Arthrospira platensis Cultivation in a Bench-Scale Helical Tubular Photobioreactor

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Abstract: Cultivations of *Arthrospira platensis* were carried out to evaluate the CO_2 capture capacity of this cyanobacterium under bench-scale conditions. For this purpose, the influence of light intensity on the microbial growth and the photosynthetic efficiency has been investigated in a helical photobioreactor. Five cultivations were performed at different photosynthetic photon flux densities ($23 \leq$ PPFD ≤ 225 µmol photons m^{-2} s^{-1}) by fed-batch pulse-feeding pure carbon dioxide from a cylinder into the helicoidal photobioreactor. In particular, a range of PPFD (82–190 µmol photons m^{-2} s^{-1}) was identified in which biomass concentration reached values (9–11 g_{DW} L^{-1}) significantly higher than those reported in the literature for other configurations of closed photobioreactors. Furthermore, as *A. platensis* suspensions behave as Newtonian and non-Newtonian (pseudoplastic) fluids at very low and high biomass concentrations, respectively, a flow analysis was carried out for evaluating the most suitable mixing conditions depending on growth. The results obtained in this study appear to be very promising and suggest the use of this helicoidal photobioreactor configuration to reduce CO_2 emissions from industrial gaseous effluents.

Keywords: carbon dioxide capture; fed-batch cultivation; *Arthrospira platensis*; helical tubular photobioreactor; light intensity

1. Introduction

Nowadays, microalgal mass cultivation in closed photobioreactors has attracted much interest [1]. Indeed, an overview of the literature published over the last two years up to the end of November 2021 has revealed that, contrary to what was observed in the last two decades, there has been an increase in the contributions related to "microalgal cultivation and closed photobioreactors", with no less than 761 scientific articles (based on the Science Direct search engine: http://www.sciencedirect.com, accessed on 27 November 2021). A substantial portion of them deals with "*Arthrospira platensis* cultivation and closed photobioreactors" (215 scientific articles). Since this topic is an active and expanding field of research, targeted studies in this area deserve further investigation.

In this regard, intense research has been carried out on plant engineering to optimize the production system as well as on microbiological aspects to improve culture productivity through the isolation, selection, and genetic transformation of strains, with the goal of expanding the range of competitive high-value microalgal compounds [2–5]. The achievement of high productivity in microalgal mass with minimum operating costs is, in

fact, fundamental to reduce the cost of valuable products and to broaden the commercial utilization of microalgae [6].

Microalgal cultivation technology has gained widespread attention because microalgae combine the renewable energy-capturing ability of photosynthesis with the high production rates of controlled microbial cultivation, which makes them potentially valuable organisms for cheap industrial production processes [5,7]. They represent the main natural source of useful and valuable molecules with numerous practical applications, for the majority of which the market is still developing; furthermore, the biotechnological use of microalgae is likely to extend to new areas. As for the application sectors, microalgae are nowadays used from human and animal nutrition [8] to aquaculture [9], pharmaceuticals [10], cosmetics [11], soil fertilization [12], environmental protection [13] including carbon dioxide fixation from industrial waste gases [14], biosorption of heavy metals [15], wastewater treatment [16], and production of biofuels [17].

Currently, the industrial priority is the development of pharmaceutical chemicals and biofuels [18]. Microalgae can, therefore, provide vast contributions on the future welfare of the planet by addressing the pressing issues of human health, food security, renewable energy sources, and global warming. Among the numerous high-quality products from biotechnology of microalgae, the most important regarding production amount and economic value is still the microalgal biomass itself. The worldwide yearly production of microalgal biomass is estimated to achieve 27,500 tons in 2024, with a turnover generation of about 1.1×10^9 US $ per year [19]. Among the tens of thousands of species that are believed to exist, just a handful are cultivated in industrial quantities (i.e., in tons per year) [20]. Due to their global presence, microalgae are generally used for human and animal nutrition. The commercial applications are restricted to four species, among which is the cyanobacterium *Arthrospira (Spirulina) platensis*, the object of this study. More than 70% of the overall production of *Arthrospira* sp. biomass is addressed to human nutrition, mainly as a nutraceutical, owing to its high protein content and its excellent nutritional value [21–24]. In addition, over 50% of the current world production of *Arthrospira* sp. is used as feed supplement for many types of animals [25].

