorella* indicates that these microalgae have been extensively investigated; however, the components of the genus *Nitzschia* have not been fully studied (Alzahrani et al. 2018). *Nitzschia* are reported as having a great eicosapentaenoic acid (EPA) production potential (Chen et al. 2007; Rodríguez-Núñez and Toledo-Agüero 2017). Several *Nitzschia* species are good and moderate biotic indicators of water quality (Martín et al. 2010) and heavy metal contamination (Guasch et al. 2009). Nevertheless, microalgal culture systems are influenced by different factors, such as light intensity, temperature, carbon dioxide, pH, and the nutrient composition of the culture media (Papapolymerou et al. 2019).

Light is the energy source that drives photosynthesis and is one of the most important factors determining the growth of photosynthetic microorganisms (Pedrosa et al. 2011). Research shows that light intensity is one of the main factors in microalgal growth and biomass accumulation (Singh and Singh 2015), due to an increase in the photosynthesis rate (Edwards et al. 2015). There are references to light intensity also influencing the biochemical composition of microalgae, especially lipids, fatty acids, proteins, and carbohydrates (e.g. Marchetti et al. 2018; Nzayisenga et al. 2020). In order to use microalgae as a product source, it is important to study the influence of the growing conditions on the biomass as well as the product generation rate (Sánchez et al. 2008).

To better estimate and optimize microalgal productivity under different conditions, it is necessary to model the process to gain useful information on the performance of the microalgae culture systems (Lee et al. 2015). According to Schwaderer et al. (2011), there are typical relationships between the growth rates and light intensity that characterize phytoplanktonic responses to this important resource. This model describes the photosynthetic processes, and those related with photoinhibition and also with photoinhibition recovery, allowing one to compare the different photosynthetic models for microalgae production (Eilers and Peeters 1988). Additionally, it can be used to characterize low light performance (α, the initial slope of the growth irradiance curve), the optimal irradiance above which growth is photoinhibited (E_{opt}), and the maximum growth rate at optimal irradiance (μ_{max}) (Edwards et al. 2015). Knowing the factors that determine the growth rate can help provide a strategy for optimizing the productivity of microalgae cultures (Kim et al. 2002). The availability of reliable models representing key phenomena affecting algae growth can help design and optimize effective production systems at the industrial level (Bernardi et al. 2014).

Various models have been developed under the assumption that the results obtained from indoor laboratory experiments can be directly extrapolated to outdoor conditions (Fernández et al. 1998). Huesemann et al. (2013) found that microalgae growth models could be useful in detecting promising strains of microalgae in the laboratory for their high biomass productivity potential in outdoor ponds under different climatic conditions. Developing a model capable of describing the behavior of a specific growth rate in response to average irradiance allows one to determine the optimal light conditions for selected microalgae and cyanobacteria, making it possible to rapidly determine the optimal growth conditions, and thus reduce the time and costs associated with culture parameter optimization (Gonçalves et al. 2016). Eilers and Peeters (1988) developed a dynamic model showing the relationship between light intensity and the photosynthesis rate in phytoplankton; this proved useful for integrating the phytoplanktonic production into the water column. Molina Grima et al. (1996) published a model for estimating the growth rate of algae. García-Malea et al. (2006) presented a model able to describe the effects of photoinhibition, light limitation, and light attenuation. The effects of light availability and temperature were presented in a general growth model, able to fit an experimental data set, which proved useful for the design and operational optimization of outdoor cultures of *Scenedesmus almeriensis* (Sánchez et al. 2008). Litchman (2000) showed the growth-irradiance curves for the diatom *Nitzschia* sp., based on the continuous light, for predicting fluctuations that affect the growth rate. Thorel et al. (2014) presented a model where the maximum *Pseudo-nitzschia australis* growth rate increased significantly, between 35 and 100 μmol photons m^{-2} s^{-1}, as a function of irradiance.

As remarked by Béchet et al. (2013), more than 40 models for predicting algal productivity have been described in the literature, but most of these were not validated against external data. Furthermore, reports published on growth models for *Nitzschia* are limited. This study presents a novel model that shows the effect of irradiance on the growth rate of the Panamanian diatom *Nitzschia aff. pellucida*, which can be used to determine the optimal culture conditions. To date, there have been no studies reporting on growth models for these microalgae that reflect the impact of light on biomass accumulation, biochemical composition, and the fatty acid profile. This information is helpful to develop industrial processes taking advantage of the potential of these microalgae.

Materials and methods

Organism

The diatom *Nitzschia aff. pellucida* was isolated from salty water by collaborators of the Microalgae Group of the Research Center for Natural Products and Biotechnology

(CIPNABIOT) at the Autonomous University of Chiriqui-UNACHI, Panama. The strain was collected at 8°20′42.98″N 80° 9′17.71″E, and cells were isolated using monoclonal culture. The strain is kept in liquid and agar cultures in the CIPNABIOT microalgae culture collection, UNACHI. It was morphologically identified by the Diatom Laboratory at the University of León (Spain). The inoculum was maintained in the laboratory using Mann and Myers medium (Mann and Myers 1968), at a constant irradiance of 200 µmol photons m^{-2} s^{-1} and temperature of 25 °C.

