Year-long production of *Scenedesmus almeriensis* in pilot-scale raceway and thin-layer cascade photobioreactors

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Abstract

Biomass of *Scenedesmus almeriensis* was produced outdoors for 12 months using three different photobioreactor designs. Optimum dilution rates to achieve the highest biomass productivities were 0.2 day^{-1} for raceways and 0.3 day^{-1} for thin-layer reactors. Biomass productivities achieved using thin-layer cascade photobioreactors during the months of higher photosynthetic activity reached 30-35 $g/m^2 \cdot day$, higher than those obtained using raceways during the same period: 20-25 $g/m^2 \cdot day$. Photosynthetic efficiency was lower in spring/summer when compared to autumn/winter, suggesting that a larger share of the solar energy that reaches the culture in spring/summer is not used for microalgal growth. During summer, culture temperature reached 40 °C in thin-layer photobioreactors, which demonstrates the importance of selecting microalgal strains able to resist these conditions. Photoinhibition was not observed at incident irradiances up to 1600 $\mu E/m^2 \cdot s$. However, dissolved oxygen values were especially high in thin-layer photobioreactors during this time of the year. They reached maximum values of 400% and showed an inhibitory effect on microalgal growth.

Keywords: microalgae, biomass, bioreactor, photosynthesis, photosynthetic efficiency.

1. Introduction

Microalgae are unicellular photosynthetic microorganisms that are naturally present in both saline and freshwater environments. These microorganisms are gaining increased importance in the context of European bioeconomy because of their potential to produce a wide variety of valuable biomolecules with diverse applications in agriculture, aquaculture, and food production, among others [1]. Despite the thousands of microalgal strains currently available in culture collections worldwide, only a limited number of species has been studied in detail, and only a few of these have achieved commercial success [2]. These include *Arthrospira platensis* and *Chlorella vulgaris*, mainly used as food [3,4], and *Dunialiella salina* and *Haematococcus pluvialis*, which are being mass-cultured for the production of β-carotene and astaxanthin respectively [2].

Key aspects that need to be considered in order to achieve commercial success include: (i) selection of a robust and highly productive strain, capable to grow under a wide range of environmental conditions; (ii) selection of a photobioreactor capable of providing the optimal conditions required by the selected strain; and (iii) the possibility to adjust the production system because of the (inevitably) changing environmental outdoor conditions, both on an hourly and a seasonal basis [5]. Several species of the *Scenedesmus* genus have certainly potential for industrial use. For example, *S. almeriensis* is a fast-growing and highly productive strain that is particularly adapted to stressful conditions. The biomass of *S. almeriensis* has been suggested as a potential source of high-value biomolecules such as lutein [6] and zeaxanthin [7]. Moreover, this microalga also showed potential for being used as fishmeal in aquaculture [8], in wastewater treatment processes [9], in agriculture as a source of biofertilisers and/or biostimulants [10], and because of its high lipid productivity – 144 mg/L·day – as a promising feedstock for biodiesel production [11].

Microalgal biomass productivity of a given production system depends on both the design of the photobioreactor and environmental conditions - and how these conditions accomplish what is needed for the microalga to be cultured. Several reports assessed the biomass productivity

of *S. almeriensis* using different reactor designs at different scales. At lab-scale level, a surface response analysis predicted a maximum productivity of 0.70 g/L·day day at 33 ºC and 1700 μE/m^2·s – confirmed *in vitro* as 0.73 g/L·day – when using bubble column photobioreactors with a capacity of 2 L [12]. In a different study, the maximum biomass productivity achieved, using a 2 L bubble column reactor, was 0.87 g/L·day and simulations performed *in silico* predicted that a productivity of 0.95 g/L·day could be achieved under outdoor conditions [13]. At a higher-scale level, studied photobioreactors include raceways and thin-layer systems. Thin-layer reactors are characterised by their low-depth culture (0.5-5.0 cm), recirculated over a flat surface by providing an adequate slope of 0.1-2.0%. They are one of the reactors with the highest areal productivity, with values ranging between 30 and 50 g/m^2·day [5,14]. In a study conducted in Almeria (Spain), it was observed that the productivity of *Scenedesmus* sp. in a thin-layer photobioreactor could reach 42 g/m^2·day while using a raceway (both reactors were of 32 m^2) the maximum productivity was barely half this value: 24 g/m^2·day [15].

Despite the high biomass productivities reported using thin-layer reactors, their use is still limited to laboratory or pilot scale - up to 50 m^2 [16]. The amount of available information about this type of reactors is very low when compared to that of raceways or tubular photobioreactors. Previous reports suggested that thin-layer reactors present some drawbacks such as pH and temperature gradients as well as inadequate mass transfer capacity and accumulation of oxygen in the culture [5]. However, more information on the characterisation of these reactors as well as their productivity is needed before scaling-up this technology to an industrial level. Thus, the aim of the current work is to evaluate the performance in terms of productivity and efficiency of pilot-scale thin layer photobioreactors (two different designs) compared to a raceway for producing *S. almeriensis* during a year.

2. Materials and methods

2.1 *Scenedesmus almeriensis* and culture conditions

Selected strain was *S. almeriensis*, which is a fast-growing and highly productive strain that is particularly adapted to stressful conditions. It was isolated for the first time within a photobioreactor in a greenhouse exposed to high temperature (45 ºC) and irradiance (2000 μE/m^2·s) conditions [12]. *S. almeriensis* is able to grow well at pH, temperature, and salinity values ranging between 7-10, 26-40 ºC, and 0-5 g NaCl/l, respectively, and shows no signs of photoinhibition up to 1625 μE/m^2·s [13]. The strain was obtained from the culture collection of the Department of Chemical Engineering of the University of Almería. Inocula of *S. almeriensis* were maintained at 23 ± 2 °C, pH 8.0 ± 0.1, and 150 μE/m^2·s in batch mode until a concentration of 1 g/L and using modified Arnon medium as described previously [15]. Once the desired concentration was achieved, the inocula were scaled-up to a final volume of 80 L using pH-controlled outdoor bubble column photobioreactors placed inside a greenhouse.

Pilot-scale production of the strain was conducted at the pilot plant facilities of the University of Almería located at IFAPA (Almería, Spain) using a culture medium that consisted of 0.90 g/L $NaNO_3$, 0.18 g/L $MgSO_4$, 0.14 g/L K_2PO_4, and 0.03 g/L of karentol® (Kenogard, Spain), which is a commercial solid mixture of micronutrients that include boron, copper, iron, manganese, molybdenum, and zinc. Chemicals used for pilot-scale production were agricultural fertilisers.

2.2 Photobioreactors and experimental conditions

Three different photobioreactor designs placed inside a greenhouse were used for the production of *S. almeriensis*: Bioreactor A, a raceway with an operating volume of 11,800 L and a land surface of 80 m^2; Bioreactor B, a single-channel thin-layer cascade photobioreactor with a total volume of 2,400 L and a land surface of 63 m^2; and Bioreactor C, a double-channel thin-layer cascade photobioreactor with a total volume 3,600 L and a land surface of 126 m^2 (Figure 1). Both thin layers were provided with a degasser with the aim to improve mass

transfer: a 250 L bubble column with continuous injection of air (75 L/min). pH and DO probes were installed at the end of the channel (where OD values are the highest) and before the degasser. Each test was conducted on semi-continuous mode at different dilution rates during the four seasons of the year, which show different ranges of temperature and incident irradiance. The pH was controlled by on-demand injection of CO_2. The three reactors were operated 24 h per day.