To grow and exploit the potential of *Arthrospira* sp., efficient photobioreactors are required [26]. Currently, the commercial production of *A. platensis* is mainly performed in open systems [27], which are cheap and easy to operate, as they use solar radiation as a free source of energy [4]. However, they do not allow for reaching high biomass productivity, owing to the difficulty of keeping the optimum temperature; thus, they are restricted to the tropical and sub-tropical regions [28]. To overcome these problems, much widespread attention is now focused on closed systems, owing to their advantages over open cultivation systems (sterility, much greater control of cultivation conditions such as light intensity, carbon dioxide and nutrient levels, temperature, and higher biomass productivity). Particularly, a great deal of work has been done to develop suitable and efficient photobioreactors, such as flat-plate, tubular, vertical-column, and internally illuminated photobioreactors. Among them, tubular photobioreactors are often considered the most suitable ones for commercial large-scale mass cultures due to their large illumination surface area [29]. Most of these reactors are usually constructed with either glass or plastic transparent tubes, inside of which the culture is re-circulated either with mechanical pumps or preferably with airlifts. Regarding the shape, they can be horizontal/serpentine, vertical, L-shaped, nearly horizontal, conical, inclined, multi-tubular, or helical [30–36]. Some studies have shown that the parameters that most influence the growth in a tubular reactor are the tube size, mixing, and culture circulation [37], which in turn depend on the reactor configuration.

The global climate change is, today, one of the greatest environmental concerns. It is mainly due to the emission into the atmosphere of greenhouse gases, such as CO_2 produced by the combustion of fossil fuels [5]. An attractive alternative to reduce carbon dioxide emissions could be the use of photosynthetic microorganisms able to utilize this pollutant as a carbon source for their growth [5,7]. From this point of view, the photoautotrophic

cultivation of the cyanobacterium *A. platensis* would allow to produce valuable biomass using the CO_2 from industrial processes and other human activities as a carbon source [38].

Light supply and distribution are undoubtedly the most important factors influencing the productivity of photoautotrophic cultures and represent the main source of energy for *A. platensis*. The first studies about the response of *Arthrospira* sp. to light were carried out by Zarrouk [39], who discovered that the growth of *Arthrospira (Spirulina) maxima* was inhibited at light intensity above 25–30 klux. Later, Vonshak [40] demonstrated that inhibition of *A. platensis* growth takes place at values of photosynthetic photon flux density (PPFD) exceeding 150–200 mmol m^{-2} s^{-1}.

Photoinhibition, which is a reduction of the photosynthetic activities caused by the exposition to high PPFD, was well studied and documented for algae as well [41,42]. When the flux of absorbed photons is too high, the concentration of high-energy electrons in the cell is excessive, and they cannot be consumed in the Calvin cycle. These electrons, by reacting with water and forming hydrogen peroxide, damage the cell structure [43]. Even in densely populated *Arthrospira* sp. outdoor cultivations, photoinhibition can be observed when light intensity is 60 to 70% of full sunlight [44]. Further studies carried out by changing light intensity in photoautotrophic cultivations suggested an optimum irradiance in the range of 30–50 W m^{-2} [43].

Based on this background, fed-batch cultivations were performed in this work in a bench-scale vertical helical photobioreactor to check the performance of this reactor configuration under different conditions of light intensity and CO_2 feeding rate.

