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Modelling *Nannochloropsis gaditana* Growth in Reactors with Different Geometries, Determination of Kinetic Parameters and Biochemical Analysis in Response to Light Intensity

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Abstract: Microalgae are unicellular and photosynthetic microorganisms which grow thanks to inorganic salts, CO_2 and light, and find applications in several fields thanks to their variety. The industrial application of microalgae has not often been fully exploited because of a lack of information about how microalgae respond to inputs and to different growth environments. In the present work a model able to predict the microalgae growth in reactors with different geometries was developed. We combined a Monod-like model for the specific growth rate with the Lambert-Beer law of homogeneous light distribution in thick photobioreactors. Kinetic parameters related to the cultivation of the microalga *Nannochloropsis gaditana* were obtained, for the first time through batch cultivation under different photon flux densities inside a quasi-isoactinic photobioreactor, in order to obtain a practically homogeneous light distribution. The maximum specific growth rate and saturation constant resulted, respectively as μ_{max} = 0.0256 h^{-1} and I_k = 15.28 μE $s^{-1}m^{-2}$. These parameters were applied to the model to obtain data on microalgae growth in different geometries. Model simulation results are presented and discussed. Furthermore, biochemical analysis was performed on the biomass obtained at the end of each batch cultivation, grown both under different light intensities and in reactors with different configurations. Results indicated that lipid content increases with increasing average photon flux density. The fatty acid and carotenoids profiles markedly shift when the average light intensity varies: the PUFA content decreases and the SFA content increases when the average light intensity rises, and an accumulation of carotenoids at lower photon flux densities is observed. In conclusion, the model resulted in a useful tool, able to predict the growth of the microalga *Nannochloropsis gaditana* in reactors with different configurations.

Keywords: *Nannochloropsis gaditana*; photobioreactor modelling; growth model; lipids; kinetic parameters

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1. Introduction

Microalgae are unicellular or pluricellular microscopic and photosynthetic microorganisms that may grow by employing solar energy in a more efficient way compared to land plants. They can be employed as a source of food, [1], in pharmaceuticals and cosmetics [2], in the bioremediation of wastewaters [3] and in general as a source of biomass.

Microalgae cultivation may occur outdoors in open ponds. Among these, raceway ponds are the most commonly employed for mass cultivation [4], as they are generally cheap (production costs for microalgal biomass in a raceway can be estimated at around 1.6–1.8 € kg^{-1} [5]), but have several disadvantages. In fact, in an outdoor cultivation facility, there is weak control of the operating parameters and risk of contamination, leading to poor mass production. Alternatively, microalgae can be grown indoors inside photobioreactors. Indoor photobioreactors are generally more expensive but ensure better control of growing conditions and, consequently, better productivity.

Several studies of the design and optimization of photobioreactors to maximize biomass productivity are available [6,7]. However, there are still several doubts about technical issues of concern and the economics of large scale microalgal cultivation. Most of the criticisms depend on the lack of reliable and reproducible parameters that strongly affect growth, productivity and, consequently, the industrial scale-up of the systems. Therefore, microalgae are usually not cultivated in their optimal conditions and this results in a gap between process operation and responses to the culture inputs, especially in an industrial environment in which productivity is largely lower than that which might be expected from lab experiments.

The light available to the cells is the main parameter in optimizing microalgal cultivation [8].

Several models describing microalgae response to light have been proposed in the literature, in some cases including investigation of mass transfer [9,10]. Béchet et al. [11] suggested classification into three categories according to how the photosynthetic productivity is computed. One of the problems is that, in order to have strict control of the kinetic parameters, one should avoid self-shading effects that cause a non-homogeneous light distribution inside the reactor.

To avoid this problem, a specific kind of reactor that allows a homogeneous light distribution inside should be employed to obtain the kinetic parameters. We refer to these kinds of reactors as quasi-isoactinic, meaning that the light distribution inside them is almost homogeneous [12]. When employing these reactors, it is fully possible to analyze the effect of a specific kind of illumination on a culture. This work proposes a Bechet type II photosynthetic model, able to predict the biomass productivity in reactors with different depths and geometries. In particular, a strain of *Nannochloropsis gaditana*, known for its lipid content, was employed for the experimental validation of the model. The kinetic parameters were obtained, for the first time, inside a "quasi-isoactinic" photobioreactor, in order to ensure an almost homogeneous light distribution. Then microalgae *Nannochloropsis gaditana* were grown in photobioreactors with three different geometries to obtain experimental data to be compared with the model prediction.

Model simulations in batch reactors are presented and discussed. Furthermore, biochemical analysis of proteins, carbohydrates, lipids, fatty acid characterization and carotenoids was performed on the biomass obtained through all the batch cultivations realized, and the results represent a complete characterization of *Nannochloropsis gaditana* grown under different average light intensities.

2. Mathematical Modelling

In microalgal research, several light models have been proposed that are able to describe the photosynthetic behavior of microalgae growing under different conditions. Béchet et al. [11] proposed classification into three types. Type I models are able to predict the rate of photosynthesis as a function of the incident or average light intensity, while type II models compute productivity as the sum of local productivities. Type III models consider, instead, the impacts of light gradients and of short light cycles. Furthermore, the distribution of light may be described in different ways, for example, through the employment of the Lambert Beer model, the two flux model or Hyperbolic Beer–Lambert law [13].

In this work a Bechet's type II light model was used to describe microalgal growth in photobioreactors with different geometries enlightened from one-side and predict the growth in reactors with variable depths. The growth is related to the light distribution inside the reactor, which takes into account the light attenuation according to the Lambert-Beer model, widely used to describe the light attenuation pattern in algal culture broth, especially in the presence of low-cell densities [13]. When using this equation, the hypothesis is that the medium inside the reactor is isotropic and that there are no scattering phenomena, which is a good approximation of reality. Furthermore, if scattering is taken into account, even smaller deviations from uniformity are predicted, with respect to the limiting "zero reflectance" case we propose [12]. Considering that the cell concentrations experimentally

obtained are below 2 g L^{-1}, the use of the Lambert-Beer model was considered to be acceptable [13].

The Lambert-Beer law describes an exponential decay of light intensity from the external surface of the cultivation system, as described in the following equation [11]:

$$I = I_0 \, e^{-k_a X z} \tag{1}$$

where I is the local light intensity of light, z is the distance of the external surface to the considered position in a flat geometry, I_0 is the intensity of the incident light, k_a is the attenuation constant and X the cellular concentration.

The attenuation constant k_a can be estimated as the ratio between the area of the cell capturing light (A_{capt}) and the mass of the cell (m_{cell}) and can be simply estimated as:

$$k_a = \frac{3}{2} \frac{d_{capt}^2}{\rho_{cell} d_{cell}^3} \tag{2}$$

where ρ_{cell} and d_{cell} are respectively the density and the diameter of a microalgal cell and d_{capt} is the diameter of the transversal section of light capture. According to the proposed model, the medium is ideally mixed, the biomass concentration is uniform inside the reactor and all the nutrients are in excess.

The growth rate μ is then expressed according to a Monod-like model [11] considering light as a substrate for algal photosynthesis, as expressed by the following expression:

$$\mu = \frac{\mu_{max} \, I}{I_K + I} \tag{3}$$

where μ is the specific growth rate, μ_{max} is the maximum specific growth rate and I_K is the light saturation constant. It is worth noting that the range of light intensities used in this work is far away from photo-inhibition phenomena [14], so that Monod's law can be applied without any limitation.

2.1. Flat-Plate Geometry

The model was first developed to describe photobioreactors with flat-plate geometry and different reactor depths, enlightened from one side only and covered with a black panel on the opposite side, as depicted in Figure 1.