2.3 Culture analysis and light availability

Biomass concentration (C_b) was measured by dry weight filtering 100 mL of culture through 1 µm filters and drying at 80 °C in an oven for 24 h. Biomass productivity (P_b) was calculated as the product of biomass concentration by the dilution rate, which varied from 0.1 to 0.3 in Bioreactor A and from 0.2 to 0.4 in Bioreactors B and C. Cell status was checked daily by measuring the chlorophyll fluorescence ratio (Fv/Fm) with an AquaPen AP 100 fluorometer (Photon System Instruments, Czech Republic). Absorbance at 400-700 nm was daily measured using a GENESYS 10S UV-Vis spectrophotometer (Thermo Fisher Scientific, Spain) and the extinction coefficient (k_a) was calculated using the equation:

$$k_a = \frac{Abs}{C_b \cdot p}$$

where Abs and C_b are the above-mentioned absorbance and dry weight biomass concentration and p is the cuvettes' light path (1 cm).

Average irradiance inside the culture (I_{av}) was calculated as a function of the irradiance at the surface of the culture (I_0), k_a, C_b, and the light path inside the reactor (p) using the equation:

$$I_{av} = \frac{I_0}{k_a \cdot C_b \cdot p} \cdot (1 - e^{-k_a \cdot c_b \cdot p})$$

The percentage of photosynthetic efficiency (Ψ) was calculated as a function of D, k_a, and I_{av} using the equation:

$$\Psi = \frac{\Psi_b' \cdot H_b}{\lambda_{550}} \cdot 100$$

where H_b is the enthalpy of the biomass (20.6 kJ/g), λ_{550} is the energy of 1 mol of photons at 550 nm (217.5 kJ/E), and Ψ_b' is the photosynthetic efficiency (g/E) calculated as:

$$\Psi_b' = \frac{D}{k_a \cdot I_{av}}$$

where D, k_a, and I_{av} were the dilution rate, the extinction coefficient, and the average irradiance inside the culture described above.

2.4 Statistical analysis

Results shown are mean values of three independent experiments ± standard deviation (SD). Differences between photobioreactor designs and culture conditions were analysed using analysis of variance (ANOVA) with JMP 13 (SAS Institute Inc., US). A Tukey pairwise comparison of the means was conducted to identify where sample differences occurred. The criterion for statistical significance was in all cases $p<0.05$. To identify relationships between different variables, bivariate Pearson's' correlation analysis was carried out.

3. Results and discussion

There are a number of aspects that need to be considered to successfully up-scale the production of microalgae. These include, but are not limited to, the selection of a robust and highly productive strain and the selection of a suitable bioreactor that is capable of both, providing the optimum growth conditions to the selected microalgal strain and that can be adjusted to the changing environmental outdoor conditions [5]. As highlighted in the introduction, a number of species of the *Scenecesmus* genus have potential for industrial use. In the current paper, the selected strain was *S. almeriensis*, which was isolated for the first time and is naturally present in Almería (Spain), and is therefore adapted to the unique weather conditions of the region where the bioreactors are located. Moreover, the current paper assessed the potential of using three bioreactor designs: a raceway reactor, a single-channel thin-layer photobioreactor, and a double-channel thin-layer photobioreactor.

3.1 Operating conditions: Optimum dilution rate for increased productivity

The first goal of this study was to identify the optimum dilution rate for each reactor design and season. This operating variable strongly influences biomass productivity. Studied dilution rates were 0.1-0.3 day^{-1} for Reactor A and 0.2-0.4 day^{-1} for Reactor B and Reactor C. This selection was based on previous experience of our research group. Higher dilution rates were studied for thin-layer cascade reactors because of their higher biomass productivity [14]. Overall, biomass concentration and productivity were significantly affected by reactor design (p<0.0001), season (p<0.0001), and dilution rate (p<0.05). Because of the weather conditions of the region (mild winter and warm spring), the productivities observed during spring/summer and autumn/winter were comparable in all three reactor designs.

When operating using the Bioreactor A, maximum biomass concentrations and productivities ranged between 0.3-0.9 g/L and 7-20 $g/m^2 \cdot day$ depending on season (solar irradiance and temperature) and the dilution rate used (Figure 2). Results were similar to those reported during the production of *Scenedesmus* spp. in raceway reactors, where productivities within

the ranges 10-15 and 20-25 g/m^2·day were achieved in winter or spring, respectively [17]. Results were slightly lower than those reported in a previous study using raceway reactors to produce *Scenedesmus* sp. where the authors achieved a biomass productivity of 24 g/m^2·day [15]. These differences can be attributed to a different reactor capacity and design, differences in the culture media composition, and environmental factors. Overall, higher biomass productivities were obtained when operating using a dilution rate of 0.2 day^{-1}. Higher biomass concentrations were also observed under this condition, especially in summer, probably due to a higher light availability when compared to other seasons and higher nutrient availability when compared to a dilution rate of 0.1 day^{-1} (p<0.05). Results were in line with those reported previously for raceway reactors in the south of Spain [15]. No major differences were observed in photosynthetic efficiency when operating under different dilution rates - although higher efficiencies were observed when using a dilution rate of 0.2 day^{-1}, these were not statistically significant. Photosynthetic efficiency values ranged between 4 and 6%, higher than that of previous reports with photosynthetic efficiencies around 2% in *Scenedesmus* cultures in raceway reactors [18].

Similar results were observed when producing *S. almeriensis* using Bioreactor B – Figure 3. Again, higher concentrations were achieved when operating at a dilution rate of 0.2 day^{-1}– the lowest studied (p<0.05). However, biomass productivity was higher in all four seasons when operating at a dilution rate of 0.3 day^{-1}. It is difficult to compare microalgae productivities reported in the literature because of differences in environmental and operating conditions, as well as algal strains used. In the current paper, all of these parameters were equivalent. The productivity of Bioreactor B was higher when compared to that of Bioreactor A, and ranged between 15 and 30 g/m^2·day depending on season (solar irradiance and temperature) and the dilution rate used. These compares well with previous studies that demonstrated the higher productivity of thin-layer reactors when compared to the traditional raceways [15]. Recently, the biomass productivity of a thin-layer reactor using urban primary wastewater as the nutrient source ranged between 25 and 50 g/m^2·day [19]. The higher productivity of these reactors has

been attributed to a higher ratio of exposed surface to total volume and higher turbulence, allowing rapid light/dark cycles [20]. The lower culture depth allows culture temperature to decrease more rapidly in thin-layer reactors and this has also been suggested as a positive effect as it minimises biomass loss during the night due to respiration [21]. Bioreactor B also led to higher photosynthetic efficiency when compared to Bioreactor A. In this case, the photosynthetic efficiency ranged between 3 and 7% depending on the season and the dilution rate used. Results compared well with previous reports using thin-layer reactors in the Czech Republic [22]. Overall, higher efficiencies were obtained during autumn/winter when compared to spring/summer, caused by the higher biomass concentrations during the latter that darken the culture and decrease light availability and also by the extreme solar radiation during spring/summer in the region, which is not all harnessed by the microorganism.