2. Materials and Methods

2.1. Microorganism and Cultivation Medium

The cyanobacterium used in this work, *Arthrospira platensis* UTEX LB 1926, was acquired from the University of Texas Culture Collection. The following information on this strain is provided by the supplier (https://utex.org/products/utex-lb-1926?variant=30992092299354#details, accessed on 20 January 2022): UTEX Number: 1926; class: Cyanophyceae; medium: Enriched Seawater Medium (ES); origin: San Diego, CA, USA; location: Del Mar Slough; isolation: R.A. Lewin in 1969; deposition: F.T. Haxo in 10/12/72; relatives: UTEX LB 1928; PCC 7345, PCC reference strain; ATCC 29408; notes: deposited as CY-42; 11/5/70 to Haxo from Lewin; renamed by R.C. Starr; taxonomy available in [45]. The inoculum for the photobioreactor was prepared in the medium of Schlösser [46], containing (g L^{-1}): NaHCO$_3$, 13.61; Na$_2$CO$_3$, 4.03; K$_2$HPO$_4$, 0.50; NaNO$_3$, 2.50; K$_2$SO$_4$, 1.00; NaCl, 1.00; MgSO$_4$·7 H$_2$O, 0.20; CaCl$_2$·2 H$_2$O, 0.04. The pH was 9.6. All nutrients were dissolved in distilled water containing (per liter): 6 mL of metal solution (97 mg FeCl$_3$·6 H$_2$O, 41 mg MnCl$_2$·4 H$_2$O, 5 mg ZnCl$_2$, 2 mg CoCl$_2$·6 H$_2$O, 4 mg Na$_2$MoO$_4$·2 H$_2$O), 1 mL of micronutrient solution (50.0 mg Na$_2$EDTA, 618 mg H$_3$BO$_3$, 19.6 mg CuSO$_4$·5 H$_2$O, 44.0 mg ZnSO$_4$·7 H$_2$O, 20.0 mg CoCl$_2$·6 H$_2$O, 12.6 mg MnCl$_2$·4 H$_2$O, 12.6 mg Na$_2$MoO$_4$·2 H$_2$O), and 0.15 mg of B12 vitamin.

Fed-batch cultivations were conducted in this medium lacking NaHCO$_3$ and Na$_2$CO$_3$ by adding daily pure carbon dioxide as a carbon source, according to the pulse-feeding mode of operation.

2.2. Culture Conditions

To avoid the need for time-consuming re-inoculation in each run, the cultivations were performed one after the next using a working volume of 4.0 L. The pH was initially adjusted and then controlled to 9.5 ± 0.2 by means of a NaOH 6.0 M solution. The continuous light intensity was ensured by a variable number (from 1 to 4) of 40 W-fluorescent lamps. The temperature was maintained at the optimum value for this microorganism (30 °C) [47] in a thermostatic chamber. CO_2 additions were performed daily by bubbling the selected amount of pure gas directly into the medium from a cylinder with a flowmeter.

2.3. Analytical Methods

The following parameters were determined during the experiments: pH, temperature, and concentration of biomass. Dry biomass concentration (X) was determined, after thrice washing and dilution with distilled water, from measurements of the culture absorbance at a wavelength of 560 nm (Abs_{560}) by means of the calibration curve $Abs_{560} = 0.0024 - 0.1129\ X$ ($R^2 = 0.9921$) [48]. Biomass concentration data have been expressed as the average of duplicate experiments. For this purpose, a Spectronic 21 UV-VIS Spectrophotometer (Milton Roy Company, Rochester, NY, USA) was used. The culture pH was measured with a pH meter, model 211 (Hanna Instruments, Woonsocket, RI, USA). Light intensity was measured with an illuminance meter, model TL-1 (Minolta, Osaka, Japan), as photosynthetic photon flux density (PPFD) and expressed as μmol photons $m^{-2}\ s^{-1}$. Measurements were performed at different points of the photobioreactor to ensure average PPFD values in the range of 23–225 μmol photons $m^{-2}\ s^{-1}$ in order to avoid either light limitation or photoinhibition.

2.4. Photobioreactor Design

The closed photobiorector (Figure 1) consisted of a cylindrical-shaped glassy helical photostage (1.5 cm in inner diameter with a glass thickness of 0.2 cm), linked via PVC tubes (10 cm, 1.6 cm, and 0.25 cm of length, inner diameter, and thickness, respectively) to a 5.0-L Erlenmeyer flask closed with a cotton cap and placed on a magnetic stirrer at 200 rpm. Circulation was granted by an airlift mechanism capable of ensuring a recycling flowrate in the range of 0.34–1.4 L min^{-1}. The vertical cylindrical photostage had a diameter of 1.6 cm and was 48 cm long. The overall volume was 4 L (0.6 L of photostage volume). The airlift system was set at a constant flowrate of 1.0 L min^{-1}, ensuring a residence time of 4 min. To avoid any atmospheric CO_2 accumulation in the photobioreactor due to the air used in the airlift device, the ambient air was first passed through two 4 L-closed vessels containing 2.0 M NaOH and distilled water, respectively, and then through a drier containing $CaCl_2$ before entering the system.
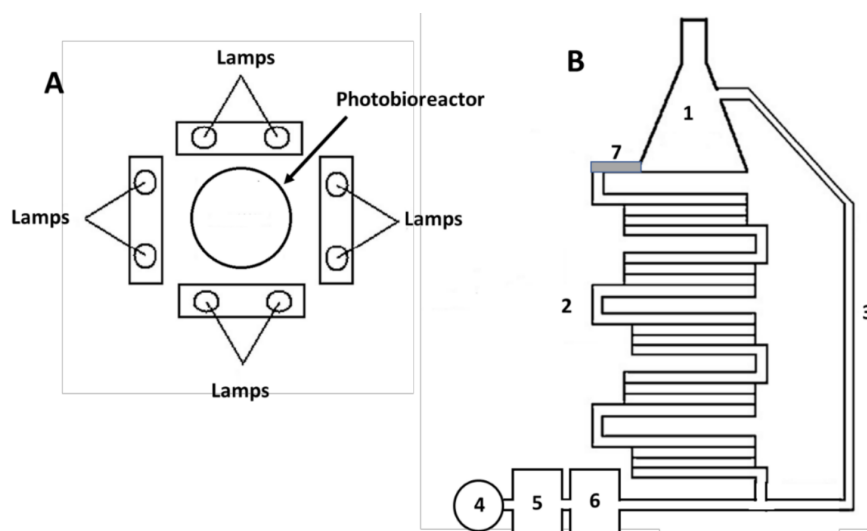