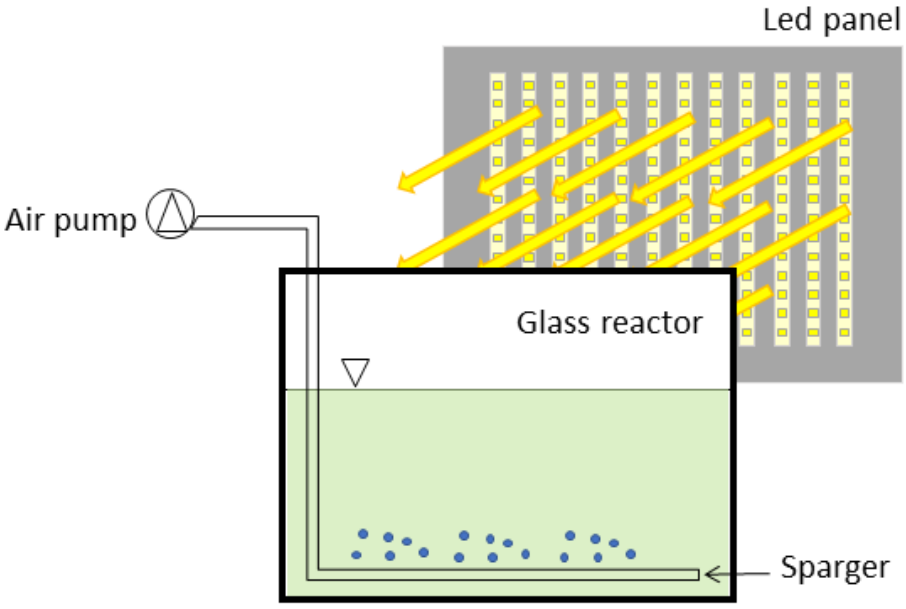

Figure 1. General scheme of the flat reactors employed in this work.

The experiments were conducted in batch mode. This means that illumination inside the PBR decreased over time as biomass concentration increased. The mass balance on the microalgal biomass inside the flat-plate reactor with volume V, concentration $X(t)$, enlightened surface A and depth L, in the transitory period can be expressed as:

$$V\frac{dX}{dt} = \int_0^L r_X A dz \tag{4}$$

where r_X is the net biomass generation rate on volume unit. By expressing the growth rate μ by means of a Monod-like equation (Equation (3)), r_X may be given by the following expression:

$$r_X = \left(\mu_{max}\frac{I}{I + I_K} - k_d\right)X \tag{5}$$

where k_d is the constant of cellular death. By substituting for I the Lambert-Beer law (Equation (1)) and by combining the Equation (4) with Equation (5) one obtains:

$$V\frac{dX}{dt} = \int_0^L \left(\mu_{max}\frac{I_0 e^{-k_a X z}}{I_0 e^{-k_a X z} + I_K} - k_d\right)X A dz \tag{6}$$

And by integrating and dividing by V, one obtains:

$$\frac{dX}{dt} = \mu_{max}X - \frac{\mu_{max}}{k_a L}\ln\left(\frac{I_K}{I_0}e^{k_a X L} + 1\right) + \frac{\mu_{max}}{k_a L}\ln\left(\frac{I_K}{I_0} + 1\right) - k_d X \tag{7}$$

The obtained differential equation can be numerically integrated to return the variation of X in time.

2.2. Annular Geometry

The model was then modified to model an annular column photobioreactor [15,16] enlightened by a central illumination lamp and placed in a chamber closed with a black cloth as depicted in Figure 2.

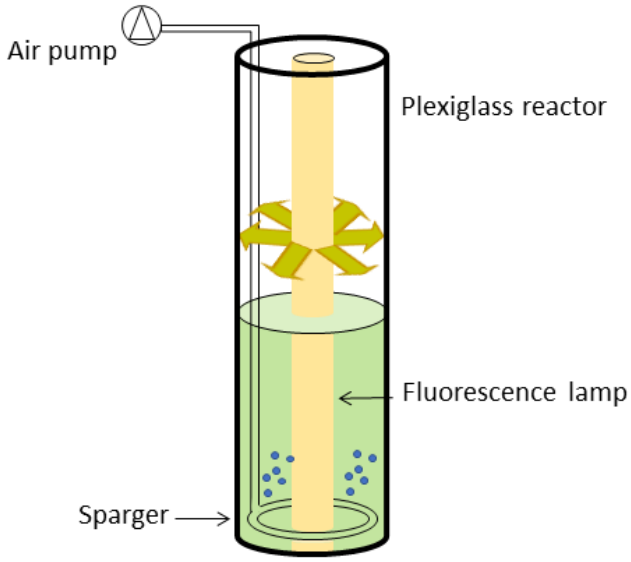

Figure 2. Schematic sketch of the annular photobioreactor employed in this work.

In this case, the mass balance during the transitory written for this geometry is more complex than that described in the previous paragraph, since it considers two contributions to light intensity attenuation:

(1) the fact that the cell concentration increases with time, with consequent mutual shading effects;

(2) the fact that, in the annular geometry, even in the absence of self-shading effects ($X = 0$), the light intensity is inversely proportional to the radius, as the transversal incident surface increases when radius increases. In this case the expression for the Lambert-Beer law can be written as:

$$I(r) = I_0 \frac{R_0}{r} e^{-k_a X(r-R_0)} \quad (8)$$

where R_0 is the internal radius of the annular section and r is the generic radius in the annular section. Based on these considerations, by substituting Equation (8) in Equation (5), and carrying out the mass balance on the microalgal biomass within an annular section with internal radius R_0 and external radius R_1, the result is:

$$\frac{dX}{dt} = \frac{1}{V} \int_{R_0}^{R_1} \left(\mu_{max} \frac{I_0 \frac{R_0}{r} e^{-k_a X(r-R_0)}}{I_0 \frac{R_0}{r} e^{-k_a X(r-R_0)} + I_k} - k_d \right) X 2\pi h r dr \quad (9)$$

where h is the height of the reactor.

The resolution of this integral is neither immediate nor traceable to a remarkable integral. Therefore, a numerical method of solving it was used, i.e., the application of Composite Simpson's 1/3 rule.

3. Materials and Methods

3.1. Quasi-Isoactinic Photobioreactor

To obtain the kinetic parameters, μ_{max} and I_k, of the species *Nannochloropsis gaditana* a quasi-isoactinic flat-plate photobioreactor, i.e., a reactor in which the local volumetric rate of photon absorption (LVRPA) can be considered uniform to a satisfactory extent, was employed [12]. This can be obtained thanks to a light superposition effect and to the very small thickness of the reactor. In this way, the specific growth rate μ will be almost constant in each part of the photobioreactor and may be expressed by the simple equation:

$$\mu = \frac{\mu_{max}\ I_0}{I_K + I_0} \quad (10)$$

where I_0 is the incident light intensity, almost constant inside the quasi-isoactinic photobioreactor, and μ_{max} and I_k are the kinetic parameters.

The quasi-isoactinic photobioreactor has a height of 200 mm, a length of 160 mm, a depth of 4 mm and a volume of 130 mL. The reactor is enlightened from two sides by two identical home-made LED panels with adjustable light intensity. Each panel is realized with LED strips (20 W/m, 24 V, IP20, Elcart), regulated through an Arduino controller. The reactor is insufflated through a micro-pored sparger by an air pump (AP 98-02, Jeneca, Huanggang, China) with a microfiltered (0.2 µm) air flow of 0.7 L min^{-1}. The system is placed inside an incubator (FTD 100, ISCO, Milan, Italy) to regulate the temperature at 25 °C.

In this way, an almost perfect light distribution is obtained, thanks to a superposition effect and to the very small thickness of the reactor. This is the most suitable way to assess the effects of a light feature to microalgal cultures. To the best of the authors' knowledge, this is the first time that the kinetic parameters (μ_{max} and I_k) of *Nannochloropsis gaditana* are obtained in a quasi-isoactinic reactor.

3.2. Flat-Plate Photobioreactors

In order to verify the goodness of the model in response to different reactor depths, another two glass flat-plate photobioreactors were employed, with a height of 105 mm, a length of 190 mm and two different depths: 15 mm with a volume of 300 mL, and 30 mm with a volume of 600 mL. The reactors are enlightened from one side only, by a single LED panel with adjustable light intensity. A black panel was put on the other side to avoid light reflection phenomena. In this case, the reactor is also insufflated through a micro-pored sparger, placed along the reactor bottom, by an air pump (AP 98-02, Jeneca, Huanggang,

China) with a microfiltered (0.2 μm) air flow of 0.7 L min^{-1} and is placed inside an incubator (FTD 100, ISCO, Milan, Italy) to maintain a fixed temperature of 25 °C. A scheme of the system is shown in Figure 1. During the cultivation inside flat photobioreactors, the cultures were periodically mixed in order to further optimize the gas-liquid mass transfer.