Culture conditions

The experiments were carried out in triplicate in 0.3 L bubble-column photobioreactors, with aeration at 0.3 vvm, pH control at 8.0 (by the on-demand injection of pure CO_2), and temperature control at 25 °C. A fertilizing culture medium prepared in salty water (NaCl 30 g L^{-1}) was used with the following components: 1500 mg L^{-1} $NaNO_3$, 300 mg L^{-1} KH_2PO_4, 250 mg L^{-1} $MgSO_4$·$7H_2O$, 90 mg L^{-1} $NaSiO_3$, 0.02 mL L^{-1} WelgroMix, 0.02 g L^{-1} Karentol, 0.1 mL L^{-1} trace elements (5.26 mg L^{-1} $C_6H_5FeO_7$·$5H_2O$, 0.024 mg L^{-1} $CoCl_2$·$6H_2O$, 0.025 mg L^{-1} $CuSO_4$·$5H_2O$, 0.152 mg L^{-1} Cr_2O_3, 1.98 mg L^{-1} $MnCl_2$·$4H_2O$, 0.24 mg L^{-1} $NaMoO_4$·$2H_2O$, 0.011 mg L^{-1} SeO_2, 0.012 mg L^{-1} EDTA), and 0.1 mL L^{-1} vitamins (Thiamin 0.035 mg L^{-1}, B12 0.03 mg L^{-1}, Biotin 0.05 mg L^{-1}). The reactors were illuminated with fluorescent lamps that automatically turned on and off to simulate the circadian solar cycle (light oscillations at time intervals, for a 12:12 L/D cycle). Different maximal irradiances were evaluated, distributed as follows: 1000, 450, 200, and 100 µmol photons m^{-2} s^{-1}. To obtain these irradiances, the reactors were coated with one, two, or three layers of a dark mesh (~ 0.5 cm in diameter) in order to reduce the light entering the reactors and allow a particular light percentage. Irradiance was measured using a ULM-500 universal light meter (Walz, Germany).

The inoculum volume supplied to the reactors at the beginning of the experiment was 10% of the total culture volume in the bubble column. The experiments were started in batch mode for 11 days. After reaching the stationary phase in batch mode, the culture mode was changed to semi-continuous till biomass concentration remain constant, i.e., for another 11 days. The dilution rates were calculated from the maximum growth rate (µ = 0.33 day^{-1}/2) of the microalgae for each of the corresponding treatments. The semi-continuous dilution rate used was 0.165 day^{-1}, calculated as the ratio of inlet flow to total volume of the culture.

Analytical methods

The biomass dry weight concentration (C_b) was determined using aliquots of culture 25 mL, filtering them

through 0.7-µm Millipore filter paper, washed with 20 mL of ammonium formate (15 g L^{-1}), and dried at 80 °C for 24 h. The biomass productivity was calculated by multiplying the biomass concentration for the growth rate measured each day. The condition of the cells was verified daily by measuring the maximum photochemical efficiency (F_v/F_m) with a fluorometer (AquaPen AP 100, Photon System Instruments, Czech Republic). Absorbance in the visible range (400 to 800 nm) was measured daily using a Genesis 10S double-beam spectrophotometer (Thomas Scientific) and the extinction coefficient (K_a) was calculated by dividing the absorbance value by the biomass concentration (C_b) and the light path of the cuvette (Eq. 1).

$$K_a = \frac{Abs}{(C_b * p)} \tag{1}$$

The average in situ irradiance of the culture (E_{av}) (Eq. 2) was calculated as a function of the irradiance at the surface (E_o), the extinction coefficient of the biomass (K_a), the concentration of the biomass (C_b), and the incident light path in the reactor (p) (Grima et al. 1997). To take into account the daily variation of irradiance the mean value of daylight period was used as external irradiance.

$$E_{av} = \frac{E_o}{(K_a * p * C_b)}(1 - \exp(-K_a * p * C_b)) \tag{2}$$

The biochemical composition was analyzed from the freeze-dried biomass at the end of the semi-continuous culture. Lipids were determined using chloroform:methanol (2:1) (v/v) for the extraction followed by the addition of HCl (0.1 N) and $MgCl_2$ (0.5%) (Kochert 1978). Briefly, centrifugation was carried out for lower phase recovery, and lipid content was determined gravimetrically. The protein content was determined using the modified Lowry method (López et al. 2010). The biomass was hydrolyzed with NaOH 1 N at 95–100 °C for 60 min. To the extract was added Lowry and Folin reagents and incubated for 30 min. The absorbance was measured at 750 nm, and the protein concentration was determined by a calibration curve using bovine serum albumin (BSA) as standard ($R^2 = 0.991$). The total ash content was determined by incinerating a 0.5 g sample in an oven at 550 °C for 6 h. The carbohydrate content of the biomass was determined as the difference from 100% of the proteins, lipids, and ash content. The fatty acids were analyzed from the lyophilized biomass obtained at the end of the semi-continuous study. The fatty acid profile was performed by direct transesterification using hexane, acetyl chloride, and methanol as extraction solvents. Gas chromatography/FID (6890 N Gas Chromatograph, Agilent Technologies, USA) and nonadecanoic acid (C 19:0) as internal standard were used as described by Rodríguez-Ruiz et al. (1998). All

the determinations were performed in triplicate to obtain mean and standard deviation values.

Statistical analysis

DaqFactory 5.0 software (Azeotech Inc., USA) was used to control the reactors. Results are the average of independent repetitions and are expressed as mean ± standard deviation (SD).