Figure 1. Schematics of the helicoidal photobioreactor utilized to cultivate *Arthrospira platensis*. Aerial (**A**) and frontal view (**B**). (1) 5.0-L Erlenmeyer flask; (2) cylindrical-shaped glassy helical photostage; (3) vertical cylindrical photostage; (4) air pump; (5) NaOH solution; (6) $CaCl_2$-containing closed vessels; (7) PVC connection.

2.5. Calculation of Growth Parameters

The start-up phase of growth was investigated since the beginning of each cultivation up to the achievement of pseudo-steady state conditions.

The maximum cell concentration (X_m) was determined at the end of start-up and the beginning of the steady state by absorbance measurements, as previously described.

The average volumetric cell productivity of the start-up (P_X) was estimated as the ratio of the difference between X_m and the initial biomass concentration (X_0) to the whole duration of the start-up (t_m):

$$P_X = \frac{X_m - X_0}{t_m} \tag{1}$$

The average specific growth rate (μ_X) of the microorganism was estimated by the equation:

$$\mu_X = \frac{1}{t_m} \ln \frac{X_m}{X_0} \tag{2}$$

The photosynthetic efficiency was calculated according to the following procedure. The PPFD, expressed in μmol photons m^{-2} s^{-1}, was converted to photosynthetic active radiation (PAR), expressed in kJ m^{-2} day^{-1}, using the conversion factor reported by Hall and Scurlock [49] for cold fluorescent light lamps (18.78 kJ s day^{-1}). Multiplying PPFD by the area exposed to the light (0.164 m^2), it was possible to obtain the input of PAR (IPAR), which allowed estimating the photosynthetic efficiency (PE) through the equation:

$$PE = \frac{r_D H_D}{IPAR} \times 100 \tag{3}$$

where r_D is the daily biomass production (g_{DW} day^{-1}) and H_D = 21.01 kJ g_{DW}^{-1} is the enthalpy of dried biomass [50,51].

3. Results and Discussion

Five cultivations of *Arthrospira platensis* were performed in duplicate at different photosynthetic photon flux densities (23 ≤ PPFD ≤ 225 μmol photons m^{-2} s^{-1}) by fed-batch pulse-feeding pure carbon dioxide from a cylinder into the helicoidal photobioreactor. In this way, it was possible to verify the influence of this variable on both the start-up profile and the pseudo-steady state biomass concentration (X_m), cell productivity (P_X), and photosynthetic efficiency (PE) of this system.

The start-up results of these cultivations in terms of biomass concentration and photosynthetic efficiency are illustrated in Figures 2 and 3, while those of parameters calculated at the beginning of pseudo-steady state conditions are summarized in Table 1.

In all the cultivations, biomass grew following the typical sigmoid behavior of batch growth curves and reached a threshold cell concentration under steady-state conditions, which may have been the result, depending on the light intensity conditions, either of lack of a limiting substrate or of possible photoinhibition occurrence. The maximum value of this growth parameter (X_m = 11.0 g_{DW} L^{-1}) was observed at PPFD = 190 μmol photons m^{-2} s^{-1}, which suggests that no photoinhibition occurred under these conditions, confirming the results of previous studies on this cyanobacterium [43,52].