3.3. Annular Column Photobioreactor

Annular column photobioreactors (AC-PBR) are widely used for microalgae growth in aquacultures thanks to their high light use efficiency [15,16]. The annular photobioreactor employed for the cultivation of *Nannochloropsis gaditana* consisted of two cylinders arranged annularly made of transparent plexiglass, with a height of 425 mm, external diameter of 125 mm, internal diameter of 54 mm and an employed volume of about 4 L. The annular interspace (photo-stage) for microalgae culturing was 35.5 mm. The central illumination 'finger' was placed in the center, inside the inner cylinder. This was made of a fluorescence lamp (Neon Tube T8 G13 15 W 6500 K, Duralamp 1586T) powered by an electronic ballast (Tronic). The light intensity supplied by the lamp is fixed at 210 μE $s^{-1}m^{-2}$. The annular interspace was insufflated through a micro-pored sparger by an air pump (AP 98-02, Jeneca, Huanggang, China) with a microfiltered (0.2 μm) air flow of 0.7 L min^{-1}. The reactor was placed on a lab bench, with controlled ambient temperature (25 °C) and covered externally by a black cloth to be sure that the only light source was the internal led lamp. The entire system is represented in Figure 2.

3.4. Algal Growth

Microalga *Nannochloropsis gaditana* (CCAP 849/5) was obtained from the Scottish Association for Marine Science. Its cultivation occurred via a modified F/2 [17] made in artificial seawater (6.3 mM KCl, 2.0 mM $NaHCO_3$, 7.1 mM KBr, 0.36 mM H_3BO_3, 0.024 M Na_2SO_4, 9 mM $CaCl_2{\cdot}2H_2O$, 0.046 M $MgCl_2{\cdot}6H_2O$, 0.35 M NaCl) with the following composition: 3.5 mM $NaNO_3$, 0.036 mM $NaH_2PO_4{\cdot}H_2O$, 0.12 μM $FeCl_3{\cdot}6H_2O$, 0.12 μM Na_2EDTA, 0.04 μM $CuSO_4{\cdot}5H_2O$, 0.076 μM $ZnSO_4{\cdot}7H_2O$, 0.042 μM $CoCl_2{\cdot}6H_2O$, 0.91 μM $MnCl_2{\cdot}4H_2O$, 0.025 μM $Na_2MoO_4{\cdot}2H_2O$. Before each cultivation, the culture medium, all the tubes and the filters were autoclave sterilized while the reactors were sterilized in an oven (TIPE) at 170 °C for 1 h. Cellular concentration was monitored daily by reading the absorbance at 750 nm (Agilent Cary 60 UV-Vis) inside 1.5 mL cuvettes and the following calibration curve was experimentally obtained from 16 data points with concentrations between 0.05 g L^{-1} and 1 g L^{-1}:

$$X[\text{g L}^{-1}] = 0.2198 \times abs_{750} \quad (11)$$

Light intensity was measured by a photometer (HD 9021, Delta OHM, Padua, Italy) with a photosynthetic active radiation (PAR) probe (LP 9021 PAR, Delta OHM, Padua, Italy).

In order to obtain the kinetic parameters of the Monod equation, μ_{max} and I_K, batch cultivations were made inside the quasi-isoactinic flat photobioreactor for 10 days at a fixed temperature (25 °C) and incident light intensities I_0 of 20, 50, 100, 140, 210, 300 and 450 μE $s^{-1}m^{-2}$.

Model results were then compared with experimental growth curves obtained for 13 days in the annular column PBR and in the two flat-plate PBRs, at an incident light intensity I_0 = 210 μE $s^{-1}m^{-2}$. The other culture conditions (temperature and air flow) were the same as those in the cultivation for the kinetic parameters.

3.5. Harvesting of Microalgal Biomass

After growth, the cell suspension was centrifuged (3600 rpm, 10 min, NEYA 10R) and the biomass frozen in liquid nitrogen and freeze-dried for 48 h in a bench lyophilizator (FreeZone 2.5 L, LABCONCO, Kansas City, MO, USA). The biomass was then stored at −20 °C for further analysis.

3.6. Extraction and Analysis of Fatty Acids

About 20 mg of lyophilized microalgae biomass was weighed in a glass tube and about 7 mg of glass beads were added. Then 5 mL of chloroform/methanol (2:1, *v/v*) and 1 mL of NaCl 1% were added and the mixture was vigorously mixed with a laboratory vortex and subsequently centrifuged until the formation of two phases. The lower phase (chloroform phase) was transferred in a pre-weighted tube and the solvent was evaporated under nitrogen stream. After complete solvent evaporation, total lipids were determined gravimetrically and subsequently trans-esterified by adding 1 mL of sodium methoxide (1 g NaOH in 100 mL MeOH) and 1 mL of hexane, and kept at 50 °C for one hour. Then 1 µL of the upper phase was analyzed by gas-chromatography using a GC 7890B System (Sigma-Aldrich, St. Louis, MO, USA) equipped with a FID detector and a capillary column Omegawax 250 (Sigma-Aldrich, St. Louis, MO, USA). Initial temperature was 50 °C, increasing to 220 °C as working temperature. Total analytic time was 79.5 min and argon was used as carrier. The quantification of lipids was performed by comparing sample chromatograms with the standard. Supelco 37-Component FAME Mix (Sigma-Aldrich, St. Louis, MO, USA) was used as standard. Measurements were carried out in duplicate and the average value was retained.

3.7. Total Carbohydrates

Total carbohydrate content was determined according to Trevelyan et al. [18]. Briefly, 10 mg of freeze-dried biomass were suspended in 6 mL HCl 2 mol L^{-1} and hydrolyzed in a water bath for 1 h at 100 °C. Subsequently, 4 mL of a fresh anthrone solution (Sigma-Aldrich, 2 mg mL^{-1} in 95–97% H_2SO_4) were added to 1 mL of sample extract. The absorbance of each sample was read at 620 nm (Cary 60 Uv-vis, Agilent technologies, Santa Clara, CA, USA). Aliquots of different glucose concentrations (0.02–0.1 mg L^{-1}) were prepared and processed in the same way as the microalgal extracts, to obtain a calibration curve. Measurements were carried out in triplicate and the average value was retained.

3.8. Total Proteins

About 50 mg of the sample was subjected to an elemental analysis of the biomass for carbon (C), hydrogen (H) and nitrogen (N), conducted (TruSpec, LECO) to assess the protein contents by multiplying the nitrogen content in the biomass (%N) by 4.78. Measurements were carried out in triplicate and the average value was retained.

3.9. Carotenoids

About 7 mg of biomass were weighed together with 0.7 g of mixed sized glass. Two drops of water were added to make the biomass wet, together with 3 mL of methanol. The contents of the tubes were mixed using a benchtop vortex and centrifuged for 10 min. Once the separation was completed, the upper phase was transferred to an aluminum wrapped tube. The extraction was repeated by adding another 2 mL to the biomass. The solutions were filtered, diluted with methanol when necessary and analysed via HPLC and spectrophotometer. All analyses were carried out under dimmed light.

The content of total carotenoids was assessed by recording a spectrum from 400 to 700 nm by means of a Cary 60 UV-Vis spectrophotometer (Cary 60 Uv-vis, Agilent technologies, Santa Clara, CA, USA). Total carotenoids were determined according to the method described by Lichtenthaler and Wellburn [19] by applying the OD measurements at 666 and 470 nm (A_{666}, A_{470}) from the methanol extracts to Equation (12):

$$\text{Total carotenoids } = (1000 \times \text{abs}_{470} - 44.76 \times \text{abs}_{666}) / 221 \quad (12)$$

The content of chlorophyll a, lutein and beta-carotene was assessed through HPLC Agilent 1220 Infinity LC, equipped with a Diode Array Detector (DAD) and column temperature set at 20 °C. The separation of the compounds was carried out using a Synergi HYDRO-RP C18 column (4 µm, 250 × 4.6 mm) and a mobile phase consisting of acetonitrile:water solution (9:1; *v/v*) as solvent A and ethyl acetate as solvent B. A constant

flow rate of 1 mL/min was used. The analysis time was set at 35 min and the following program was used: (i) 0–16 min, 0–60% B; (ii) 16–30 min, 60% B; (iii) 30–32 min 100% B and (iv) 32–35 min 100%. The sample injection volume was 100 μL. The chromatograms were recorded at 450 nm. Quantifications of chlorophyll a, lutein and β-carotene were performed through calibration curves of pigment standards (Sigma-Aldrich).