Results and discussion

In this study, the growth of the diatom *Nitzschia aff. pellucida* was evaluated at different irradiances. The biomass concentration at different external irradiances (100 to 1000 µmol photons m^{-2} s^{-1}) during the first growth phase (batch mode) is presented in Fig. 1a. The results show an increase in the biomass concentration that is proportional to the increase in the external irradiance. The maximum biomass accumulation (> 1.4 g L^{-1}) was achieved at irradiances ≥ 450 µmol photons m^{-2} s^{-1}, whereas lower biomass concentrations (< 1 g L^{-1}) were found at low irradiances ≤ 200 µmol photons m^{-2} s^{-1}. Culture medium provided contains 247 mg N L^{-1} and 17 mg P L^{-1}, enough to achieve biomass concentrations higher than 2.5 and 1.8 g L^{-1} respectively, thus confirming the excess of nutrient availability. This demonstrated that an increase in external irradiance provided more light

availability, thus promoting biomass accumulation in the *N. aff. pellucida* cultures. Liang et al. (2001) presented similar results, in which the *Nitzschia closterium* biomass concentration increased from 0.49 to 0.72 g L^{-1} with increasing light. Two further studies reported an increase in biomass concentrations in *Chlorella minutissima,* where the biomass content rose from 0.49 to 1.03 g L^{-1} in 7 days when the light increased from 100 to 400 µmol photons m^{-2} s^{-1} (Tang et al. 2011), and in *Nannochloropsis* sp., where the biomass content increased from 2.8 to 3.3 g L^{-1} in 7 days when the light increased from 170 to 700 µmol photons m^{-2} s^{-1} (Pal et al. 2011).

It has been reported that light intensity plays an important role in algal growth and biomass accumulation (Singh and Singh 2015), but some microalgae have different light requirements for these parameters (Khan et al. 2018). In the present study, there were notable differences in the biomass production of *N. aff. pellucida* cultures in response to irradiance (Fig. 1b). The biomass productivity increased proportionally with increased irradiance up to 450 µmol photons m^{-2} s^{-1} in the first days of the culture. The highest value (0.25 g L^{-1} day^{-1}) was achieved at 1000 µmol photons m^{-2} s^{-1}, but this decreased drastically over the culture period. However, at 450 µmol photons m^{-2} s^{-1}, there was an increase in biomass productivity in the final days of the batch. This showed that, over the irradiance limit, the biomass production reduced due to the impact of light on the microalgae cells. The cell growth of *Scenedesmus obliquus*
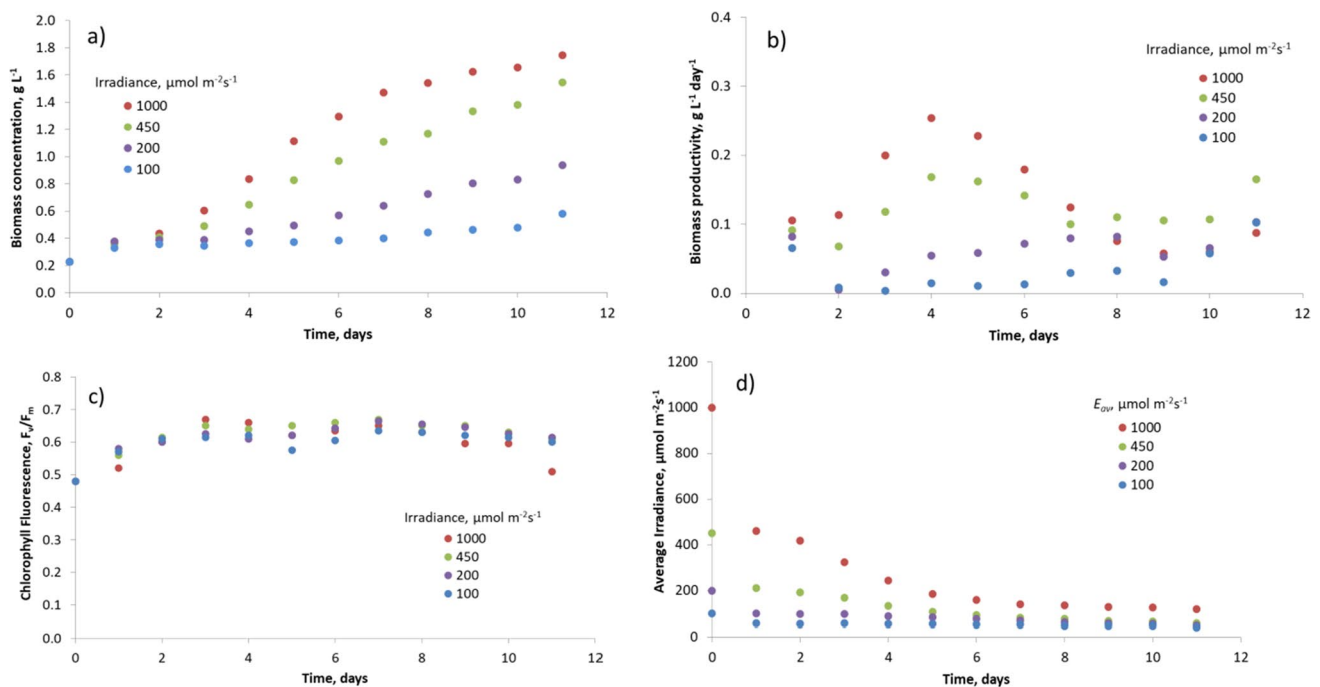

Fig. 1 Influence of external irradiance on **a** biomass concentration, **b** biomass productivity, **c** chlorophyll fluorescence, and **d** average irradiance (E_{av}) in batch cultures of *Nitzschia aff. pellucida*