The specific growth rate (μ_X) also increased with PPFD, but it reached a maximum value (0.62 day^{-1}) at quite lower PPFD (61 μmol photons m^{-2} s^{-1}), beyond which a quick decline was observed. This result suggests that the lack of a carbon source rather than photoinhibition may have been the growth limiting factor up to PPFD = 190 μmol photons m^{-2} s^{-1}. However, by further increasing the light intensity (PPFD = 225 μmol photons m^{-2} s^{-1}), the photoinhibition likely became the predominant factor because of the excess photon flux on both photosystems of the cyanobacterium with their consequent damage [43].

The volumetric cell productivity appeared to be influenced by PPFD, according to the following empirical second-degree polynomial equation:

$$P_X = 6.5 \times 10^{-3} \, PPFD - 2.0 \times 10^{-5} \, PPFD^2 \; (R^2 = 0.8064) \tag{4}$$

while the photosynthetic efficiency clearly decreased with increasing PPFD. However, despite the highest value of PE occurred at the lowest light intensity, these conditions led to the lowest X_m value.
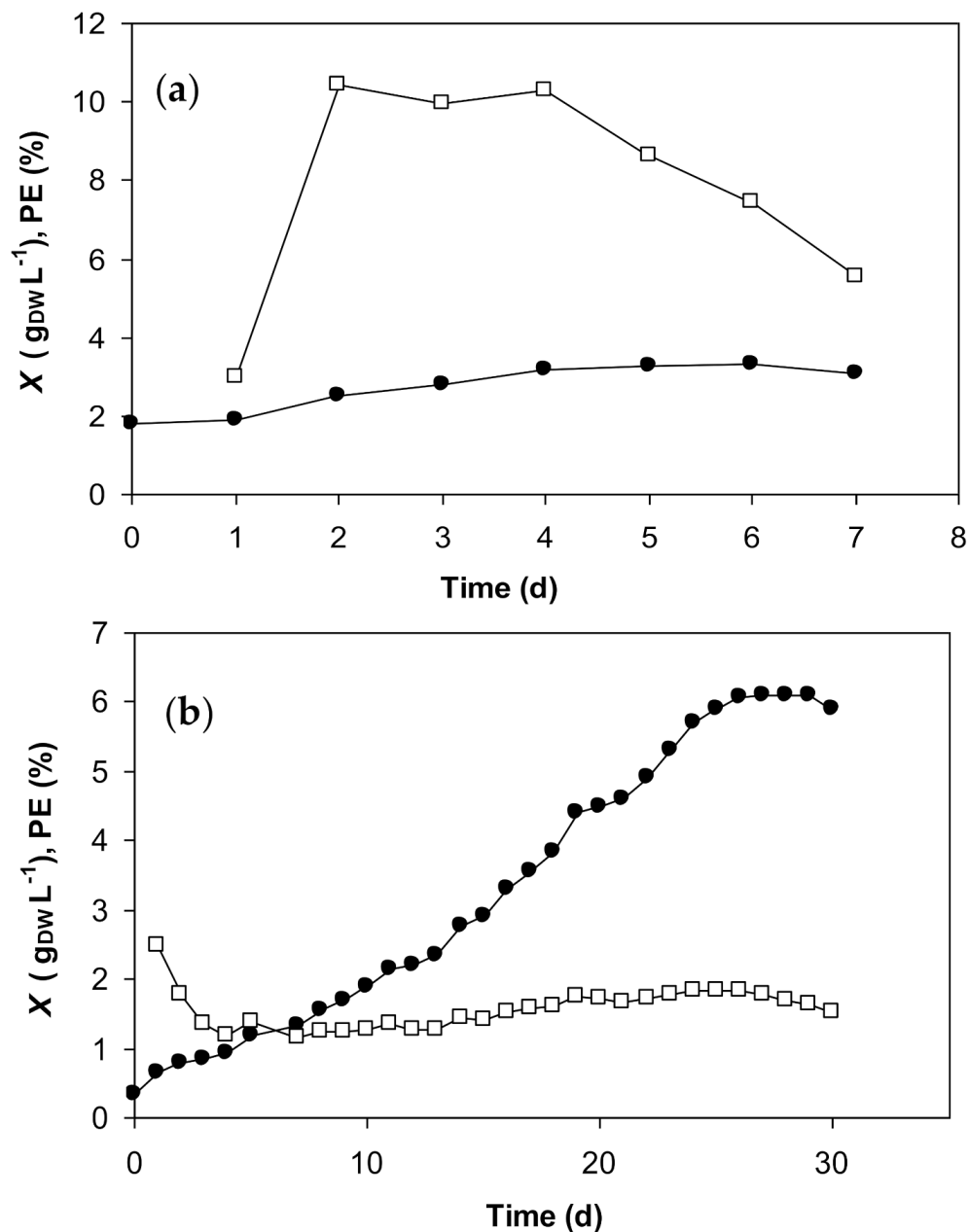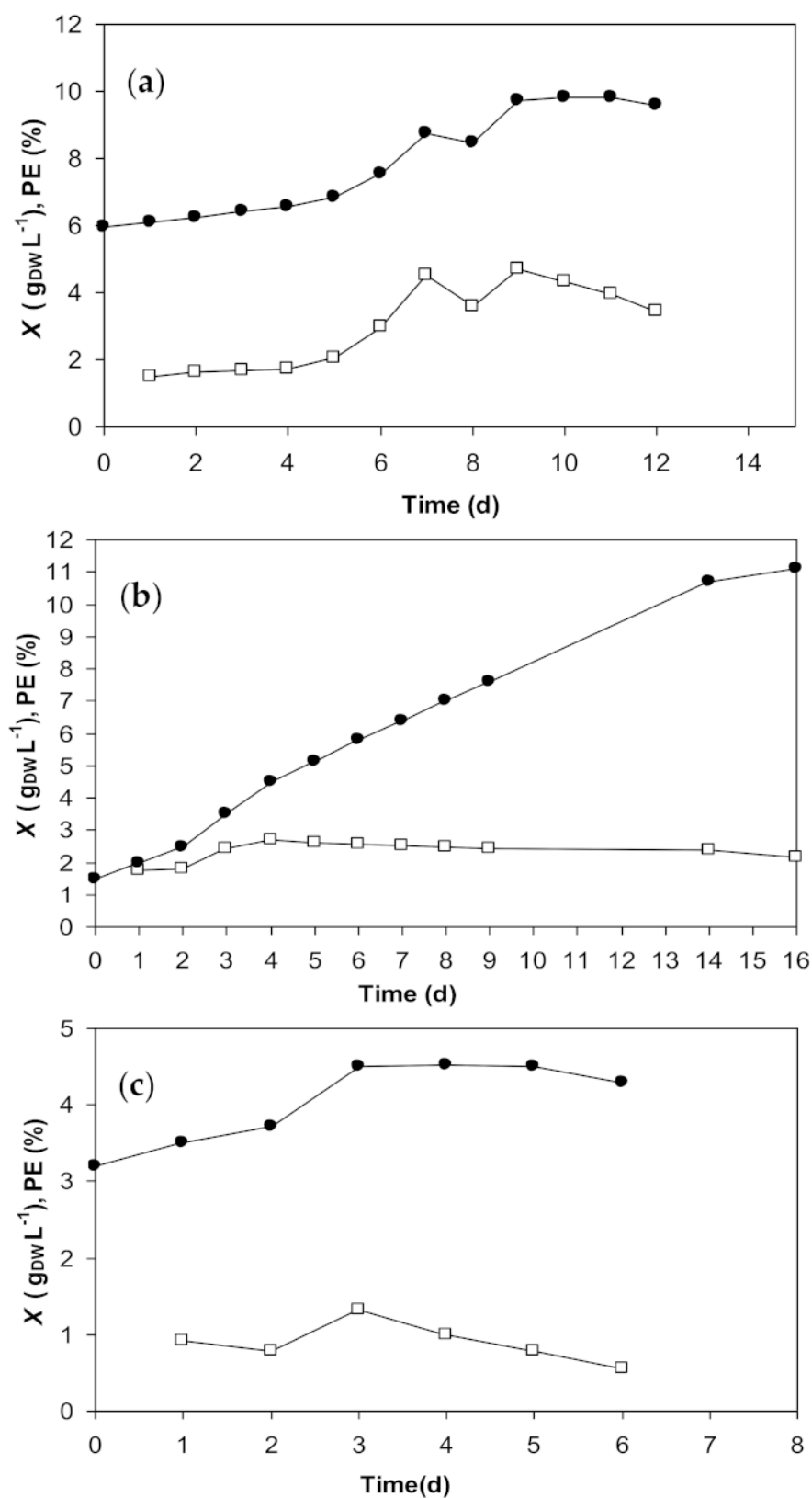

Figure 2. Time behaviors of biomass concentration (X, ●) and photosynthetic efficiency (PE, □) along fed-batch pulse-feeding cultivation of *Arthrospira platensis* in a helical photobioreactor at a PPFD of (**a**) 23 μmol photons m^{-2} s^{-1} and (**b**) 61 μmol photons m^{-2} s^{-1}.