4. Results and Discussion

4.1. Kinetic Parameters

In order to obtain the kinetic parameters of the Monod equation, batch cultivations were carried out inside the quasi-isoactinic photobioreactor for 10 days at a fixed temperature (25 °C) and incident light intensities of 20, 50, 100, 140, 210, 300 and 450 μE $s^{-1}m^{-2}$. The growth of microalga *Nannochloropsis gaditana* at 25 °C and at different light intensities is shown in Figure 3.

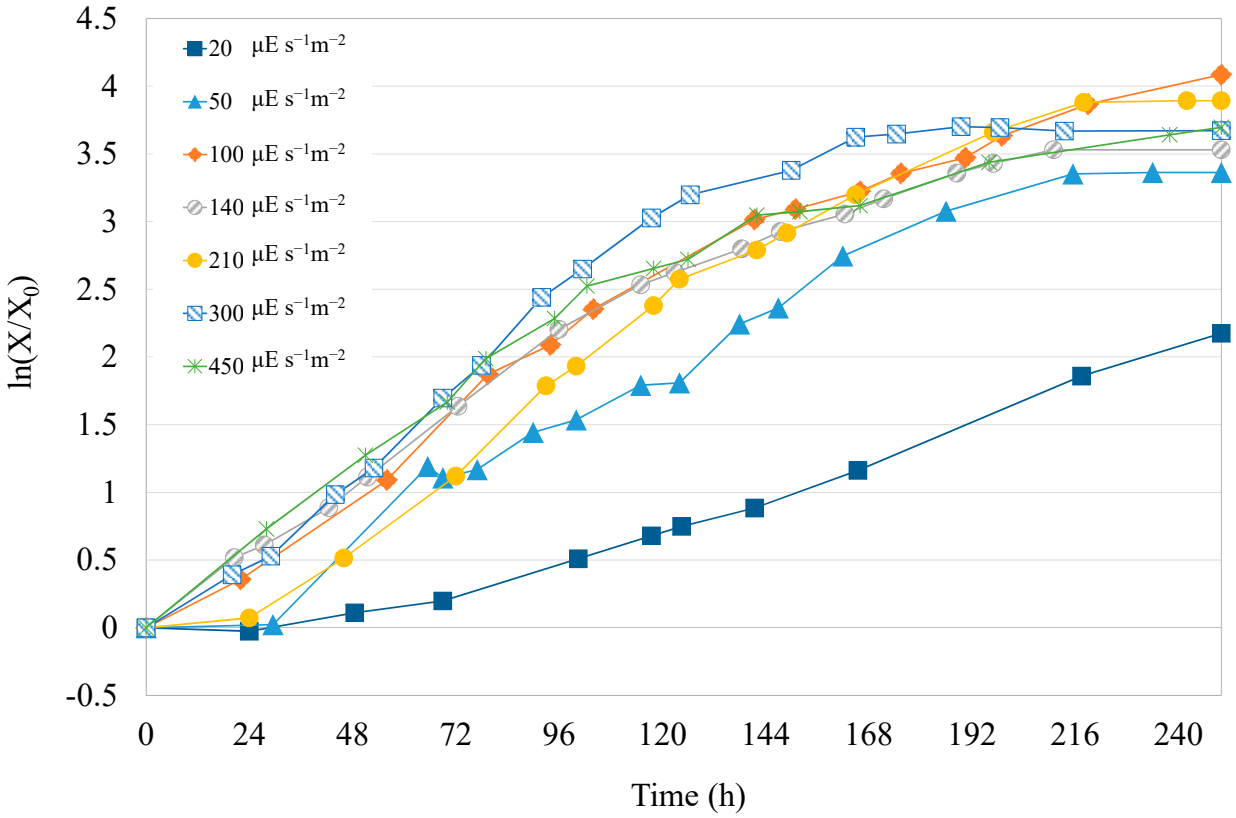

Figure 3. Growth of microalga *Nannochloropsis gaditana* at 25 °C in the quasi-isoactinic photobioreactor under different light intensities.

As can be seen, the growth is well related to the light intensity: the slowest growth is observed under I = 20 μE $s^{-1}m^{-2}$ and it gradually increases with increasing light intensity. The best growth performance is shown by cultures grown between I = 300 μE $s^{-1}m^{-2}$ and I = 450 μE $s^{-1}m^{-2}$. There is no, in any case, very high difference relatively to growth performance from I = 100 μE $s^{-1}m^{-2}$ to I = 450 μE $s^{-1}m^{-2}$, while between I = 50 μE $s^{-1}m^{-2}$ and I = 100 μE $s^{-1}m^{-2}$ there is a distinct difference in accordance to that predicted by the Monod equation [20,21].

In a batch photobioreactor, during the exponential growth phase, the specific growth rate μ can be expressed as:

$$\mu = \frac{\ln X - \ln X_0}{t - t_0} \quad (13)$$

where X_0 is the biomass concentration at time t_0 and X is the biomass concentration at time t. By employing a linear regression from the data in the exponential phases of Figure 3,

the specific growth rates under each light intensity were obtained and reported in Table 1, together with the values of R^2 and of the relevant I_0/μ.

The observed growth rates are in the same range as that previously found for the same strain by other authors [22].

Table 1. Values of specific growth rates and R^2 calculated at different light intensities at T = 25 °C in *Nannochloropsis gaditana*.

I_0 (μE $s^{-1}m^{-2}$)	μ (h^{-1})	I_0/μ (μE h $s^{-1}m^{-2}$)	R^2
20	0.0117	1709	0.9956
50	0.0184	2717	0.9912
100	0.0228	4386	0.9817
140	0.0237	5907	0.9976
210	0.0249	8434	0.9891
300	0.0246	12195	0.9859
450	0.0243	18519	0.9988

To obtain the kinetic parameters, μ_{max} and I_k, Equation (10) can be rewritten according to Langmuir [20] as:

$$\frac{I_0}{\mu} = \frac{1}{\mu_{max}} I_0 + \frac{I_K}{\mu_{max}} \quad (14)$$

Therefore, a Langmuir plot of I_0/μ versus I_0 should give a straight line with slope $1/\mu_{max}$ and intercept I_K/μ_{max} as reported in Figure 4. From the intercept and the slope of the linear regression of Figure 4, the kinetic parameters $\mu_{max} = 0.0256\ h^{-1}$ (0.61 day^{-1}) and $I_K = 15.28$ μE $s^{-1}m^{-2}$ were obtained.