Bioreactor C led to the higher photosynthetic efficiency, which ranged between 4 and 8% depending on the season in which the microalga was produced and the dilution rate used. Photosynthetic efficiency depends largely on solar irradiance. Although it is relevant for microalgal production and for the design of photobioreactors, as the main goal is to produce large quantities of biomass, it is more valuable to optimise biomass productivity rather than photosynthetic efficiency. When operating using Bioreactor C, productivities were lower than those expected and ranged between 10 and 20 $g/m^2 \cdot day$. In this case, no differences were observed when operating at a dilution rate of 0.3 or 0.4 day^{-1}, which were higher than those obtained at 0.2 day^{-1} ($p<0.05$; Figure 4). Previous studies algo suggested the optimum dilution rate for thin-layer reactors in the south of Spain to be 0.3 day^{-1} [15]. Although Bioreactor C is also a thin-layer cascade, productivities obtained in spring and summer were lower when compared to those obtained when using Bioreactor B ($p<0.05$; Figure 3). These lower productivities were caused by the high productivity of thin-layer reactors and an inefficient removal of dissolved oxygen in this reactor, which will be discussed in the next section.

All three bioreactors were located inside the same greenhouse and therefore had comparable environmental conditions. The average irradiance inside the culture ranged between 20 and

60 μE/m^2·s in the raceway (Bioreactor A) and between 50 and 100 μE/m^2·s in both thin-layer designs. The effect of solar irradiance on productivity and efficiency will be discussed in the next section. In the current study, no differences were observed in Fv/Fm and k_a values between seasons and dilution rates, suggesting that the robustness of the productive process and the stability of the biological system under the studied conditions in all three bioreactors. Previous studies obtained reduced Fv/Fm values during summer because of photoinhibition caused by (reversible) damage of key PSII components in indoor and outdoor cultures [23]. However, as discussed above, *S. almeriensis* is highly resistant to harsh environmental conditions especially in terms of solar irradiance [13] and although a slight decrease in Fv/Fm values was observed during spring and summer, this was not statistically relevant and demonstrate the high tolerance of *Scenedesmus* species to seasonal temperature and radiation oscillations.

3.2 Influence of environmental and associated parameters on productivity and efficiency

Once the optimum operation conditions were selected, in this case a dilution rate of 0.2 day^{-1} for Bioreactor A (raceway) and 0.3 day^{-1} for Bioreactor B and Bioreactor C (thin-layer), the current section will discuss in depth the effect of different parameters on biomass productivity and photosynthetic efficiency. Figure 5 shows the effect of environmental and culture temperature, solar radiation, oxygen saturation, and average irradiance inside the culture on both biomass productivity and photosynthetic efficiency for Bioreactor A. Both biomass productivity and photosynthetic efficiency were influenced by month of production (p<0.001) and therefore influenced by solar irradiance (p<0.001) and temperature (p<0.001). Spring and summer were the most productive seasons as biomass productivity was positively correlated with average irradiation (R^2=0.830; p<0.05) and the average culture media temperature (R^2=0.849; p<0.05). Although irradiance constitutes the major growth limitation, temperature also affects the biomass output of microalgae production [22]. These two parameters are linked, as higher temperatures are achieved during those months of higher solar irradiance.

Indeed, in the current study, a positive correlation was also observed between maximum irradiance and average (R^2=0.853; p<0.05) and maximum (R^2=0.913; p<0.05) temperature of the culture medium. During these months, the maximum daily values of solar radiation were within 1500 and 1600 μE/m^2·s and the maximum temperature reached in the culture media was in the range 30-35 °C, which was within the same range of temperature reached inside the greenhouse (no overheating of the culture was observed). These conditions are suitable for production of *S. almeriensis*¸ which is able to grow under temperatures within the range 26-40 ºC and showed no signs of photoinhibition at 1625 μE/m^2·s previously [13]. The average oxygen saturation values for Bioreactor A were stable along the whole year around 100% and showed daily peaks of nearly 200% during summer at mid-day. Moreover, photosynthetic efficiency was negatively correlated with I_{av} (R^2=0.803; p<0.05) and average temperature of the culture medium (R^2=0.805; p<0.05). These results demonstrate that photosynthetic efficiency in outdoor cultures is not as important as other parameters as higher productivities were associated with lower efficiencies.

The productivity and the photosynthetic efficiency of Bioreactor B were also affected by month (p<0.001), solar irradiance (p<0.001), and temperature (p<0.001) – Figure 6. Productivity of Bioreactor B was higher when compared to Bioreactor A in all the studied months (p<0.05), except for June, and was especially higher in summer July-September. As it happened in Bioreactor A, a positive correlation was observed between maximum irradiance and average (R^2=0.895; p<0.05) and maximum temperature (R^2=0.916; p<0.05) of the culture in Bioreactor B. However, in this case, because of the lower depth of the culture in this reactor (0.02 m against 0.13 m in Bioreactor A on average), the maximum temperature reached in the culture was extremely high during summer, surpassing peaks of 40 °C in August. Because of the high resistance of the selected strain to temperature, these peaks did not affect productivity as July, August, and September were the most productive months (p<0.05) – with average culture temperature of 25-30 °C. Photosynthetic efficiency of Bioreactor B was negatively correlated with I_{av} (R^2=0.871; p<0.05) and average temperature of the culture (R^2=0.867; p<0.05). Higher

efficiencies were achieved in the months where maximum and average solar irradiation were lower (Figure 6). In the current study, a major difference between thin-layer and raceway reactors was the extremely high oxygen saturation values measured in the former. Maximum dissolved oxygen concentration during the months of lower photosynthetic activity was within 200 and 250% saturation. However, maximum peaks of 400% were measured in summer (average daily values were over 350%), which probably limited growth in August-October (Figure 6). Although it is not commonly addressed in lab-scale cultures, dissolved oxygen accumulation in the culture has long been considered as one of the key factors limiting mass cultivation of microalgae, especially in highly productive and closed photobioreactors [24]. It has been suggested that high concentrations of dissolved oxygen may cause damage to the photosynthetic apparatus, membrane, and/or components of microalgal cells and therefore it has been associated with decreased cell growth rate and lower productivities [25,26]. Concentrations higher than 1.1 mM (480% saturation at 20 °C) can even become toxic to most microalgae [27]. In our pilot plant facilities, a degasser, which consists of a continuous injection of air (75 L/min) in a 250 L bubble column is used to reduce dissolved oxygen concentration in the culture. However, it was not enough to achieve more efficient values in highly productive months. Recently, the use of substances such as perfluorocarbon nanoemulsions as oxygen scavengers has been suggested [27]. Future studies regarding Bioreactor B will include the optimisation of oxygen removal, which could probably promote biomass production, especially during the months of higher photosynthetic activity.