Figure 3. Time behaviors of biomass concentration (*X*, ●) and photosynthetic efficiency (PE, □) along fed-batch pulse-feeding cultivation of *Arthrospira platensis* in a helical photobioreactor at PPFD of (**a**) 82 μmol photons m^{-2} s^{-1}, (**b**) 190 μmol photons m^{-2} s^{-1}, and (**c**) 225 μmol photons m^{-2} s^{-1}.

Table 1. Results of batch *Arthrospira platensis* cultivations carried out in the helical photobioreactor under different light intensities.

PPFD [1] (μmol photons m^{-2} s^{-1})	X_m [2] (g$_{DW}$ L^{-1})	P_X [3] (g$_{DW}$ L^{-1} day^{-1})	μ_X [4] (day^{-1})	PE [5] (%)
23	3.3 ± 0.3	0.21 ± 0.03	0.16	10.4 ± 0.94
61	6.1 ± 0.5	0.23 ± 0.02	0.62	2.49 ± 0.20
82	9.8 ± 0.7	0.42 ± 0.03	0.55	4.70 ± 0.34
190	11.0 ± 0.9	0.59 ± 0.06	0.28	2.62 ± 0.21
225	4.5 ± 0.5	0.43 ± 0.05	0.11	1.31 ± 0.14

[1] Photosynthetic photon flux density; [2] maximum cell concentration; [3] maximum cell productivity; [4] specific growth rate; [5] photosynthetic efficiency. Values of X_m, P_X, and PE are expressed as the average values of duplicate runs ± standard deviation.

Considering that, on an industrial scale, free sunlight is used as an energy source, to optimize the system for stable long term-production of biomass, the best conditions to ensure the maximum cell growth in the absence of any photoinhibition should be searched.

As mentioned earlier, these conditions were ensured in the proposed photobioreactor configuration at PPFD = 190 μmol photons m^{-2} s^{-1}, under which we obtained X_m = 11.0 g$_{DW}$ L^{-1}, whereas the maximum photosynthetic efficiency (PE = 10.4%) was obtained at the lowest light intensity (PPFD = 23 μmol photons m^{-2} s^{-1}). This finding demonstrates that an increase in PPFD, under non-photoinhibiting conditions, results in an increased dissipation of energy, which is not utilized for photosynthesis. Soletto et al. [53] observed a slightly lower PE in a similar horizontally arranged helical reactor (PE = 9.4%) but at a higher PPFD (125 μmol photons m^{-2} s^{-1}); however, the photoinhibition threshold was substantially lower (PPFD = 166 μmol photons m^{-2} s^{-1}).

A comparison with literature data shows that the helical configuration under consideration was able to guarantee a cell concentration substantially higher than those reported for open ponds (X_m = 0.84–1.21 g$_{DW}$ L^{-1}) [22] and two-plane tubular reactors (X_m = 4.2 g$_{DW}$ L^{-1}) [54]. In addition, although Duarte et al. [55] obtained the best growth parameters for *Arthrospira* sp. LEB 18 cultures in a tubular photobioreactor with a maximum biomass concentration about 63% higher (1.22 g$_{DW}$ L^{-1}) than in a raceway pond (0.72 g$_{DW}$ L^{-1}), these values were lower than those found in the present study.

From the results of μ_X listed in Table 1, one can propose the following empirical second-degree polynomial equation relating this kinetic parameter to PPFD:

$$\mu_X = 0.011 \, \text{PPFD} - 5.0 \times 10^{-5} \, \text{PPFD}^2 \quad (R^2 = 0.8498) \tag{5}$$

which, together with Equation (4), may be usefully employed to optimize this process on an industrial scale.