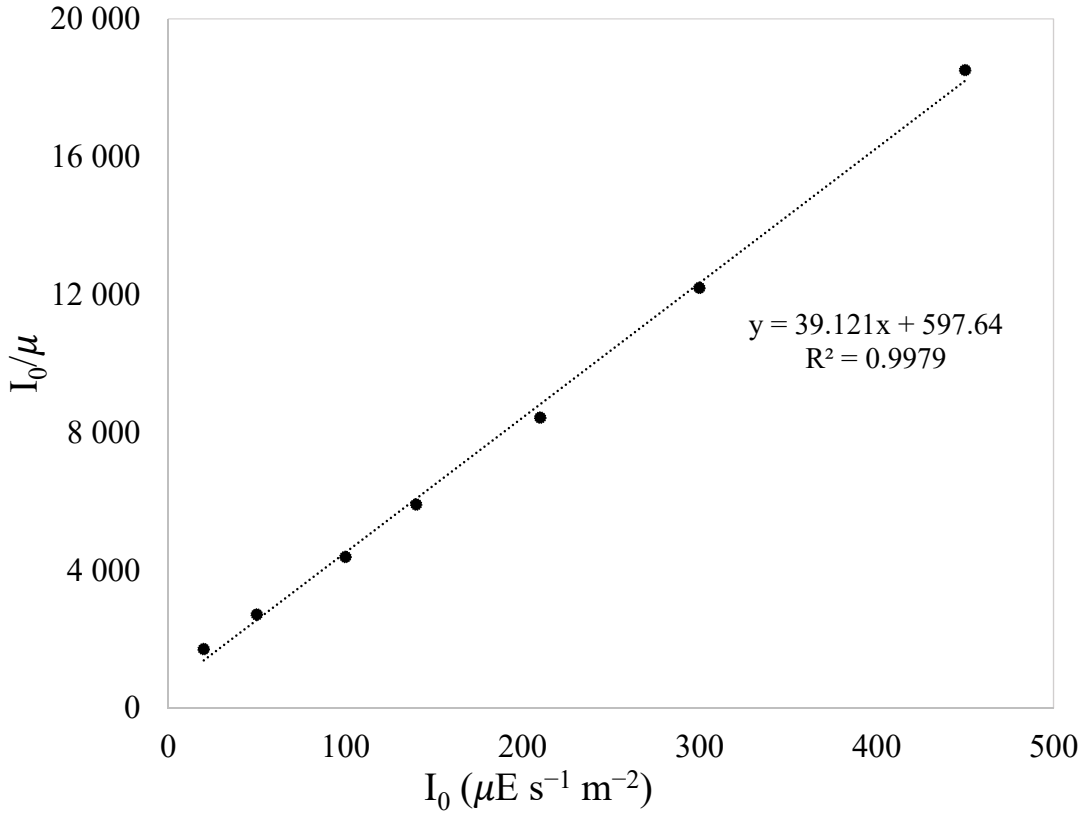

Figure 4. "Langmuir plot" of experimental values obtained at different light intensities.

By using these kinetic parameters, the specific growth rate μ as a function of incident light I_0 can be plotted, as reported in Figure 5, for the quasi-isoactinic photobioreactor. As

can be seen, the typical Monod curve trend may be easily observed, and the saturation constant (I_k) corresponds to the value of I at which the growth rate is half of the maximal ($\mu = 0.5 \cdot \mu_{max}$).

It is worth noting that the calculated maximum growth rate μ_{max} (0.61 day^{-1}) is comparable to typical *Nannochloropsis* growth rates found by other researchers [23,24]. Polishchuk et al. found that *Nannochloropsis oculata* grown in a saline medium has a maximum growth rate of 0.365 day^{-1} [23], while Huesemann et al. found that *Nannochloropsis salina* has a maximum specific growth rate of about 1.0 day^{-1} when cultivated at 26 °C under a photon flux density of 250 µE $s^{-1}m^{-2}$ [24]. In the literature, the only work showing the specific growth rate (μ) in the exponential phase as a function of the mean integral photon flux density in cultures of *Nannochloropsis gaditana* reports a maximum growth rate μ_{max} of about 0.0370 h^{-1} (about 0.89 day^{-1}) and a saturation constant I_k of 43 ± 6 µE $s^{-1}m^{-2}$ [14]. The small differences in the values found by these authors may be due to different growth conditions, experimental set-up and light distribution inside the reactor.

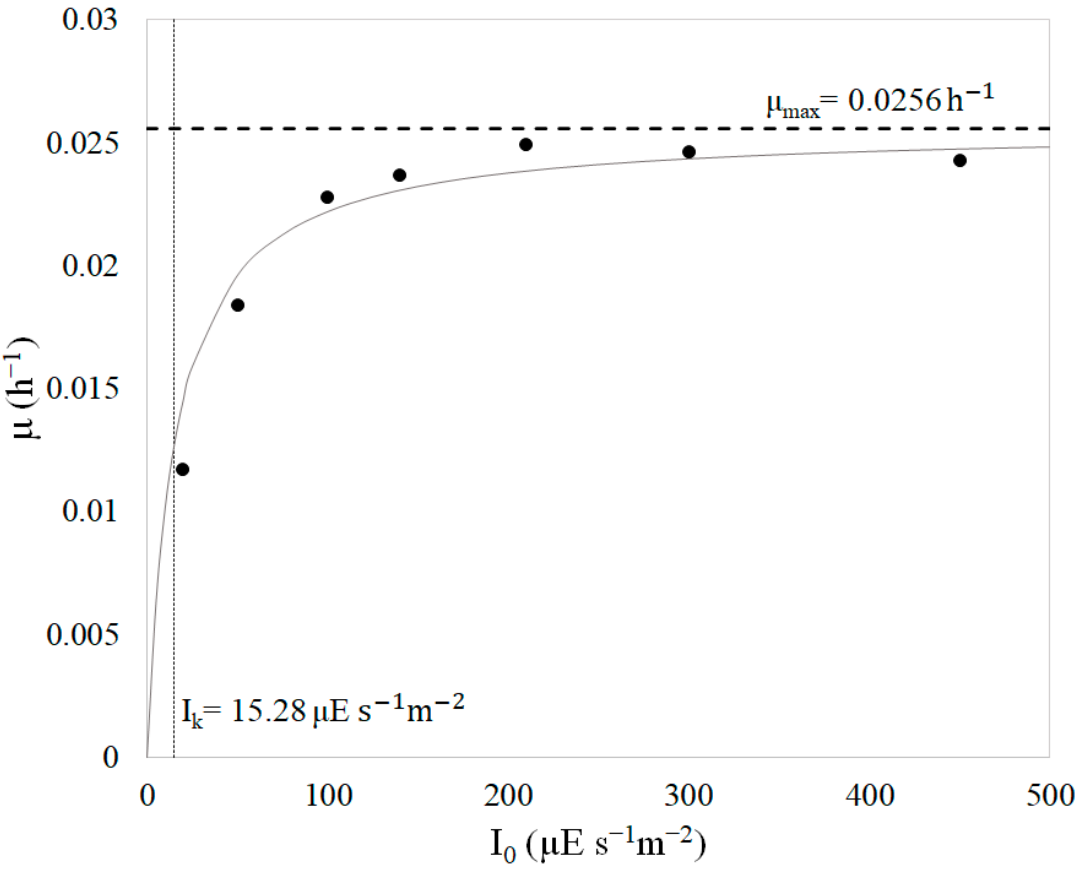

Figure 5. Specific growth rate versus different irradiation intensities of the microalga *Nannochloropsis gaditana* in the quasi-isoactinic photobioreactor: black circles, experimental data; line, Equation (10).

4.2. Model Validation

The equations shown in the "Mathematical modelling" section were implemented in MatLab® for the "Flat-plate geometry model" and the "Annular section geometry model". The kinetic parameters μ_{max} and I_k were integrated in both "Flat-plate geometry model" and the "Annular section geometry model", while the adjustable parameters k_d and k_a were instead obtained by fitting model results with experimental concentration data. These cultures were conducted under a photon flux density of 210 µE $s^{-1}m^{-2}$ at 25 °C. The data and parameters employed and obtained from the modelling are shown in Table 2.

Table 2. Data and parameters employed for the model.

Parameter	Value			Unit
	Flat type 15	Flat type 30	Annular	
L	15	30	35.5	mm
μ_{max}	0.0256	0.0256	0.0256	h^{-1}
I_K	15.28	15.28	15.28	$\mu E\ s^{-1}m^{-2}$
k_d	0.0046	0.0046	0.0046	h^{-1}
k_a	0.38	0.38	0.20	$m^2\ g^{-1}$

The two adjustable parameters were first changed in the flat geometry model, obtaining the best fit for both reactors' thickness with k_d = 0.0046 h^{-1} and k_a = 0.38 $m^2\ g^{-1}$. When using these two parameters in the annular geometry model, the best fit is obtained with the same value of k_d = 0.0046 h^{-1}, and this is sound as the microalgae strain did not change, while the value of k_a had to be set at 0.20 $m^2\ g^{-1}$, meaning that, in the case of the annular reactor, the light attenuation factor is a little smaller than in the flat geometry. Considering that the cellular size (cellular diameter) does not significantly vary in the three different cultivations (data not shown), this variation may be due to the cells creating agglomerates differently in the three cultivations. In fact, we observed that the cells in the annular reactor showed bigger agglomerates than in the other two cultivations. A bigger agglomerate reduces the transversal section of light capture (because some cells are not exposed to the light) and therefore reduces the attenuation constant k_a. In Figure 6 the growth curves obtained from the model are compared with the relevant experimental data.