Photosynthetic efficiency of Bioreactor C was negatively correlated with maximum irradiance (R^2=0.730; p<0.05) and average temperature of the culture medium (R^2=0.706; p<0.05) demonstrating that a large amount of the solar energy that reaches the culture in spring/summer was not used for microalgal growth. Photosynthetic efficiency of both thin-layer cascade reactors was higher than that of the raceway, highlighting the importance of the surface to volume ratio to achieve high photosynthetic efficiencies, as reported by other authors previously [28]. Again, those months where the biomass productivity was higher

showed a lower photosynthetic efficiency. As highlighted in the previous section, the productivity of Bioreactor C was lower than that expected, especially during spring/summer (Figure 7). Solar radiation and temperature both inside the greenhouse and inside the culture were similar to those of Bioreactor B. However, because of the (potentially) high productivity of the reactor, oxygen saturation in Bioreactor C was very high and reached maximum daily values of 250-300% in autumn/winter and in the range 300-400% in spring/summer. Again, it is likely that the inefficient removal of oxygen in this reactor limited the maximum biomass productivity. Previous studies also highlighted the problem encountered while operating highly productive reactors with the accumulation of photosynthetic oxygen in the culture, with values reaching 350% in spring/summer [17]. The inhibitory effect of oxygen in Bioreactor C was higher than in Bioreactor B because both reactors share the same degasser design (and air flow injection), but the channel surface and culture volume of Bioreactor C almost doubles that of Bioreactor B. Moreover, as the same injector is used to inject air and CO_2 (the latter used to control the pH), at highly productive seasons, especially at midday, the air injection (75 L/min) is substituted by CO_2 injection (10-12 L/min) and therefore oxygen removal is reduced. Future studies solving the low removal of oxygen from the culture are ongoing.

Conclusions

Optimum dilution rates to maximise biomass productivity were 0.2 and 0.3 day^{-1} for the raceway and thin-layer photobioreactors, respectively. Overall, these conditions led to higher biomass productivities during the 12 months of a year. As expected, the months with higher solar radiation showed higher photosynthetic activity and therefore higher biomass productivities. Once the optimum operating conditions were selected, thin-layer cascade photobioreactors led to higher biomass productivities when compared to the raceway design. The thin-layer photobioreactor with a smaller surface and volume led to the highest biomass productivities. Annual productivity of the raceway (80 m^2), single-channel thin-layer cascade (63 m^2), and double-channel thin-layer cascade (126 m^2) was 5100, 5600, and 9100 kg/year.

The lower areal productivity of the double-channel thin-layer reactor, when compared to the single-channel reactor, was caused by an inefficient removal of dissolved oxygen in the former. Although the optimisation of photosynthetic efficiency is relevant for the design of bioreactors, it must be considered that photosynthetic efficiency was lower in all three studied reactors in those months where solar irradiation was higher, and was therefore negatively related with biomass productivity. Results reported in the current study, which have been collected during a year, are relevant to improve photobioreactors design and microalgal biomass productivity as they demonstrate not only the potential to produce *S. almeriensis* outdoors, but also the striking effect of an inefficient oxygen removal, which can limit biomass production. Further studies will up-scale the degasser of thin-layer photobioreactors and assess the potential of these highly-productive reactors to produce biomass using both, wastewater and seawater.

Acknowledgements

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Conflict of interests

None

Statement of informed consent, human/anima rights

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

Figure legends

Figure 1. Photobioreactors design characteristics

All of the reactors were located inside the same greenhouse and thus exposed to identical environmental conditions. Reactor A was a standard raceway reactor, while reactors B and C were 1-line and 2-lines thin-layer reactors.

Figure 2. Bioreactor A (raceway): Effect of dilution rate and season on the *S. almeriensis* culture

Data shown represents mean values ± SD. Different capital letters indicate significant differences between dilution rates. Different lower-case letters indicate differences between seasons. The criterion for statistical significance was $p<0.05$.

Figure 3. Bioreactor B (single-channel thin layer cascade): Effect of dilution rate and season on the *S. almeriensis* culture

Data shown represents mean values ± SD. Different capital letters indicate significant differences between dilution rates. Different lower-case letters indicate differences between seasons. The criterion for statistical significance was $p<0.05$.

Figure 4. Bioreactor C (double-channel thin layer cascade): Effect of dilution rate and season on the *S. almeriensis* culture

Data shown represents mean values ± SD. Different capital letters indicate significant differences between dilution rates. Different lower-case letters indicate differences between seasons. The criterion for statistical significance was $p<0.05$.

Figure 5. Bioreactor A (raceway): Effect of solar irradiance, dissolved oxygen concentration, and temperature inside the greenhouse and of the culture on biomass productivity

Data shown represents mean values ± SD. Maximum/minimum values are the average of the maximum/minimum measurements obtained per day during one month (1 maximum and 1 minimum per day). Average values are the average of all the daily measurements (1 measurement per second), except for the average daily irradiance where only 12 h of sunlight were considered.

Figure 6. Bioreactor B (single-channel thin layer cascade): Effect of solar irradiance, dissolved oxygen concentration, and temperature inside the greenhouse and of the culture on biomass productivity

Data shown represents mean values ± SD. Maximum/minimum values are the average of the maximum/minimum measurements obtained per day during one month (1 maximum and 1 minimum per day). Average values are the average of all the daily measurements (1 measurement per second), except for the average daily irradiance where only 12 h of sunlight were considered.

Figure 7. Bioreactor C (double-channel thin layer cascade): Effect of solar irradiance, dissolved oxygen concentration, and temperature inside the greenhouse and of the culture on biomass productivity

Data shown represents mean values ± SD. Maximum/minimum values are the average of the maximum/minimum measurements obtained per day during one month (1 maximum and 1 minimum per day). Average values are the average of all the daily measurements (1

measurement per second), except for the average daily irradiance where only 12 h of sunlight were considered.