Equations (4) and (5) allowed for estimating two different PPFD ranges (100–120 and 155–170 μmol photons m^{-2} s^{-1}) within which cell productivity and specific growth rate reached maximum values of 0.527–0.528 g$_{DW}$ L^{-1} day^{-1} and 0.600–0.605 day^{-1}, respectively.

A flow analysis was finally done to check the suitability of mixing in the photobioreactor because *A. platensis* suspensions behave as Newtonian and non-Newtonian (pseudoplastic) fluids at low (<2 g L^{-1}) and high (>4 g L^{-1}) biomass concentrations [56], respectively. For this purpose, to investigate all the mixing conditions possibly occurring in the photobioreactor, the Reynolds number (N_{Re}) was estimated, according to that study, at culture flowrates in the range of the selected airlift system (0.33–1.4 L min^{-1}) using two biomass concentrations (0.05 and 10 g$_{DW}$ L^{-1}) representative of poor and excellent growth conditions, which were below and over the above threshold values, respectively.

As can be seen in Figure 4, the N_{Re} would range from about 100 and 600 under the former growth conditions and about 600 and 2500 under the latter, with respective values of about 400 and 1900 at the recycling flowrate selected for this study (1.0 L min^{-1}), corresponding to a residence time of 4 min and a culture speed of about 6.0 m min^{-1}. Even

though the higher value (1900) is about 5% lower than that (2100) required to ensure a turbulence regime [56], which is often considered preferable for satisfactory mixing, it is worth reminding that the mechanical stress under these conditions could lead to the damage of cyanobacterial thricomes [57].
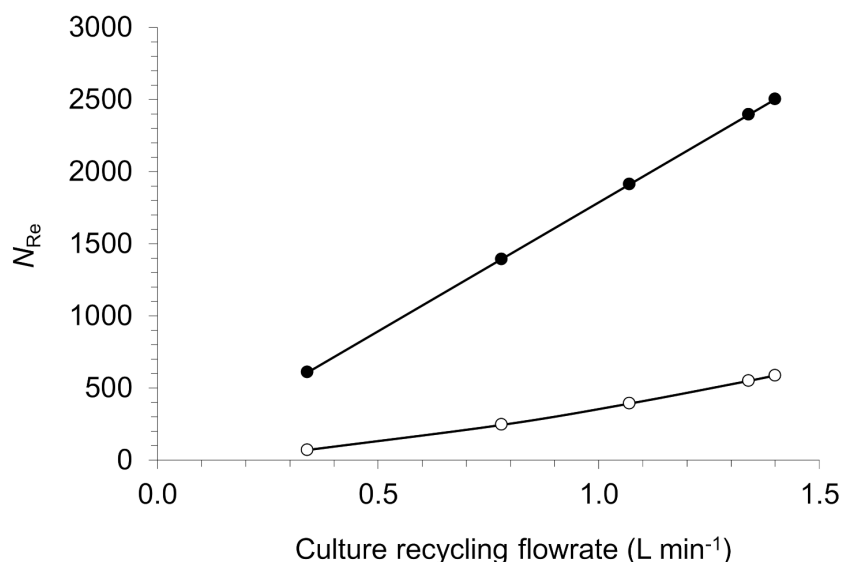

Figure 4. Reynolds number calculated for the helical photobioreactor as a function of the culture recycling flowrate at two different cell concentrations ($g_{DW} L^{-1}$): (○) 0.05; (●) 10.0.

4. Conclusions

Overall, the results obtained in this study appear very promising and suggest the use of this helical photobioreactor configuration to reduce CO_2 emissions from industrial gaseous effluents. A wide range of light intensity (82–190 µmol photons $m^{-2} s^{-1}$) was identified in which biomass concentration reached values (9–11 $g_{DW} L^{-1}$) significantly higher than those reported in the literature for other configurations of closed photobioreactors, which constitutes a further advantage of the system, considering the natural variability of this parameter in nature. A flow analysis that was performed to check the suitability of mixing within the photobioreactor suggested that such a good performance may be ascribed to the ability of the airlift system to minimize mechanical stress to cells. Remembering that the optimal results of this work were obtained under lower light intensity than sunlight, with the due precautions they can be useful for designing a real-scale plant to reduce carbon dioxide emissions from industrial gaseous effluents. Future efforts in this field will deal with continuous application of this photobioreactor configuration, either for the autotrophic capture of CO_2 from gaseous emissions or for the mixotrophic treatment of industrial wastewater.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

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