CNW-N under different irradiances presented a comparable situation; the biomass productivity increased at between 60 and 540 μmol photons m^{-2} s^{-1}, then decreased notably above this irradiance level (Ho et al. 2012). Singh and Singh (2015) found that algal growth could improve with increasing irradiance up to a point, but that excessive illumination would inhibit the biomass production and CO_2 fixation ability; this is recognized as a common photoinhibition effect. Gutierrez-Wing et al. (2014) also suggested that the relationship between irradiance and biomass productivity is complex and not a constant, whereby low light could promote light limitation and excessive light could induce photoinhibition and damage the photosynthetic pigments. These reports support the chlorophyll fluorescence data for *N. aff. pellucida* shown in Fig. 1c, where irradiance influenced the physiological state of this microalga. A decrease in F_v/F_m was observed in *N. aff. pellucida* cultures illuminated at 1000 μmol photons m^{-2} s^{-1}, while cultures exposed to irradiances between 100 and 450 μmol photons m^{-2} s^{-1} presented optimal chlorophyll fluorescence values ($F_v/F_m \geq 0.60$). Similarly, Solovchenko et al. (2008) observed a slight reduction in the F_v/F_m values for *Parietochloris incisa* cultures when the irradiance increased from 35 to 400 μmol photons m^{-2} s^{-1}. An increase in irradiance has been reported to produce a decrease in the photosynthetic pigment content, such as in chlorophyll *a* (Guihéneuf et al. 2009; Guedes et al. 2010). McMinn et al. (2005) found that a decrease in the F_v/F_m values of different algae species was associated with the closing of the reaction centers; this was because of photoinhibitory damage to the D1 proteins of PSII due to increased reactive oxygen production. This explanation does not consider the different kinetics of reactions taking place during activation and deactivation of PSII, thus a deeper analysis of biochemical reactions taking place being needed to confirm this phenomena. Decrease of F_v/F_m is also supported in the work by Ugwu et al. (2007) on *Chlorella sorokiniana*, where the data indicated a decrease in F_v/F_m and biomass productivity for cultures exposed to between 50 and 400 μmol photons m^{-2} s^{-1}, suggesting that a high dissolved oxygen content caused photochemical damage to the photosynthetic apparatus. The production of ROS (reactive oxygen species) depends on the level of excitation pressure absorbed in the antennas in relation to the capacity of the photosynthetic apparatus (electron transport system and enzymatic processes) to absorb these electrons. This contrasts with the variation of average irradiance (E_{av}) during the culture (Fig. 1d). According to these results, the average irradiance is an indicator of light availability, and this decreased with increasing external irradiance as the microalgae started to accumulate biomass.

On the other hand, a relationship was found between the external irradiance and the specific growth rate and average irradiance (E_{av}) inside the cultures. Consistent with the experimental data, the growth rate was a function of the average irradiance inside the cultures, and it fitted a light-dependent model (Fig. 2). Average irradiance in the mean daylight period was used as the most representative variable describing the light availability, more than average irradiance at noon or daylight integral average irradiance, anyway all of them providing a similar explanation of the overall light provisioning. This model was calculated using Eq. 3, considering the maximum growth rate $\mu_{max} = 0.33$ day^{-1}, the saturation constant for irradiance $E_k = 115$ μmol photons m^{-2} s^{-1}, and the shape parameter $n = 2$. The model fitted a hyperbolic function between the variation in the growth rate and the average irradiance measured during batch cultures. Our results indicate that the *N. aff. pellucida* growth rate was a function of increasing irradiance through a hyperbolic relationship, as established by Molina Grima et al. (1994) (Eq. 3). Hall et al. (2003) observed a hyperbolic relationship between the growth rate and the average irradiance in *Phaeodactylum tricornutum* cultures, as the biomass productivity increased with average irradiance to a maximum value and then dropped as the irradiance surpassed that level. This was also observed in our study, where biomass productivity decreased at an external irradiance of 1000 μmol photons m^{-2} s^{-1}. Bouterfas et al. (2002) studied the influence of irradiance on growth rate in three microalgae species, finding that their growth rate increased with irradiance up to a maximum point but then decreased after optimum irradiance was achieved.

$$\mu = \frac{\mu_{max} \cdot E_{av}^n}{E_k^n + E_{av}^n} \tag{3}$$

The experimental data from the growth model suggested the cultures were light dependent; these data were used to design semi-continuous experiments which considered
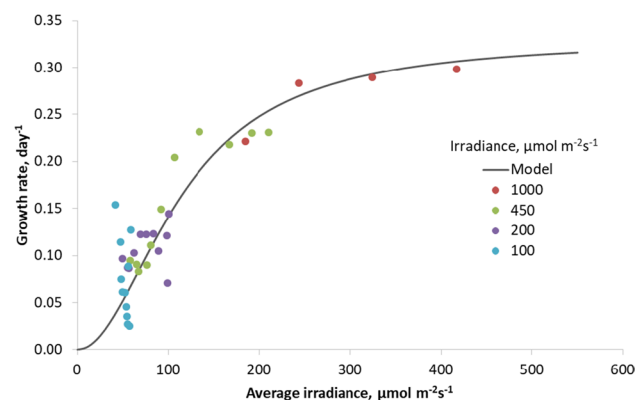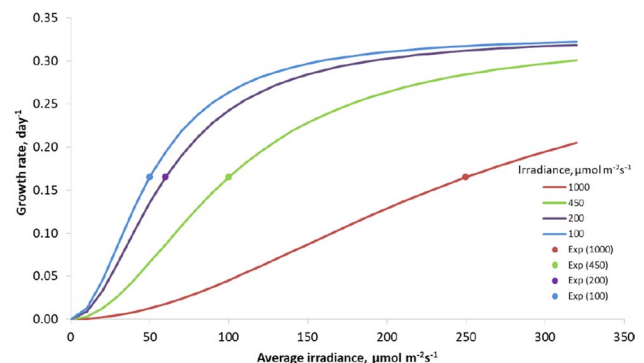