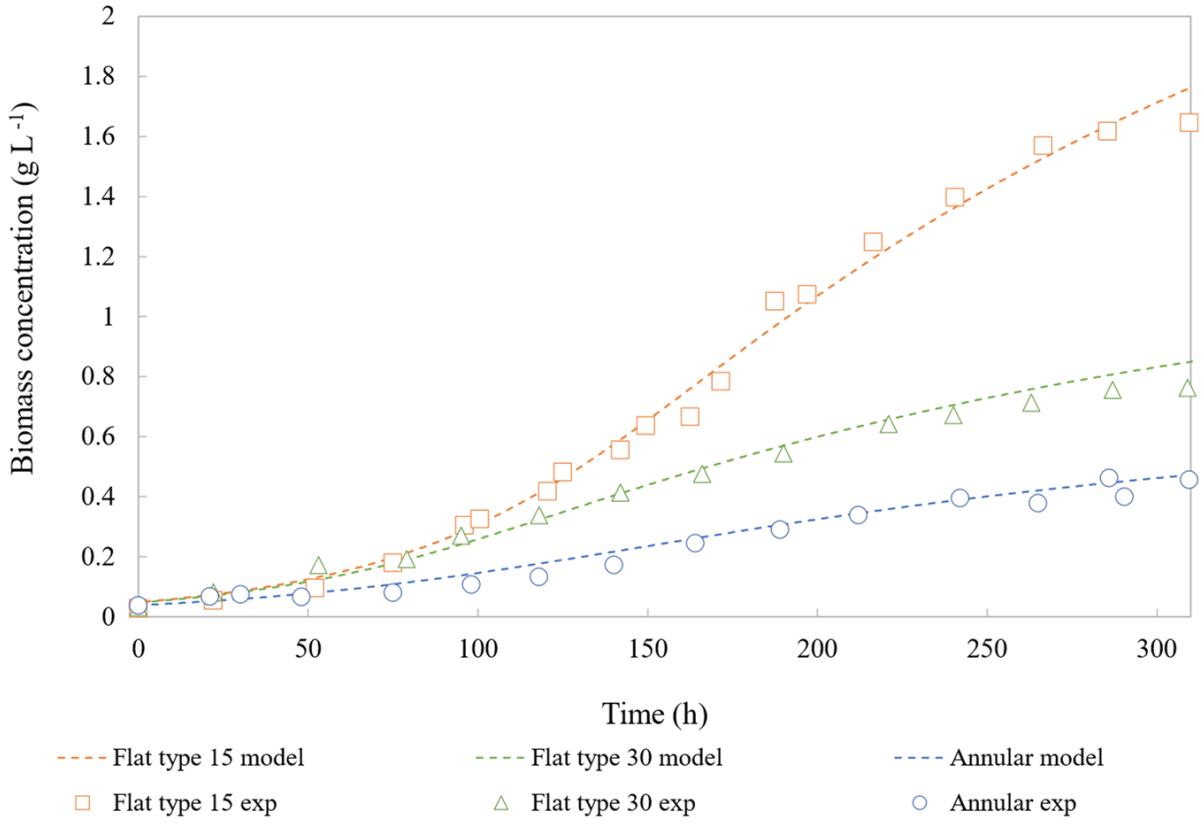

Figure 6. Comparison between model prediction and experimental data of *Nannochloropsis gaditana* growth curves in batch photobioreactors: "orange" Flat-plate 15 mm, "green" Flat-plate 30 mm, "blue" Annular photobioreactor. Dotted lines, model predictions; symbols, experimental data.

As can be seen, the modelled growth curves show a good fit with the experimental data, meaning that the model can predict the growth in flat-plate reactors of differ-

ent depths and in an annular geometry, once the attenuation constant parameter k_a has been adjusted.

The data obtained through this model may be of interest in order to create a link between the metabolic response of *Nannochloropsis gaditana* and the action of inputs such as the incident light intensity, the reactor thickness and the geometry. Furthermore, this model may be easily implemented on other microalgae, after experimentally measuring the kinetic parameters.

4.3. Biochemical Analysis

After growth, all the batch cultures were harvested, lyophilized and the biomass was employed to perform biochemical analysis. Results are shown in Figures 7–9.

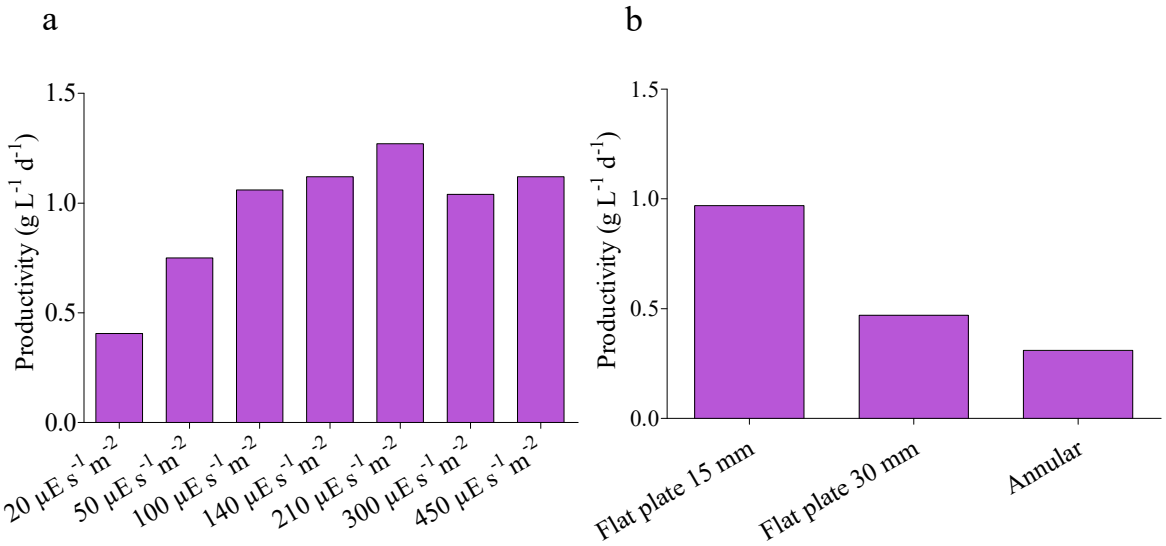

Figure 7. Batch productivity of the cultures grown in different conditions: (**a**) quasi-isoactinic reactor with different photon flux densities; (**b**) reactors with different configurations at incident light intensity I_0 = 210 µE $s^{-1}m^{-2}$.

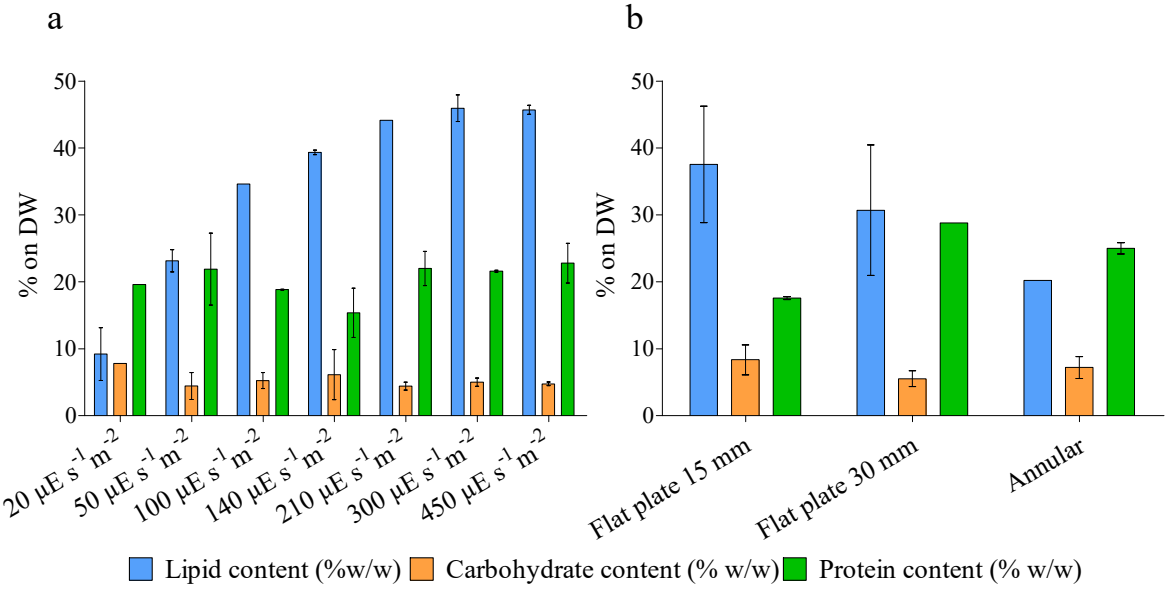

Figure 8. Biochemical composition of the biomass obtained at the end of the batch cultivations grown under different conditions: (**a**) quasi-isoactinic reactor with different photon flux densities; (**b**) reactors with different configurations at incident light intensity I_0 = 210 µE $s^{-1}m^{-2}$.