Figure 1

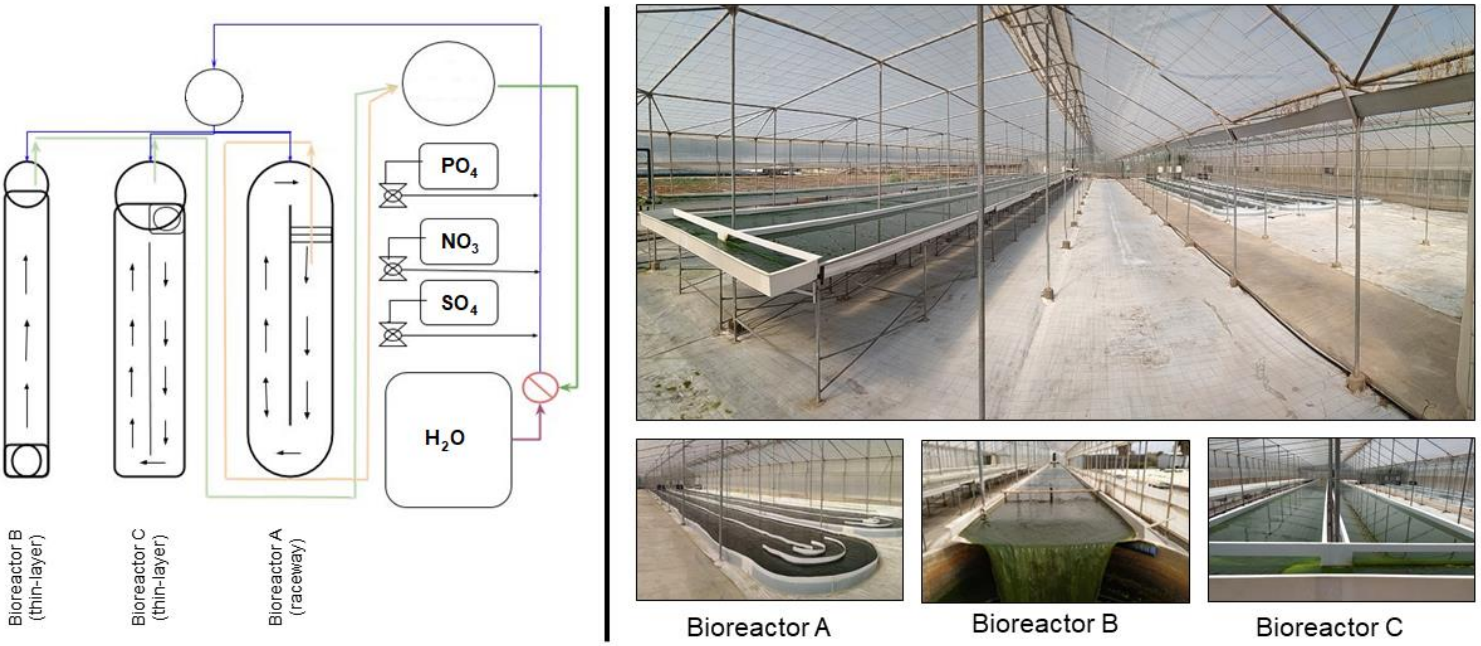

PO4
NO3
SO4
H2O
Bioreactor B (thin-layer)
Bioreactor C (thin-layer)
Bioreactor A (raceway)
Bioreactor A
Bioreactor B
Bioreactor C

Figure 2

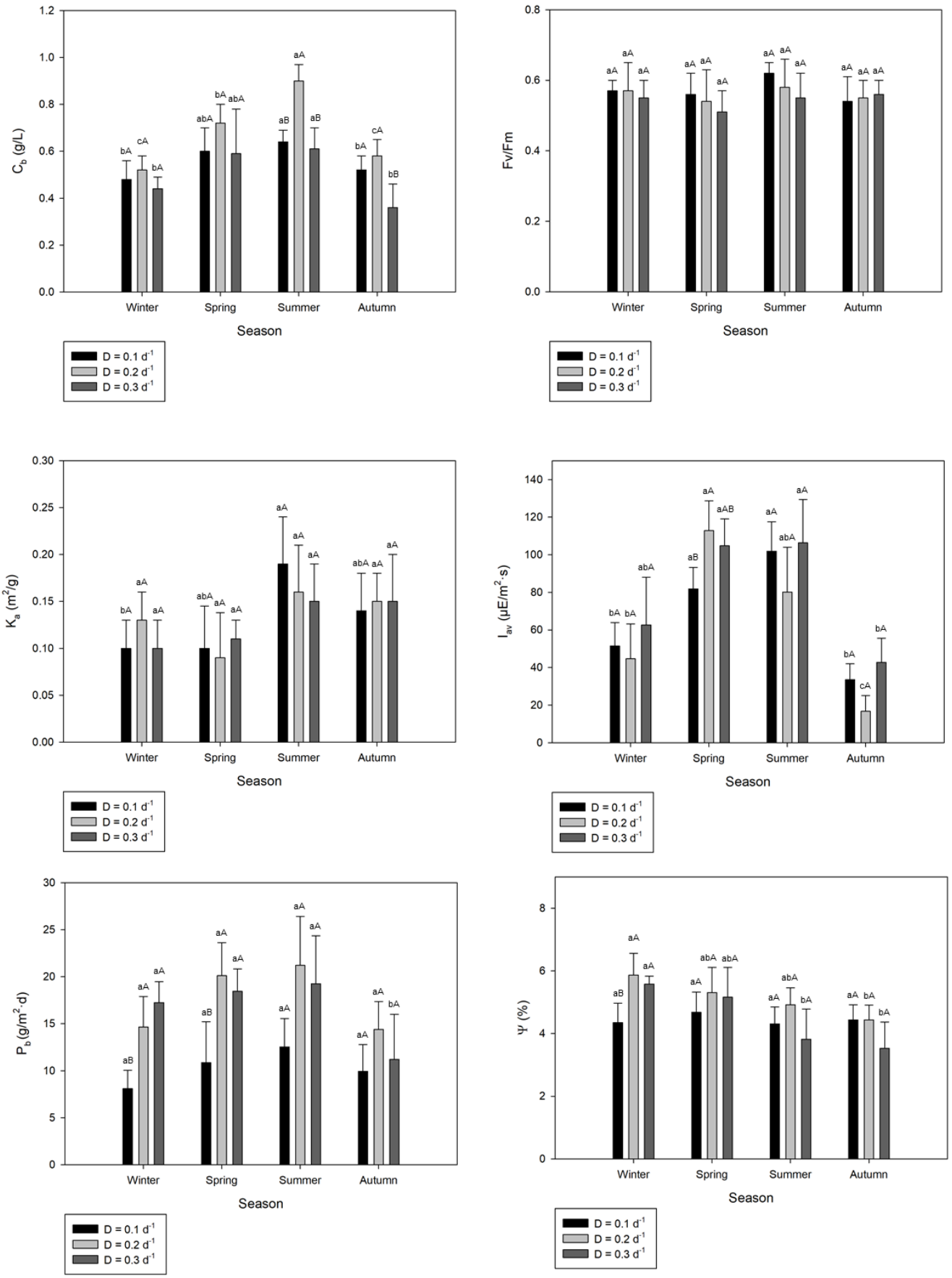

C_b (g/L)
Fv/Fm
K_a (m^2/g)
I_{av} ($\mu E/m^2 \cdot s$)
P_b ($g/m^2 \cdot d$)
Ψ (%)
Winter
Spring
Summer
Autumn
Season
D = 0.1 d^{-1}
D = 0.2 d^{-1}
D = 0.3 d^{-1}