Fig. 2 Variation in the specific growth rate as a function of the average irradiance inside the culture and the external irradiance. The points represent experimental values and the line corresponds to the model

Table 1 Culture parameters in semi-continuous experiments at different irradiances (mean ± SD, $n = 3$)

Irradiance, μmol photons m^{-2} s^{-1}	D, day^{-1}	E_{av}, μmol photons m^{-2} s^{-1}	C_b, g L^{-1}	F_v/F_m	P_b, g L^{-1} day^{-1}
1000	0.165	254.13 ± 30.79	0.80 ± 0.10	0.52 ± 0.01	0.13 ± 0.01
450	0.165	97.57 ± 9.31	0.92 ± 0.09	0.61 ± 0.01	0.15 ± 0.01
200	0.165	55.23 ± 2.93	0.83 ± 0.05	0.60 ± 0.02	0.14 ± 0.01
100	0.165	45.36 ± 0.94	0.51 ± 0.02	0.61 ± 0.01	0.08 ± 0.0

the optimal dilution (D) to be 0.165 day^{-1} for irradiances between 100 and 1000 μmol photons m^{-2} s^{-1}. The results showed differences in the biomass concentration and productivity that were not proportional to the irradiance increase, contrary to the batch experiments (Table 1). The maximum biomass concentration (0.92 g L^{-1}) and biomass productivity (0.15 g L^{-1} day^{-1}) were detected at 450 μmol photons m^{-2} s^{-1}, and these parameters decreased at other irradiances. At 200 μmol photons m^{-2} s^{-1}, the biomass concentration dropped to ~ 0.1 g L^{-1}, but the biomass productivity (0.14 g L^{-1} day^{-1}) was similar to that achieved at 450 μmol photons m^{-2} s^{-1}. Although an irradiance of 1000 μmol photons m^{-2} s^{-1} produced results comparable to other treatments, the biomass productivity tended to decrease due to high irradiance conditions. However, the chlorophyll fluorescence data were constant ($F_v/F_m \geq 0.60$) at irradiances ≤ 450 μmol photons m^{-2} s^{-1}, but they decreased to 0.52 at 1000 μmol photons m^{-2} s^{-1}. Jebali et al. (2019) reported a decrease in F_v/F_m (as low as 0.57) for *Nitzschia* sp. associated with increased dilution allowing more light into the cultures, resulting in cell photoinhibition. The F_v/F_m ratio decreased in *Nitzschia alexandrina* cultures from 100 to 400 μmol photons m^{-2} s^{-1} (Cointet et al. 2019). These data were useful in supporting our previous experimental information and confirmed the negative impact of high irradiance on photosynthetic processes stimulating stress over time (Béchet et al. 2013).

The average irradiance (E_{av}) did not remain constant in the cultures at different irradiances (Fig. 3). In this situation, the average irradiance must have been constant with irradiance increasing at the same dilution due to the growth-irradiance relationship present in the batch cultures. Similar average irradiance values were identified at irradiances ≤ 200 μmol m^{-2} s^{-1}, but at irradiances ≥ 450 μmol photons m^{-2} s^{-1}, the average irradiance values changed. When the cultures were exposed to an external irradiance between 450 and 1000 μmol photons m^{-2} s^{-1}, they needed more average irradiance, and this led to photoinhibition. No other factors such as nutrient (C, N, P) limitation can justify this phenomena; thus, only higher costs of repair of the photosynthetic apparatus at the highest irradiance levels would explain the impact of high light on cell growth at these conditions. Photoinhibition occurred in these cultures because the irradiance was
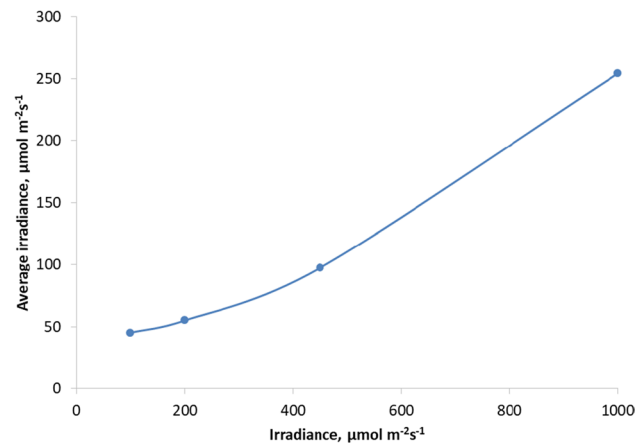
Fig. 3 Average irradiance (E_{av}) as a function of the external irradiance in semi-continuous cultures

Fig. 4 Variation in the growth rate as a function of the average irradiance and different saturation constant values (E_k). Lines represent the growth model, and points represent the experimental data

above the saturation point, thus inhibiting cell growth (Lee et al. 2015).