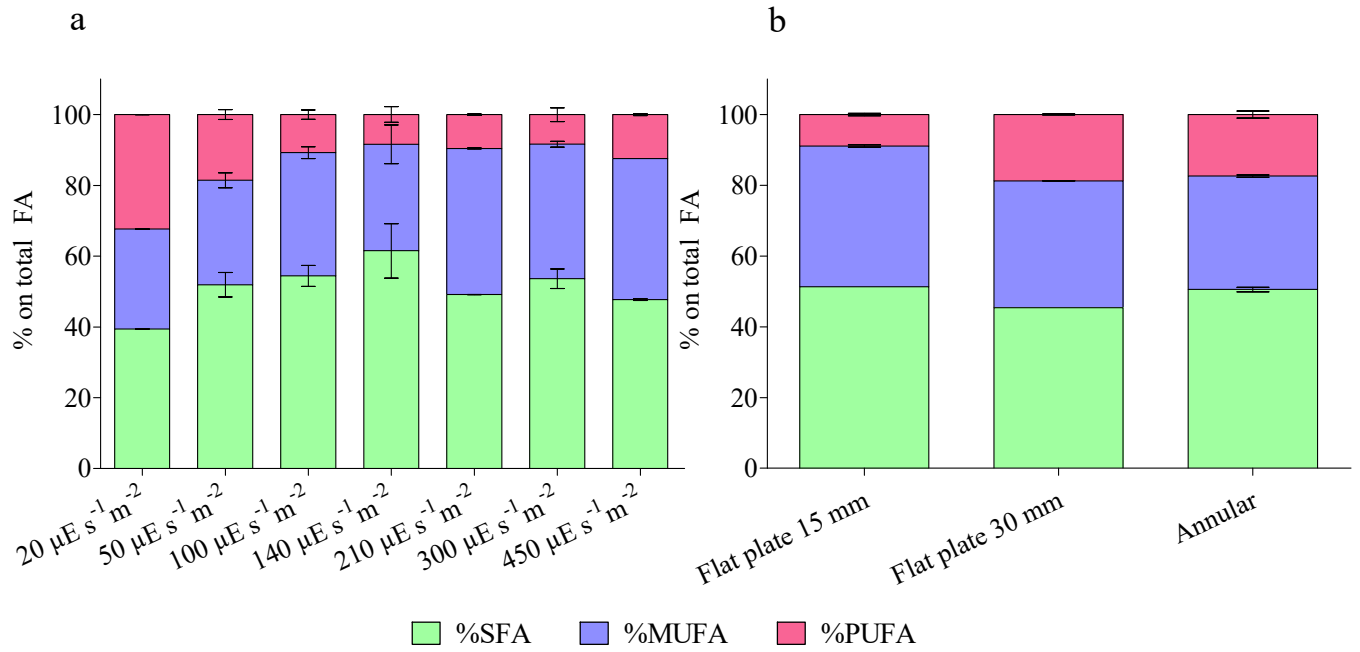

Figure 9. Fatty acid analysis as percentage of total fatty acid content in the biomass obtained at the end of the batch cultivations grown under different conditions: (**a**) quasi-isoactinic reactor with different photon flux densities; (**b**) reactors with different configurations at incident light intensity I_0 = 210 µE $s^{-1}m^{-2}$. SFA, saturated fatty acid; MUFA, monounsaturated fatty acids; PUFA: polyunsaturated fatty acid.

Figure 7 reports the batch productivity in terms of grams of dry weight per liter per day of cultivation. The biomass productivity ranges from 0.3 g L^{-1} d^{-1} to 1.2 g L^{-1} d^{-1} of the culture grown under 210 µE $s^{-1}m^{-2}$. As expected, the productivity increases with the incident photon flux density, as shown in Figure 7a. At the same time, the productivity decreases with the reactor thickness, as shown in Figure 7b. This is a consequence of the fact that the average light intensity is lower when the reactor thickness increases, and therefore the volumetric productivity diminishes because of the lack of light as substrate.

Other studies in the literature report batch productivity of *Nannochloropsis* in different cultivation conditions [25,26]. The productivities reported in the present study are in general significantly higher and this is due to the high average light intensity distributed inside the flat and the annular reactors employed in the present work.

Figure 8 shows the biochemical composition of the biomass obtained at the end of the batch cultivations and grown under different photon flux densities (Figure 8a) and in reactors with different configurations (Figure 8b). Lipid content shows a defined trend between the analysed conditions, while carbohydrate and protein content do not. Carbohydrate content is on average around 8% of the dry weight, while protein content is on average around 20% of the dry weight, as previously determined in the same strain [27]. *Nannochloropsis* is considered to be one of the most promising genera of microalgae in the production of lipids because of the possibility of accumulating high contents of up to 60% [28]. In this work, *Nannochloropsis gaditana* accumulated lipids from 9% of dry weight of the biomass grown under 20 µE $s^{-1}m^{-2}$ up to 45% of the dry weight of the biomass grown under 450 µE $s^{-1}m^{-2}$, similarly to that previously reported for the same strain [22,27]. The content of lipids increases with the average light intensity inside the reactor, both when cells were cultivated in the quasi-isoactinic reactor with increasing light intensity (Figure 8a) and when cells were cultivated in reactors with different geometries (Figure 8b). This trend was already observed before but, in this case, we report an accurate analysis with several different changing inputs in the cultivation [26,29]. This is the first time that a complete biochemical analysis was performed on biomass cultivated in quasi-isoactinic conditions.

Analysis of the fatty acids content was performed in the same way and results are shown in Figure 9 and in Table 3.

Table 3. Specific fatty acid classes in total fatty acids (TFAs) of the biomass obtained at the end of the batch cultivations grown under different photon flux densities and in reactors with different configurations with I_0 = 210 μE $s^{-1}m^{-2}$. n-3 and n-6 represent classes of fatty acids with the first unsaturation at the carbon 3 or 6. EPA: eicosapentaenoic acid.

	20 μE $s^{-1}m^{-2}$	50 μE $s^{-1}m^{-2}$	100 μE $s^{-1}m^{-2}$	140 μE $s^{-1}m^{-2}$	210 μE $s^{-1}m^{-2}$	300 μE $s^{-1}m^{-2}$	450 μE $s^{-1}m^{-2}$	15 mm	30 mm	Annular
% of n-3 on TFAs	29 ± 0.1	15.9 ± 0.9	7 ± 0.3	6 ± 1.6	6.1 ± 1.6	8.3 ± 0.3	5.9 ± 0.3	6.4 ± 0.1	15.6 ± 0.9	12.7 ± 0
% of n-6 on TFAs	3.4 ± 0.1	2.7 ± 0.5	3.2 ± 0.8	2.4 ± 0.6	2.3 ± 0.3	4.1 ± 0	3.6 ± 0.1	2.5 ± 0	3.1 ± 0	4.6 ± 0
n3/n6 ratio	8.5 ± 0.2	6 ± 0.8	2.3 ± 0.6	2.4 ± 0.1	2.6 ± 0.4	2 ± 0.1	1.6 ± 0	2.5 ± 0	5 ± 0.2	2.7 ± 0
% of C20:5 n-3 (EPA) on TFAs	26 ± 0.1	13.3 ± 0.5	4.9 ± 0.3	4 ± 1.1	4.2 ± 1	5.6 ± 0.2	0 ± 0	4.4 ± 0	10.4 ± 0.6	9 ± 0