Figure 3

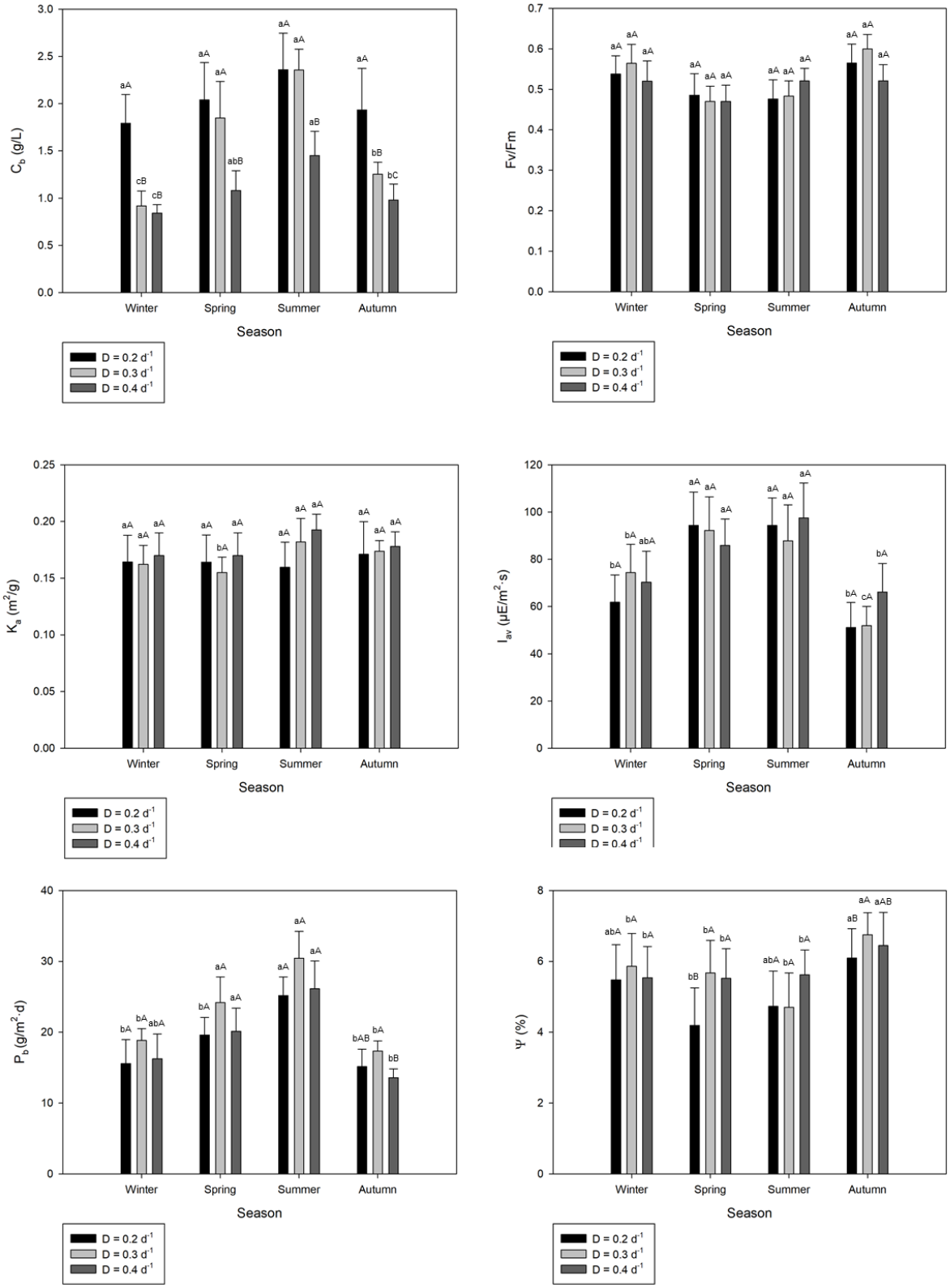

C_b (g/L)
Fv/Fm
K_a (m^2/g)
I_{av} ($\mu E/m^2 \cdot s$)
P_b ($g/m^2 \cdot d$)
Ψ (%)
Winter
Spring
Summer
Autumn
Season
D = 0.2 d^{-1}
D = 0.3 d^{-1}
D = 0.4 d^{-1}

Figure 4

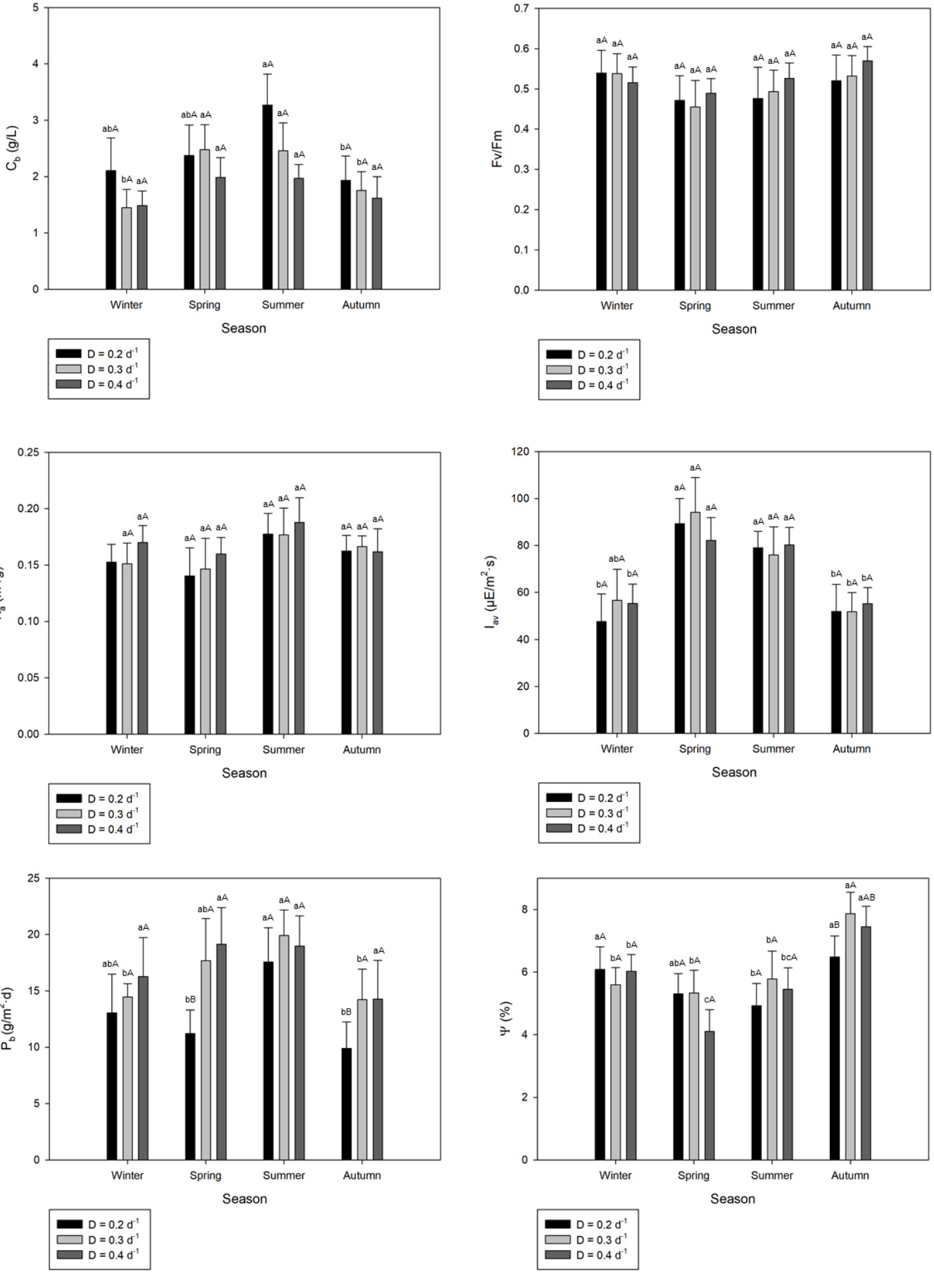

C_b (g/L)
Fv/Fm
K_a (m^2/g)
I_{av} ($\mu E/m^2 \cdot s$)
P_b ($g/m^2 \cdot d$)
Ψ (%)
Winter
Spring
Summer
Autumn
Season
D = 0.2 d^{-1}
D = 0.3 d^{-1}
D = 0.4 d^{-1}