Based on these results, a new growth model was proposed that considered the different saturation constant (E_k) and E_{av} values. The growth rate model was adjusted to different E_k for each irradiance (Fig. 4). In this diagram, the lines represent the growth-average irradiance model, and the points represent the experimental E_{av} data. The red line (1000 μmol photons m^{-2} s^{-1}) and green line (450 μmol photons m^{-2} s^{-1}) confirm that these treatments were photoinhibited. However,

the purple (200 μmol photons m^{-2} s^{-1}) and blue lines (100 μmol photons m^{-2} s^{-1}) show similar stable growth results in the semi-continuous cultures and represent a hyperbolic relationship between the variables, as indicated in Fig. 2. This supports the fact that the E_k parameter changed with the E_o data and that irradiances ≥ 450 μmol photons m^{-2} s^{-1} could affect the metabolism of *N. aff. pellucida*, whereas irradiances ≤ 200 μmol photons m^{-2} s^{-1} proved to be better for culture growth. Researchers have indicated that a hyperbolic function is useful for describing growth in a specific light regime, but, at high irradiances, growth might decrease, and so the hyperbolic function cannot be extrapolated (Gutierrez-Wing et al. 2014). As shown in Fig. 4, hyperbolic functions were presented at irradiances between 100 and 200 μmol photons m^{-2} s^{-1}. Bill et al. (2012) found a link between growth and irradiance in *Pseudo-nitzschia australis* and *Pseudo-nitzschia turgidula* cultures up to a saturation point of 200 μmol photons m^{-2} s^{-1} and 100 μmol photons m^{-2} s^{-1}, respectively, for each species. However, *Skeletonema costatum, Olisthodiscus luteus*, and *Gonyaulax tamarensis* cultures showed a direct growth-irradiance relationship between 5 and 450 μmol photons m^{-2} s^{-1} (Langdon 1987). According to Béchet et al. (2013), the photosynthetic processes depend on irradiance at low irradiances, and this might be because they are associated with the photon capture rate. From our model, we showed the variation in growth related with E_{av} at different E_k values and established a global growth model. Various works have presented E_k data variations related to irradiance. For example, an irradiance increase from 105 to 1100 μmol photons m^{-2} s^{-1} was observed with an increase in the E_k data from 140 to 415 μmol photons m^{-2} s^{-1} in *Nitzschia pungens f. multiseries* cultures (Pan et al. 1991). Cruz and Serôdio (2008) reported an increase in E_k values from 44.3 to 162.4 μmol photons m^{-2} s^{-1} in *Nitzschia palea* between 20 and 400 μmol m^{-2} s^{-1}. As with the photoinhibition process indicated in some cultures of *N. aff. pellucida*, the experimental E_k values and external irradiances were adjusted to an exponential model ($y = 42.558e^{0.0018x}$), as shown in Fig. 5.

Analysis of the biochemical composition

The analysis of the biochemical composition of *N. aff. pellucida* was carried out on biomass obtained at steady state in semi-continuous mode using different irradiances (Table 2). The results show an increase in the lipid portions from 10 to 19% as the irradiance increases from 100 to 1000 μmol photons m^{-2} s^{-1}. Liang et al. (2001) reported increases in total lipids for *Nitzschia closterium* (4.1 to 5.4%) and for *P. tricornutum* (3.6 to 4.3%), which are proportional to the increase in irradiance. Orcutt and Patterson (1974) reported an increase
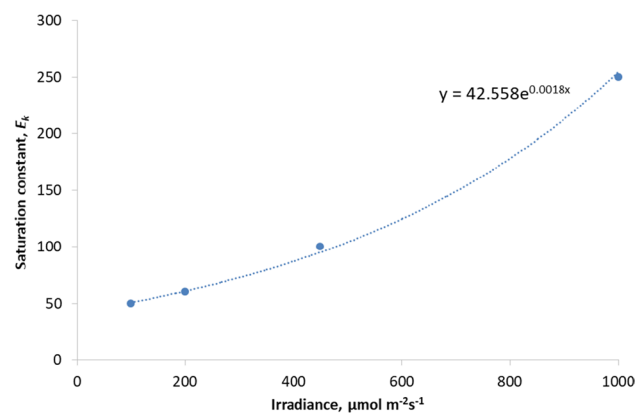
Fig. 5 Saturation constant for irradiance (E_k) related to the external irradiance

of 2% in the total lipid content between a high light and low light intensity in cultures of *N. closterium*. The effect of light intensity on lipid biosynthesis is positive because of the proportional increase in lipid content with irradiance (Carvalho and Malcata 2005). Likewise, in our study, the increase in the lipid free-ash content supports the impact of increasing irradiance on lipid production. According to Khotimchenko and Yakovleva (2005), marine microalgae can acclimatize to light variation by changing the content of their structural and storage substances, such as lipids. In contrast, the protein content in *N. aff. pellucida* cultures decreased with increasing irradiance. The highest protein content (11%) was found at the low irradiance of 100 μmol photons m^{-2} s^{-1}, while at high irradiance (1000 μmol photons m^{-2} s^{-1}), the protein content reduced to ~ 9%.

These results support the fact that cultures were photoinhibited at 1000 μmol photons m^{-2} s^{-1}. Similar data were published on *Scenedesmus* sp. 11–1 cultures, in which the highest protein contents were observed at the lowest light intensities, while the highest lipid contents were obtained at the highest light intensities (Liu et al. 2012). In *P. tricornutum* cultures, the protein content decreased, and the lipid content increased with irradiance, indicating that the microalgae produced storage products such as lipids (KaiXian and Borowitzka 1993). Grima et al. (1994) noted an inverse relationship between the protein and lipid contents for *Isochrysis galbana* as the irradiance increased, but a proportional increase in the lipid and protein contents for *Tetraselmis* sp.. The last point, regarding the effect of light on the algal lipid and protein contents being variable, might be because the effect is species-specific (Chrismadha and Borowitzka 1994), given that some diatoms, such as *C. gracilis* (10.8 to 7%) and *C. fusiformis* (15.9 to 13%), showed a slight decrease in their lipid contents as irradiance increased (Liang et al. 2001). Nzayisenga et al. (2020) stated that the increase in the amount of lipids was directly related to the