The fatty acid class composition varies when growing microalgae under different photon flux densities and in different geometries. Saturated fatty acids (SFAs) vary from 39% of algae grown under 20 μE $s^{-1}m^{-2}$ to 50% of those grown inside the annular reactor; Monounsaturated fatty acids (MUFAs) vary between 28% to 41% of algae grown under 20 and 210 μE $s^{-1}m^{-2}$, respectively: Polyunsaturated fatty acids (PUFAs) vary from 8–9% in algae grown under 140, 210, 300 μE $s^{-1}m^{-2}$ and in a 150 mm reactor to 32% of algae grown under 20 μE $s^{-1}m^{-2}$. Looking at Figure 9, the most interesting aspect is that the PUFA content is highest when the cells were cultivated under low photon flux densities (20 μE $s^{-1}m^{-2}$), then gradually decreases until 140 μE $s^{-1}m^{-2}$ and is stable under the highest tested photon flux densities. The same trend is shown when the thickness of the reactor decreases, meaning that the PUFA content is inversely related to the average photon density. An opposite trend may be observed for the SFA, while the MUFAs do not vary significantly. Table 3 reports in detail the composition in n-3 and in n-6 fatty acids, the ratio between n-3 and n-6 and the percentage composition of the eicosapentaenoic acid (EPA), a fatty acid of interest from an industrial point of view. The fatty acids of the n-3 series decrease with an increase in the average photon flux density, in an opposite way to those of the n-6 series. The content of EPA is again highest under the lowest average light intensities employed. In general, the fatty acid profile is coherent with that found by other authors [27], as well as the change in composition with light intensity [30]. The increase in PUFA and in n-3 fatty acids in cultures exposed to low-light conditions is related to a low-light adaption, because cells increase their photosynthetic active membranes quantities [31,32]. It is worth highlighting, in any case, that the total lipid content is lower in cultures grown under low photon flux densities. It may be possible that the increase of lipids in algae grown under higher photon flux densities is due to an accumulation of storage lipids as SFAs and MUFAs, and that actually the PUFAs concentration is stable. Figure 10 reports an analysis of the carotenoids content of the biomass obtained at the end of the batch cultivations grown under different photon flux densities and in reactors with different configurations.

The content of chlorophyll a (Figure 10a), total carotenoids (Figure 10b), beta-carotene (Figure 10c) and lutein (Figure 10d) varies from a minimum of 1.7, 1.7, 0.05 and 0.08 μg mg^{-1} of dry weight, respectively, of the biomass grown under 450 μE $s^{-1}m^{-2}$ to a maximum of 29.4, 8.71, 1.55, and 0.60 μg mg^{-1} of dry weight, respectively, of the biomass grown under 20 μE $s^{-1}m^{-2}$. The contents are in the same range observed earlier [27]. The content of all the analysed compounds shows the same trend: it is higher in conditions where the average light intensity is lower (20 μE $s^{-1}m^{-2}$ and annular reactor) and coherently decreases along with increases in average light intensity. This response is caused by a photo-acclimatization mechanism, already known, through which the cells grown under low photon flux densities increase the number of photoactive systems, and consequently need higher quantities of carotenoids and chlorophyll [33,34].

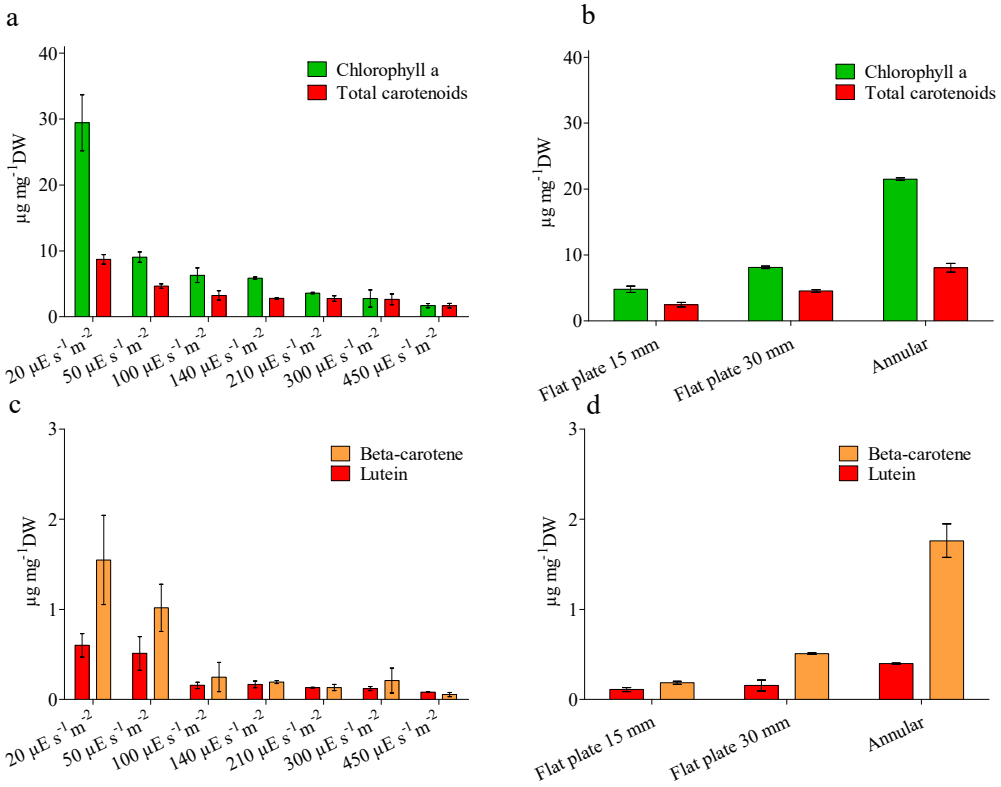

Figure 10. Carotenoid analysis of the biomass obtained at the end of the batch cultivations grown under different conditions: (**a**,**c**), quasi-isoactinic reactor with different photon flux densities; (**b**,**d**), reactors with different configurations at incident light intensity I_0 = 210 µE $s^{-1}m^{-2}$. DW: dry weight.

5. Conclusions

In the present work a model able to predict the microalgae growth in reactors with different geometries was developed. We combined a Monod-like model for the specific growth rate with the Lambert-Beer law for non-homogeneous light distribution in thick photobioreactors. Kinetic parameters relating to the cultivation of the microalga *Nannochloropsis gaditana* were obtained, for the first time, through batch cultivation under different photon flux densities inside a quasi-isoactinic photobioreactor in order to obtain a practically homogeneous light distribution. The maximum specific growth rate and saturation constant resulted, respectively, as μ_{max} = 0.0256 h^{-1} and I_k = 15.28 µE $s^{-1}m^{-2}$.

These parameters were applied to the model to obtain microalgae growth curves in reactors with three different geometries: two flat-plate photobioreactors (FP-PBR) with different thicknesses (15 mm and 30 mm), and one annular column photobioreactor (AC-PBR). Simulation results showed good agreement with experimental growth curves of *Nannochloropsis gaditana* obtained in the three photobioreactors.

All the cultures cultivated in this work were also employed to perform biochemical analysis of the content of proteins, carbohydrates and lipids, and for the characterization of the fatty acid profile and the carotenoids content. The results show that the carbohydrate and protein content do not vary significantly with changing average light intensity, while the lipid content does. In particular, lipid content increases with increasing average photon flux density. The fatty acid profile also shows a marked shift when the average light intensity varies: the PUFA content decreases and the SFA content increases when the average light intensity increases. The carotenoids content is markedly shifted towards an accumulation at lower photon flux densities. These last responses indicate a strong low-light adaption of *Nannochloropsis gaditana* grown under low average light intensities.

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Nomenclature

I = local light intensity, µE $s^{-1}m^{-2}$
I_0 = incident light intensity, µE $s^{-1}m^{-2}$
k_a = attenuation constant, m^2 g^{-1}
X = biomass concentration, g L^{-1}
z = distance from the surface to the considered position, m
A_{capt} = area of the cell capturing light, m^2
d_{capt} = diameter of the transversal section of light capture, m
d_{cell} = cell diameter, m
ρ_{cell} = cell density, g m^{-3}
μ = growth rate, h^{-1}
μ_{max} = maximum growth rate, h^{-1}
I_k = light saturation constant, µE $s^{-1}m^{-2}$
V = volume of the reactor, m^3
A = enlightened surface of the reactor, m^2
L = depth of the reactor, m
k_d = constant of cellular death, h^{-1}
r_x = rate of biomass production, g m^{-3} h^{-1}
r = radius of the annular section, m
h = height of the reactor, m

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