Figure 5

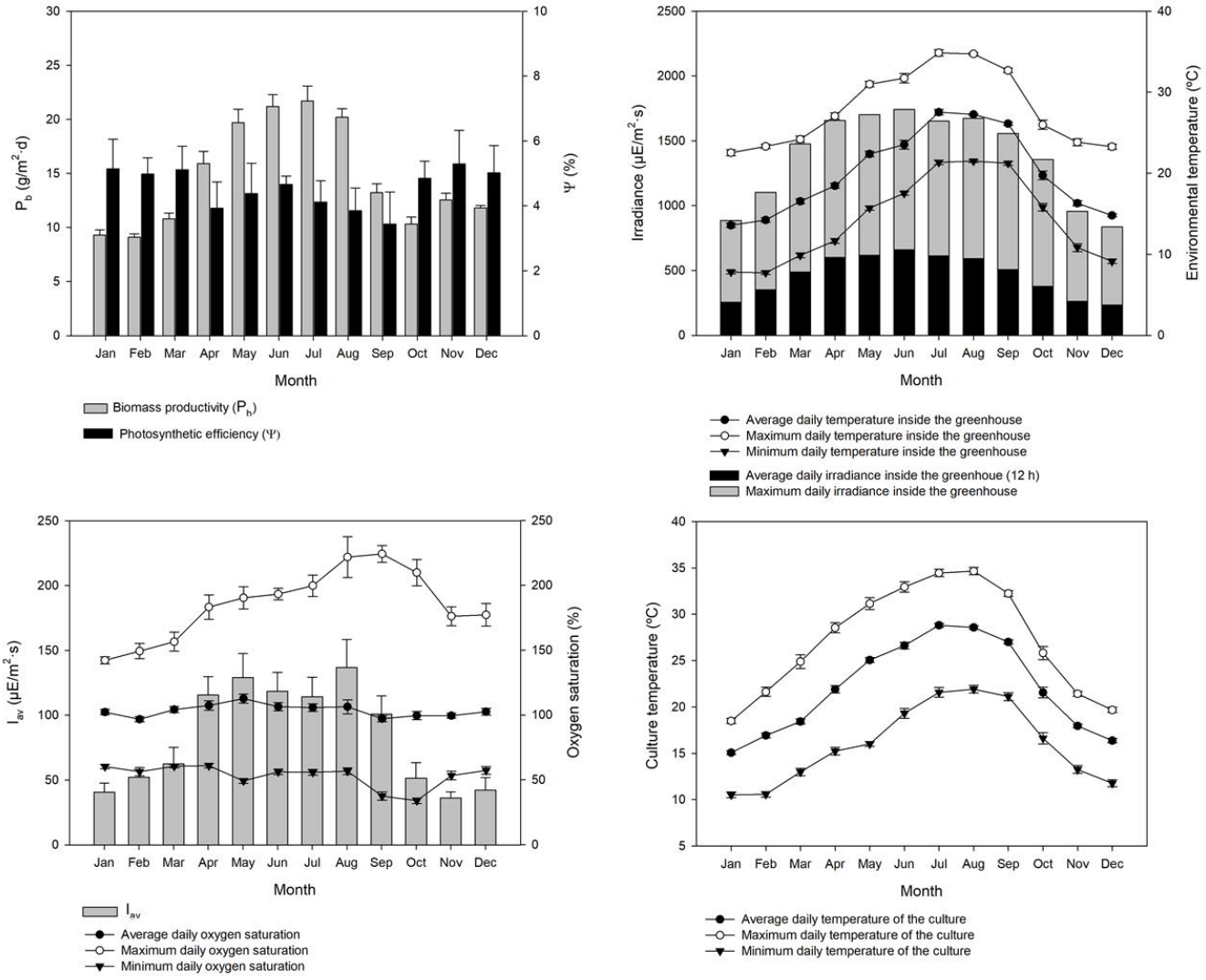

Pb (g/m2·d)
Ψ (%)
Month
Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec
Biomass productivity (Ph)
Photosynthetic efficiency (Ψ)
Irradiance (μE/m2·s)
Environmental temperature (ºC)
Average daily temperature inside the greenhouse
Maximum daily temperature inside the greenhouse
Minimum daily temperature inside the greenhouse
Average daily irradiance inside the greenhoue (12 h)
Maximum daily irradiance inside the greenhouse
Iav (μE/m2·s)
Oxygen saturation (%)
Iav
Average daily oxygen saturation
Maximum daily oxygen saturation
Minimum daily oxygen saturation
Culture temperature (ºC)
Average daily temperature of the culture
Maximum daily temperature of the culture
Minimum daily temperature of the culture