Table 2 Biochemical composition (%) of the *Nitzschia aff. pellucida* biomass (mean $\pm$ SD, $n = 3$)

Treatment, μmol photons m^{-2} s^{-1}	Lipids, %	Proteins, %	Carbohydrates, %	Lipid/carbo-hydrate	Lipids free ash, %
1000	19.3 ± 0.5	8.9 ± 0.5	41.7 ± 0.7	0.46	30.7
450	18.7 ± 0.5	7.7 ± 0.5	44.5 ± 0.7	0.42	27.4
200	12.6 ± 0.5	8.8 ± 0.5	34.9 ± 0.7	0.36	27.8
100	10.6 ± 0.5	11.0 ± 0.5	39.7 ± 0.7	0.27	17.8

decrease in the amount of proteins (based on their results), concluding that lipid synthesis could be dependent on protein degradation, or that it inhibited protein synthesis due to increasing irradiance.

The protein content and growth model results highlight the effect of high irradiance on cellular metabolism degradation in *N. aff. pellucida*. The highest carbohydrate contents ($> 40\%$) were reported at irradiances above 450 μmol photons m^{-2} s^{-1}, whereas the carbohydrate portions ($> 34\%$) were reported at low irradiances between 100 and 200 μmol photons m^{-2} s^{-1}. According to Pal et al. (2011), under unfavorable conditions, microalgal growth is limited, photosynthetic activity decreases, and the microalgae increase their accumulation of energy-rich storage products such as lipids and/or carbohydrates. This fact was observed in our work on *N aff. pellucida* cultures, with the highest lipid and carbohydrate levels reported at irradiances above 450 μmol photons m^{-2} s^{-1}. Irradiances ≥ 450 μmol photons m^{-2} s^{-1} were found to promote photoinhibition on the cultures, although they led to lipid and carbohydrate accumulation. As with *P. tricornutum* cultures, the carbohydrate and lipid contents increased with increasing irradiance (Chrismadha and

Borowitzka 1994). However, there is a relationship between irradiance and the lipid and carbohydrate ratios. These ratios increased from 0.27 to 0.46 between 100 and 1000 μmol photons m^{-2} s^{-1}. The lipid/carbohydrate content and ratio are extremely important for liquid biofuel production (Ho et al. 2012; Chiu et al. 2017).

Fatty acid analysis

Table 3 summarizes the dry weight percentages of fatty acids identified at different irradiances. A higher production of saturated (SFA) and monounsaturated (MUFA) fatty acids was observed in the *N. aff. pellucida* cultures compared to the polyunsaturated (PUFA) fatty acid portions. Several *Scenedesmus* strains presented higher SFA and MUFA portions than PUFA (Ördög et al. 2013). In our study, we found a variable relationship for some fatty acids as a function of the different irradiances. The SFA portions varied between treatments, with the highest amounts at 450 μmol photons m^{-2} s^{-1}, C16:0 ($\sim 63\%$) being the most abundant fatty acid. A study on *Chlorella minutissima* found that the

Table 3 Fatty acids present in the *Nitzschia aff. pellucida* biomass according to irradiance (mean $\pm$ SD, $n = 3$)

Fatty acids as percentage of total fatty acids				
	1000 μmol photons m^{-2} s^{-1}	450 μmol photons m^{-2} s^{-1}	200 μmol photons m^{-2} s^{-1}	100 μmol photons m^{-2} s^{-1}
14:0	13.36 ± 0.50	14.57 ± 0.05	12.26 ± 0.07	14.04 ± 0.23
16:0	48.95 ± 9.78	62.95 ± 0.13	43.75 ± 0.45	50.31 ± 0.20
16:1 n-7	4.26 ± 5.74	3.68 ± 0.01	26.20 ± 0.22	14.95 ± 0.09
18:1 n-9	11.11 ± 0.99	10.91 ± 0.96	7.43 ± 0.01	14.75 ± 0.31
18:2 n-6	5.78 ± 0.73	7.27 ± 1.12	3.91 ± 0.16	6.40 ± 0.45
18:3 n-3	0.36 ± 0.09	0.61 ± 0.02	0.92 ± 0.02	n.d
20:4 n-6	n.d	n.d	4.82 ± 0.10	n.d
20:5 n-3	n.d	n.d	1.41 ± 1.00	n.d
ΣSFA	62.31	77.52	56.01	64.35
ΣMUFA	15.37	14.60	33.63	29.71
ΣPUFA	6.15	7.89	11.07	6.40
Σn-6	5.8	7.3	8.7	6.4
Σn-3	0.4	0.6	2.3	-

n.d. not detected, *SFA* saturated fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids

SFA fraction increased with light intensity between 100 and 400 μmol photons m^{-2} s^{-1} (~22 to ~31%) and that this was related to the increase in C16:0 at high light intensity (Tang et al. 2011). Orcutt and Patterson (1974) reported on *N. closterium* cultures, where the saturated and C16:0 fatty acids increased at high light intensity rather than at low light intensity. In our *N. aff. pellucida* cultures, there was a predominance of palmitic acid (C16:0) over palmitoleic acid (C16:1 n-7) at all the irradiance levels; this contrasts with other studies that reported a predominance of C16:1 n-7 over C16:0 fatty acids in *Nitzschia* species (Orcutt and Patterson 1974; Wen and Chen 2001).