Figure 6

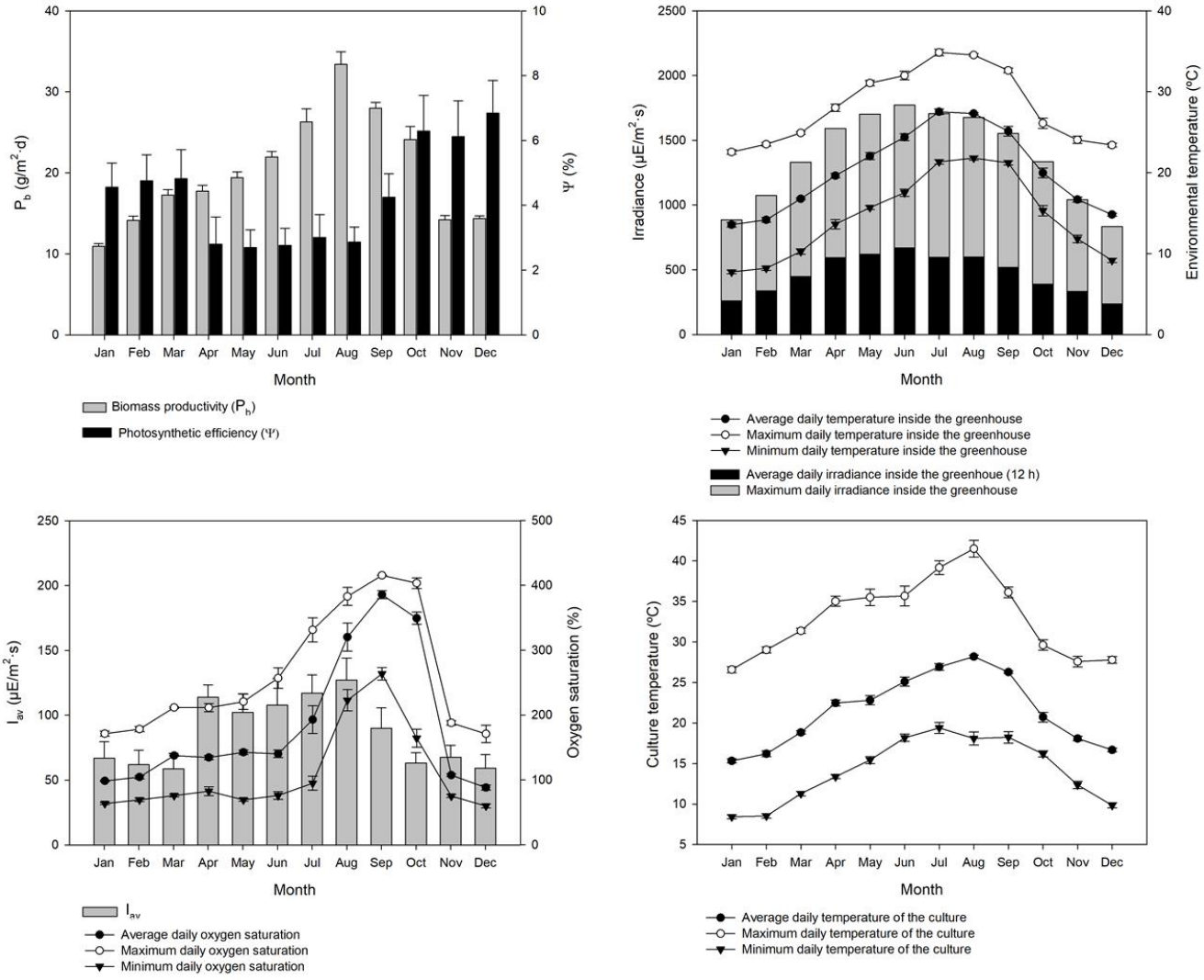
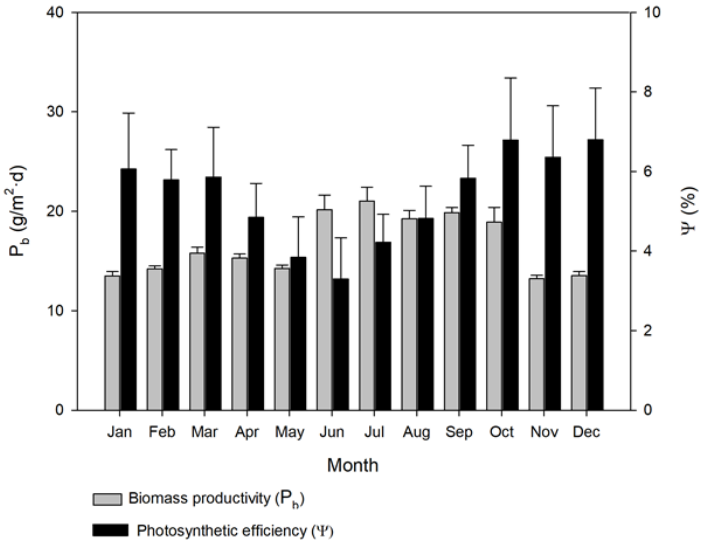
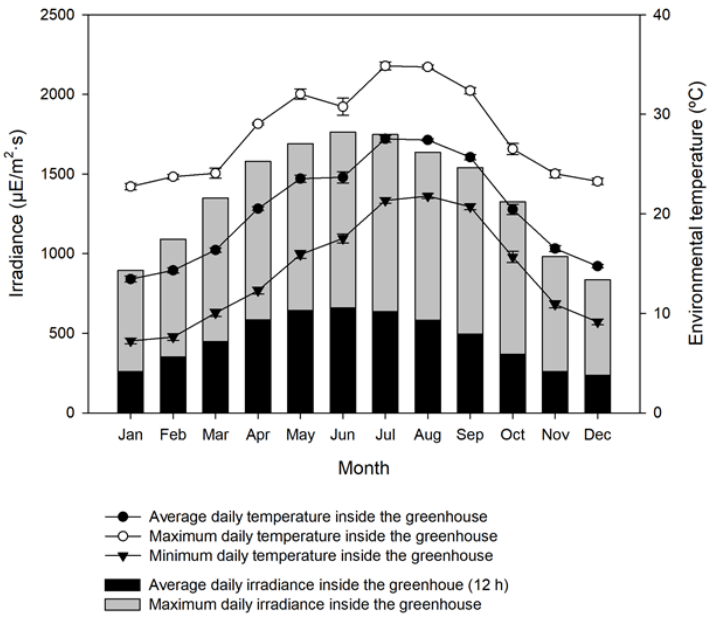
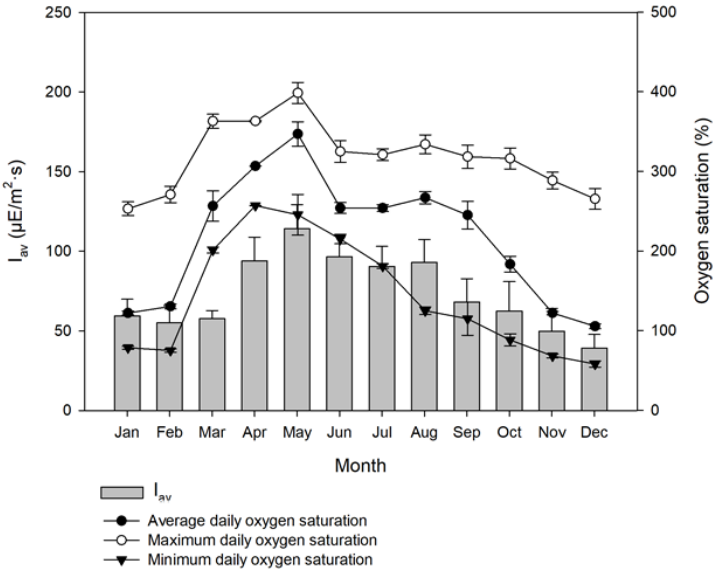
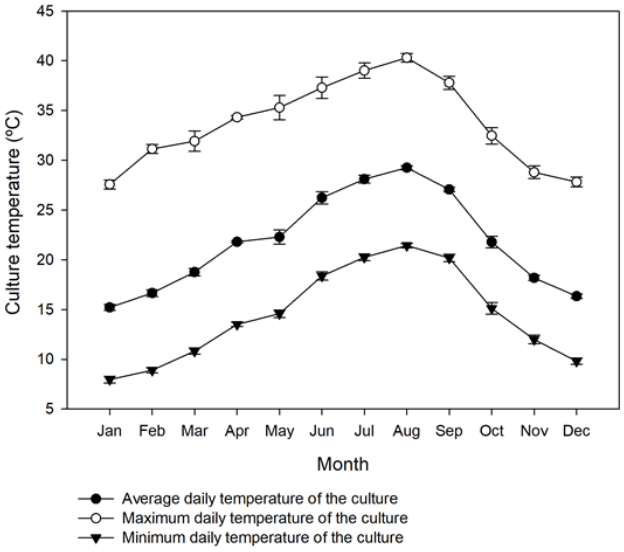

Pb (g/m2·d)
Ψ (%)
Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec
Month
Biomass productivity (Pb)
Photosynthetic efficiency (Ψ)
Irradiance (μE/m2·s)
Environmental temperature (ºC)
Average daily temperature inside the greenhouse
Maximum daily temperature inside the greenhouse
Minimum daily temperature inside the greenhouse
Average daily irradiance inside the greenhoue (12 h)
Maximum daily irradiance inside the greenhouse
Iav (μE/m2·s)
Oxygen saturation (%)
Iav
Average daily oxygen saturation
Maximum daily oxygen saturation
Minimum daily oxygen saturation
Culture temperature (ºC)
Average daily temperature of the culture
Maximum daily temperature of the culture
Minimum daily temperature of the culture

Figure 7


Pb (g/m2·d)
Ψ (%)
Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec
Month
Biomass productivity (Pb)
Photosynthetic efficiency (Ψ)


Irradiance (μE/m2·s)
Environmental temperature (ºC)
Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec
Month
Average daily temperature inside the greenhouse
Maximum daily temperature inside the greenhouse
Minimum daily temperature inside the greenhouse
Average daily irradiance inside the greenhoue (12 h)
Maximum daily irradiance inside the greenhouse


Iav (μE/m2·s)
Oxygen saturation (%)
Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec
Month
Iav
Average daily oxygen saturation
Maximum daily oxygen saturation
Minimum daily oxygen saturation


Culture temperature (ºC)
Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec
Month
Average daily temperature of the culture
Maximum daily temperature of the culture
Minimum daily temperature of the culture

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