The highest MUFA (~33%) and PUFA (~11%) contents were detected at a low irradiance (200 μmol photons m^{-2} s^{-1}), and these were increased compared to high irradiance (1000 μmol photons m^{-2} s^{-1}). The highest unsaturated fatty acid contents were identified at ≤620 μmol photons m^{-2} s^{-1} for *Isochrysis* sp. and ≤490 μmol photons m^{-2} s^{-1} for *N. oculata* (Renaud et al. 1991). High PUFA levels were observed at a low light intensity, showing that the biochemical composition of the chloroplasts was adapting to the low irradiances by increasing the PUFA content (Brown et al. 1993). This physiological acclimation could be a regulating mechanism to establish membrane integrity (Mock and Kroon 2002). The increase in the PUFA portions at 200 μmol photons m^{-2} s^{-1} in *N. aff. pellucida* led to a high accumulation of n-3 and n-6 fatty acids and to the production of EPA (20:5 n-3) and ARA (20:4 n-6), which were not detected at other irradiances. This reflects the physiological impact of irradiance on the production of biologically interesting fatty acids in cultures that are not photoinhibited. Orcutt and Patterson (1974) also found more EPA produced at low light intensities in *N. closterium* cultures. Similar results were found in *N. grossestriata* and *N. panduriformis* cultures, where there was more n-3 PUFA and EPA accumulation in those microalgae grown in an incubator under low intensity light compared to summer or winter greenhouse cultures grown under high intensity light (Chen 2012). Khotimchenko and Yakovleva (2005) reported that the exposure of algae to low light increased the EPA content yet slightly reduced the ARA content. At 170 μmol photons m^{-2} s^{-1}, *Nannochloropsis* sp. cells contained higher EPA levels (17.8%) than at 700 μmol photons m^{-2} s^{-1} (10.8%), indicating that under stressful conditions, LC-PUFAs such as EPA, reduced, suggesting more efficient light use at low levels (Pal et al. 2011). Nevertheless, the highest α-linolenic fatty acid (18:3 n-3) content (0.92%) was found at 200 μmol photons m^{-2} s^{-1} rather than at 450 μmol photons m^{-2} s^{-1} (0.61%) or 1000 μmol photons m^{-2} s^{-1} (0.36%) in the *N. aff. pellucida* cultures. According to Bird et al. (2018), 18:3 n-3 is an essential fatty acid that acts as a precursor for the synthesis of long-chain fatty acids like EPA. Finding the highest amount of 18:3 n-3 at 200 μmol m^{-2} s^{-1} might explain the exclusive presence of EPA and ARA in this treatment and

not in the others. There were similar results for *N. closterium* cultures, where the amount of 18:3 n-3 and 20:5 n-3 fatty acids increased as the light intensity decreased (Liang et al. 2001). The effect of reduced light intensity led to an increase in the 18:3 n-3 and 20:5 n-3 portions compared to a high light intensity at low phosphorus concentrations (Hill et al. 2011). Other results obtained for *P. lutheri* cultures, grown using different carbon sources, showed increasing 18:3 n-3 and EPA fatty acid contents with decreasing light intensity (340 to 100 μmol photons m^{-2} s^{-1}) (Guihéneuf et al. 2009).

Our results with *N. aff. pellucida* support the idea that the optimum light intensity for most microalgae is located between 200 and 400 μmol photons m^{-2} s^{-1} (Radakovits et al. 2010). This would explain the differences in the growth model, biomass accumulation, biochemical composition, and fatty acid profile for treatments at high (1000 μmol photons m^{-2} s^{-1}) and low (100 μmol photons m^{-2} s^{-1}) irradiances compared to other irradiances. As in our study, some *Nitzschia* species, such as *Nitzschia cylindrus* and *Nitzschia kerguelensis*, exhibited optimal growth at 200 μmol photons m^{-2} s^{-1}, while *Nitzschia turgiduloides* did so at 155 μmol photons m^{-2} s^{-1} (Fiala and Oriol 1990). Bouterfas et al. (2002) reported on the appropriate light intensity for *Selenastrum minutum* (190–400 μmol photons m^{-2} s^{-1}) and *Coelastrum microporum f. astroidea* (125–400 μmol photons m^{-2} s^{-1}) in the 15 to 35 °C temperature range. Likewise, Imaizumi et al. (2014) reported that the optimum light intensity for *Chlorella zofingiensis* was between 250 and 1000 μmol photons m^{-2} s^{-1}. The light intensities required by microalgae to grow vary greatly (Han et al. 2015). Some researchers stated that low light could not penetrate the dark areas formed by the cells and the medium and that this might limit microalgae cell proliferation (Han et al. 2015), while others stated that high light intensities are harmful to algal photosynthetic systems (Brown et al. 1993).

Conclusions

A direct relationship between growth and irradiance was shown, a hyperbolic relationship being found. It was shown that irradiance affected the growth rate, biomass accumulation and productivity, and chlorophyll fluorescence. Variations in irradiance resulted in different saturation constants (E_k); these were fitted to an exponential model for external irradiance and were included in the growth model. Variations in light availability affected the biochemical composition. It was determined that increased irradiance in the cultures had a positive impact on the accumulation of lipids and carbohydrates, whereas the highest protein content was reported at low irradiance. Irradiances ≥450 μmol m^{-2} s^{-1} affected the microalga's metabolism and caused photoinhibition. As the

irradiance values decreased, the MUFA, n-6, and n-3 portions increased, with EPA and ARA fatty acids also being reported. This, together with the growth model results, suggested that an irradiance of 200 μmol m^{-2} s^{-1} was best for the *Nitzschia aff. pellucida* culture. It was also demonstrated that light availability served as an important "nutrient" for the microalgae culture, especially in terms of biomass production, biochemical components, and high-value fatty acids. This information is highly useful to design industrial processes based on *N. aff. pellucida,* especially on the aspects related with the design of the reactor.

Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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